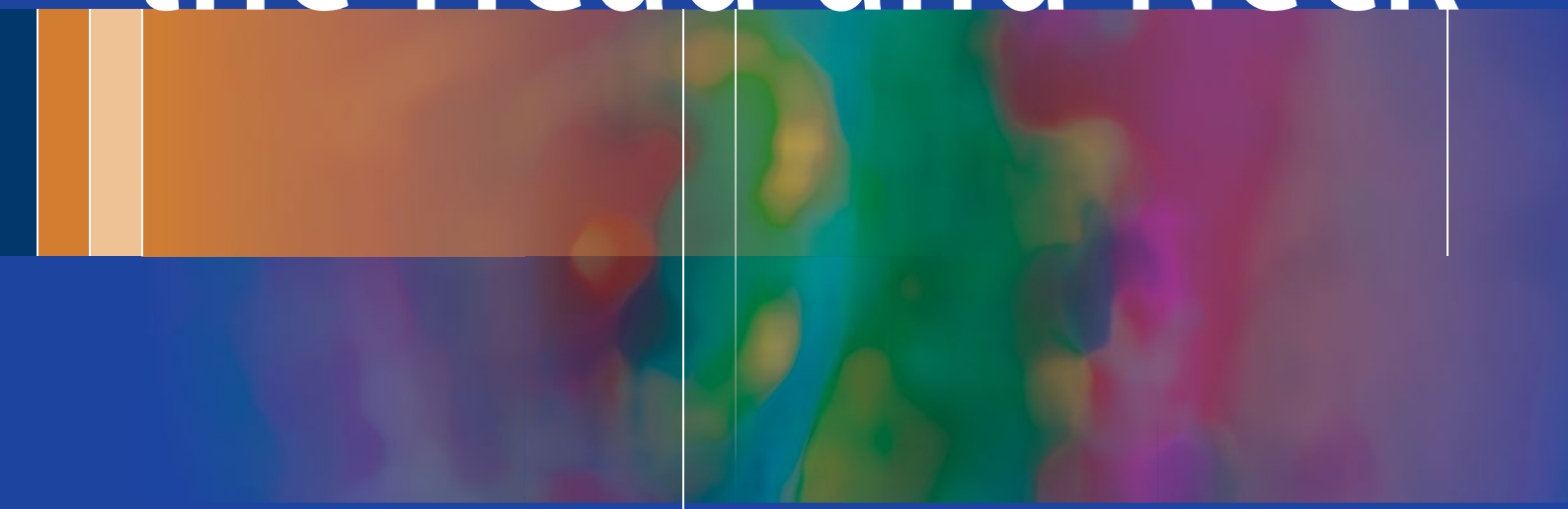
Antonio Cardesa
Pieter J. Slootweg
Nina Gale
Alessandro Franchi
Editors

Pathology of the Head and Neck



Second Edition

Springer

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Editors
Antonio Cardesa
University of Barcelona
Anatomic Pathology Hospital Clínic
Barcelona
Spain

Pieter J. Slootweg
Radboud University Nijmegen Medical Center
Pathology
Nijmegen
The Netherlands

Nina Gale
University of Ljubljana
Institute of Pathology, Faculty of Medicine
Ljubljana
Slovenia

Alessandro Franchi
University of Florence
Department of Surgery and Translational Medicine
Florence
Italy

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To Our Families:
For all the time and attention we have taken away from them while writing and editing this book.
Their patient support and understanding was a source of energy.
Their positive attitude to life has been a continuous stimulus to improve.
Their warm surrounding was a well of creative inspiration.

Preface

The head and neck is a remarkable territory that, the encephalon excepted, conventionally encompasses all the anatomic structures extending proximally from the frontal sinuses, orbits, roof of the sphenoidal sinuses and clivus to distally the upper borders of the sternal manubrium, clavicles, and first ribs. Central to this region, stand out the complex and vital organs where the upper respiratory airway and the upper digestive tract meet and cross.

To cover in detail the pathology of this intricate part of the body, the new edition, while retaining the ten initial chapters, all updated and improved, contains seven entirely new chapters that expand the knowledge on additional organs, systems, and techniques not previously covered, as well as on multifocal and systemic diseases that, although having their main focus in other territories, present distinctive features when involving the head and neck.

From the 17 chapters of this second edition, the first covers the spectrum of precursor and neoplastic lesions of the squamous epithelium. It is followed by chapters devoted to nasal cavities and paranasal sinuses, oral cavity, maxillofacial skeleton and teeth, salivary glands, nasopharynx and oropharynx, larynx and hypopharynx, ear and temporal bone, neck and neck dissection, eye and ocular adnexa, neuroendocrine neoplasms and paraganglioma, soft tissue tumors, lymphoid lesions, thyroid and parathyroid, skin tumors, cytology, as well as gross examination, dissection, evaluation, reporting, and staging.

Since the publication of the first edition in 2006, important progress in knowledge of diseases and in technical developments has taken place throughout. Therefore, attention has been paid to current correlations of pathology with epidemiology, clinical features, pathogenesis, biomarkers, and molecular genetics. Timely information is provided on advances in differential diagnoses, staging, prognosis, and therapy. New entities and lesions not addressed in the original edition are also incorporated. The number of illustrations has been substantially increased.

The authors selected for writing the different chapters are international experts and senior members or invitees of the Working Group on Head and Neck Pathology of the European Society of Pathology. Our best thanks to all of them, for their dedication and excellent work. Our great thanks to Leslie Michaels, a foremost leader of the pathology of the ear, who being unable to participate this time in the authorship, he generously permitted to use a part of his text and figures of the previous edition in the current one. The thanks are added to those colleagues who kindly provided the authors with unique illustrations, as well as to those secretaries, photographers, and others who helped them. We want also to express our special thanks to the publisher Springer for their stimulating support and permanent trust.

Finally, we have to deeply regret the recent loss of two dear and unforgettable members of our Working Group, Gerhard Seifert and Mario A. Luna, both great champions of the

pathology of the salivary glands, the former a founding father of our group and the latter author of one of the chapters of this book. Their seminal contributions to the pathology of the head and neck will remain in our memory forever.

Barcelona, Spain — Antonio Cardesa
Nijmegen, The Netherlands — Pieter J. Slootweg
Ljubljana, Slovenia — Nina Gale
Florence, Italy — Alessandro Franchi
May 2016

Contents

Benign and Potentially Malignant Lesions of the Squamous Epithelium and Squamous Cell Carcinoma

1

Nina Gale, Nina Zidar, Antonio Cardesa, and Alfons Nadal

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N. Gale (✉) • N. Zidar, MD, PhD
Faculty of Medicine, Institute of Pathology, University of Ljubljana, Korytkova 2, 1000 Ljubljana, Slovenia
e-mail: nina.gale@mf.uni-lj.si; nina.zidar@mf.uni-lj.si

A. Cardesa • A. Nadal
Department of Anatomic Pathology, Hospital Clinic, University of Barcelona, Villarroel 170, 8036 Barcelona, Spain
e-mail: acardesa@clinic.ub.es; anadal@clinic.ub.es

1.1 Introduction

The chapter is focused on the three main groups of lesions of the covering squamous epithelium of the oral cavity and larynx. The first part treats squamous cell papillomas and related, viral-induced lesions with the main stress on laryngeal recurrent papillomatosis.

The second part is dedicated to squamous intraepithelial lesions (SILs), which still represent one of the most controversial topics in oral and laryngeal pathology. The modified Ljubljana classification provides clear morphological criteria for defining the prognostic groups of SILs squamous intraepithelial lesions and could acts as a model to a unified classification of the head and neck region. The third and the most extensive part is devoted to invasive squamous cell carcinoma (SCC) and its nine variants, including spindle cell carcinoma, verrucous carcinoma, papillary SCC, basaloid squamous cell carcinoma, non-keratinizing human papillomavirus positive SCC, adenoid squamous cell carcinoma, adenosquamous carcinoma and lymphoepithelial carcinoma. The second primary SCCs, which have a much lower 5-year survival than the primary tumors in the head and neck region, are also discussed. All manners of spreading and metastasising of the SCCs are widely discussed with the point on significant predictors of

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patients' survival. The chapter concludes with a comprehensive review of the already known molecular events in carcinogenesis of head and neck SCC.

1.2 Squamous Cell Papilloma and Related Lesions

General considerations Benign, exophytic, papillary or verrucous lesions of the squamous epithelium of the oral cavity, oropharynx and larynx include similar entities, such as squamous cell papilloma (SCP), verruca vulgaris (VV), condyloma acuminatum (CA) and focal epithelial hyperplasia (FEH), also known as Heck's disease. However, not every papillary lesion in these areas can be reliably placed into one of the listed categories. It seems that the majority of lesions are similar variants of papillary proliferations, all induced by infections with different genotypes of human papillomaviruses (HPV), showing more or less overlapping clinical and morphological attributes but different biological behaviour, ranging from rather inconspicuous to potentially life threatening. Classification of these changes into infectious (VV, CA, FEH) and neoplastic (SCP) is thought to be fairly inconsistent and not well founded. Papillary lesions, except laryngeal papillomatosis, generally have a favourable outcome.

1.2.1 Oral Squamous Cell Papilloma, Verruca Vulgaris, Condyloma Acuminatum and Focal Epithelial Hyperplasia

Definition SCP, the most frequent papillary lesion of the oral cavity and oropharynx, is characterised as an exophytic papillary lesion, composed of fibrovascular projections covered by a benign proliferation of the squamous epithelium and induced by HPV infection.

VV is a rare intraoral lesion resembling its dermal counterpart, characterised as a solitary or multiple papules with verrucous surface and histologically classified as a wart-like hyperplasia of the squamous epithelium.

CA are usually larger than SCP, multiple, dome-shaped nodular lesions, resembling anogenital CA, which mainly appear on the lips and soft palate.

FEH is a rare oral lesion of children characterised by multiple sessile or elevated papules, usually distributed over the buccal, labial and tongue mucosa.

Epidemiology SCP is most frequently located on the tongue and soft palate but may appear on any epithelial surface of the oral cavity [1, 2]. It occurs most commonly between 30 and 50 years but can also be seen over a broad spectrum of ages. Males are slightly more often affected than females [1, 3].

VV rarely occurs in the oral cavity; frequently affected sites are the labial mucosa of the lower lip and the vermilion border of both lips. The lesions, which are seen mainly in children, result from autoinoculation of HPV from VV on the fingers [2].

CA is a rare, sexually transmitted lesion of adults. Common locations of CAs are the lips, tongue and gingival.

FEH is a rare, HPV-induced, contagious disease, initially described among the Native American population. FEH have also recently been published from other parts of the world. Small multiple lesions occur on the labial and buccal mucosa and tongue. The disease commonly occurs in children and young adults [2, 4].

Etiology and pathogenesis Oral mucosa can be contaminated by HPV by various pathways, including sexual contacts, autoinfection and perinatal infection. Low-risk HPVs, which mainly induce the whole spectrum of oral papillary lesions, are also present in healthy persons; the prevalence of HPV detection in normal oral mucosa ranges from 0.6 to 81 % [5, 6]. Several low-risk HPV genotypes have been detected in oral papillary lesions, although it is not easy to establish an accurate HPV type for each separate papillary lesion due to variations in tissue samplings, various ethnic and geographic origins of patients and the use of non-molecular vs. molecular methods for HPV detection, with different levels of sensitivity and specificity. SCPs are mainly related to HPV genotypes 6 and 11 [2], VV to HPV genotypes 2 and 4 [7], CA to HPV genotypes 6, 11, 16 and 18 [8] and FEH to HPV genotypes 13 and 32 [2]. The pathogenesis of papillary lesions caused by low-risk HPV has not yet been elucidated [9].

Macroscopy SCP is usually a single, pedunculated, white or pink lesion, consisting of fingerlike projections of the oral mucosa (Fig. 1.1a). It may be sessile with a granular or verrucous surface. The lesion, usually smaller than 1 cm, grows rapidly and has a predilection for the hard and soft palate and lateral border of the tongue [1, 2, 10].

VVs are frequently multiple, rough-surfaced sessile lesions of whitish colour.

CAs are characterised as small, sessile pink papules, which can combine into a larger cauliflower lesion.

FEHs are sessile, well-demarcated, round or ovoid flat lesions; they can appear in clusters and measure up to 10 mm in diameter;

Microscopy SCP is composed of narrow papillary projections of soft fibrous stroma covered by keratotic or parakeratotic, hyperplastic squamous epithelium, usually with normal maturation. Rarely, basal–parabasal cell hyperplasia is seen, as well as an increased number of mitoses (Fig. 1.1b).

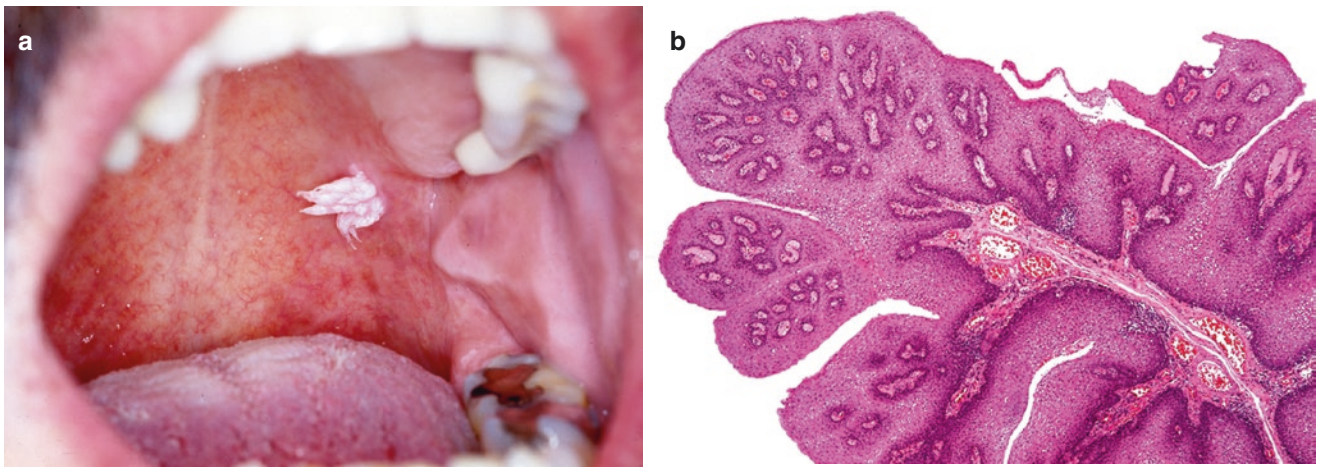

Fig. 1.1 Oral squamous cell papilloma. (**a**) Whitish papillary lesion of the palate (Courtesy of Dr. J. Fischinger, Ljubljana, Slovenia). (**b**) Projections of fibrovascular stroma are covered by parakeratotic squamous epithelium

Koilocytosis, the only visible cytopathic effect of HPV infection, caused by viral replication in the upper intermediate and superficial zone of the squamous epithelium, is rarely visible in SCPs. The characteristics of koilocytes are described in the paragraph on laryngeal papillomatosis. VV shows similar histological features, but peripheral papillary projections are usually centrally inverted and koilocytosis and the granular layer are prominent. The base of the lesion is usually broad and flat. CA is histologically described as a broad papillary proliferation with koilocytosis and parakeratosis on the surface of the epithelium and bulbous rete ridges with a possible extension into the ducts of the minor salivary glands [11]. Papillary projections in FEH are blunt; the hyperplastic and acanthotic epithelium shows numerous koilocytosis and apoptotic bodies, and prominent rete ridges are frequently fused (Fig. 1.2).

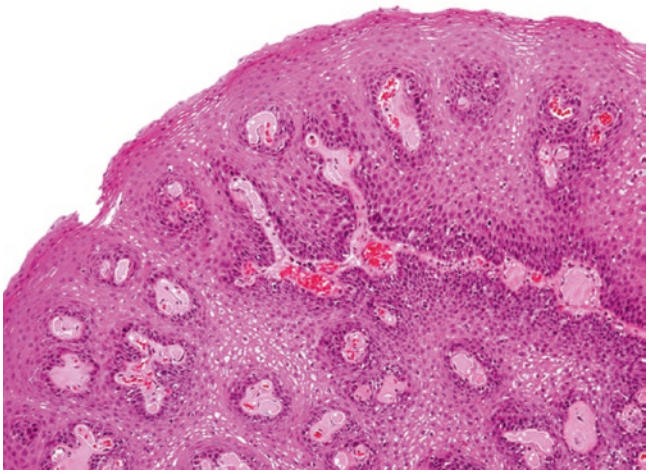

Fig. 1.2 Focal epithelial hyperplasia (Heck's disease). Prominent acanthosis of the squamous epithelium with broad rete ridges, papillary surface is not present

Molecular genetic methods for HPV detection They are described in the paragraph on LSCPs.

Differential diagnosis SCPs can be distinguished from other benign HPV-induced papillary lesions on the basis of site of occurrence, age of patients and morphological differences. However, it is important to distinguish SCPs from a papillary variant of SCC, which is characterised by fibrovascular projections covered by a neoplastic squamous epithelium with or without invasion.

Verrucous carcinoma (VC), as an additional differential diagnostic possibility, also displays a prominent papillary surface, usually with abundant keratinization, but an evident downgrowth of bulbous epithelial projections without atypias favours a diagnosis of VC. SCPs in patients with acquired immunodeficiency syndrome (AIDS) are usually multiple papillary lesions with prominent epithelial atypias. In these cases, SCPs have to be differentiated from SCC [12].

It is also important to distinguish FEH from CA when there is concern about possible sexual abuse of children. Both lesions are induced by HPV infection, although the former lesion is not a sexually transmitted disease, caused by HPV 13 and 32; the latter is a result of direct orogenital contact or self-inoculation and associated with HPV 6 or 11 [13].

HPV-induced papillary lesions of the oral cavity have to be distinguished from a rare congenital disease, systemic epidermal nevus syndrome, which can also involve oral mucosa in children, with confluent papulous and verrucous lesions (Fig. 1.3). Histologically they resemble SCPs but without koilocytes; genetically, the syndrome represents mosaicism

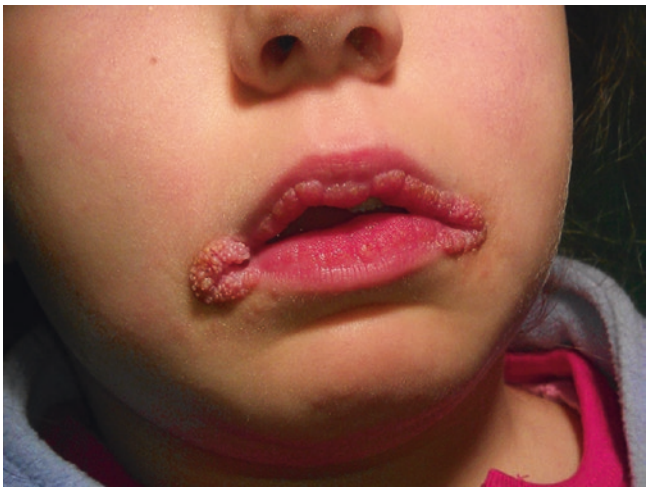

Fig. 1.3 Congenital systemic epidermal nevus syndrome. A confluent papulous and verrucous lesion has to be separated from HPV-induced papillary lesions of the oral cavity (Courtesy of Dr. T. Dovšak, Ljubljana, Slovenia)

of the skin and mutations in the *FGFR3* gene with possible oral and other extracutaneous manifestations [14].

Treatment and prognosis The treatment of SCPs and related papillary lesions is surgical removal. The infectivity of HPV in SCPs is very low and recurrences uncommon, except in lesions associated with human immunodeficiency virus (HIV) infections. Recurrences are more common in CA. No special treatment is required for FEH unless the lesions are extensive because spontaneous resolution may occur in a few months or years. Recurrences of the disease, even following spontaneous regression, are common [13].

1.2.2 Laryngeal Squamous Cell Papilloma/ Papillomatosis

Definition Laryngeal squamous cell papillomas (LSCPs) are the most common benign laryngeal epithelial tumors. They are induced by infection with low-risk HPV, types 6 and 11 [15–22]. LSCPs are composed of branching fibrovascular cores, covered by squamous epithelium. Because of their clinical specificities, such as multiplicity, recurrences and propensity to spread to adjacent areas, it has been suggested that LSCP be renamed recurrent respiratory papillomatosis. Due to a characteristic bimodal age of distribution, LSCPs are additionally divided into juvenile and adult groups [9, 23–27].

Epidemiology The incidence of LSCPs is low compared to the risk of exposure to HPV 6 and HPV 11, which happens frequently during life. It has been reported that HPV DNA can be detected in the upper airway mucosa in as many as 25 % of normal non-affected children and adults [28, 29].

The increase of prevalence of HPV cervical infection in women has been reflected in an increase of juvenile LSCPs; it is estimated that juvenile LSCPs are present in 4.3/100,000 children and 1.8/100,000 adults in the USA; the incidence of LSCPs has been reported as higher in patients of lower socioeconomic status [9, 25]. In a Danish study incorporating 50 % of the population of the country, the overall incidence of LSCPs was 3.84/100,000 [30].

LSCPs are a well-known disease historically, having first been recognised as a distinct disease of children by Sir Morell Mackenzie in 1880 [27]. It has subsequently become obvious that the disease affects persons of all ages, although on the basis of a characteristic bimodal age distribution, LSCPs are usually divided into juvenile and adult group [19, 20, 31–35]. The first incidence peak appears before the age of 5 years, with no gender predominance. The second incidence peak is between 20 and 40 years of ages, with a slight male predominance [20, 31–35].

Etiology and pathogenesis LSCPs are aetiologically related to HPV infection, which is considered a common sexually transmitted disease. HPV 6 and 11 are the most frequent genotypes associated with LSCP [15–21]. Perinatal transmission from an affected mother to a child is traditionally accepted. A history of maternal condylomata during pregnancy has been associated with a 200-fold risk of LSCPs in children [36]. Patients with the juvenile form were more likely to have been born to teenage mothers and to be the firstborn child, compared to controls. Infection of adults is more likely to be related to sexual transmission [27].

The mechanism, by which HPV 6 and HPV 11 alter cellular growth and cause papillary lesions, has only been partially elucidated [9]. A microtrauma of the laryngeal epithelium enables entrance of HPV types 6 or 11 into basal epithelial cells. The receptor has not been definitely identified, but α6-integrin and heparan sulphate may play important roles in virus entry [27]. Viral persistence in extrachromosomal (episomal) maintenance alters cellular growth and viral replication, which contributes to the protraction and spread of the disease. Considerable information is available concerning the pathogenesis of high-risk HPV types producing SCC, but little is known about the life cycle of alpha low-risk HPV types, such as HPV 6 and HPV 11 [37–39]. The two key viral genes and their proteins, E6 and E7, of high-risk HPV 16 are responsible for the immortal growth of cells in SCC by inactivating the two key apoptotic proteins, p53 and Rb [38]. On the other hand, many interactions that are seen in high-risk oncoproteins either do not occur or are much weaker in low-risk HPV 6 and 11 [40]. Nevertheless, HPV 6 and HPV 11 fulfil three vital postulates that contribute to the development of RRP: (1) persistence, causing a protracted disease course, (2) altered cellular growth and (3) viral replication, allowing a spread of SCPs [24]. It has also been discovered that children with SCPs

have a compromised cell-mediated immune response, which may be associated with repeated and persistent HPV infections. The CD4/CD8 ratio and weaker lymphocytic response to mitogen stimulation were significantly reduced in comparison to healthy children. A reduction in lymphocyte response to mitogen stimulation significantly correlated to a higher number of SCPs and more frequent recurrences [41, 42]. In addition, important results about the nature of HPV infection in recurrent SCPs have recently been detected: the presence of an identical and unique HPV genomic variant within an individual patient with SCPs in initial and follow-up samples obtained from 1 to 22 years later supports the hypothesis that frequent recurrences of SCPs are a consequence of the long-term persistence of a single viral genomic variant, rather than of repeated reinfection with novel HPV strains [43].

Clinical aspects SCPs almost invariably involve the larynx, especially the true and false vocal cords, subglottic areas and ventricles [17, 44]. An extralaryngeal spread has been identified in approximately 30 % of children and in 16 % of adults with SCPs. The most frequent extralaryngeal spread occurs successively to the oral cavity, trachea, bronchi and oesophagus [25]. Although SCPs have been traditionally divided into juvenile and adult groups [19, 30, 45], the prevailing opinion is that the disease is a unified biological entity with differences in clinical course, caused by HPV genotypes 6 or 11 [19, 20, 32]. For children, multiple and extensive growth with rapid recurrences after excision are characteristic. The small diameter of the airways in children may cause dangerous or even fatal airway obstruction. The clinical course in adults is usually not so dramatic, although SCPs can be aggressive, with multiple recurrences [21]. Most children present with dysphonia and stridor, less commonly with chronic cough, recurrent pneumonia, dyspnea and acute life-threatening events [25, 33]. The disease in adults presents mostly with dysphonia and hoarseness. From a clinical point of view, a new staging system, which is helpful for tracking the disease in an individual patient, represents the extent of SCPs at specific sites along the aerodigestive tract, as well as functional parameters, and assigns a final numerical score to the extent of disease at each assessment [25].

Macroscopy Grossly, papillomas are exophytic, branching, pedunculated or sessile, cauliflower-like masses, pink or reddish in colour, with a finely lobulated surface, presenting either singly or, more frequently, in clusters. These neoplasms are prone to haemorrhage on touch because of their fragility (Figs. 1.4 and 1.5a) [46].

Microscopy Histologically, SCPs are composed of finger-like, branching projections of the squamous epithelium, covering thin fibrovascular cores. A basal and parabasal hyperplasia of the squamous epithelium is frequently seen,

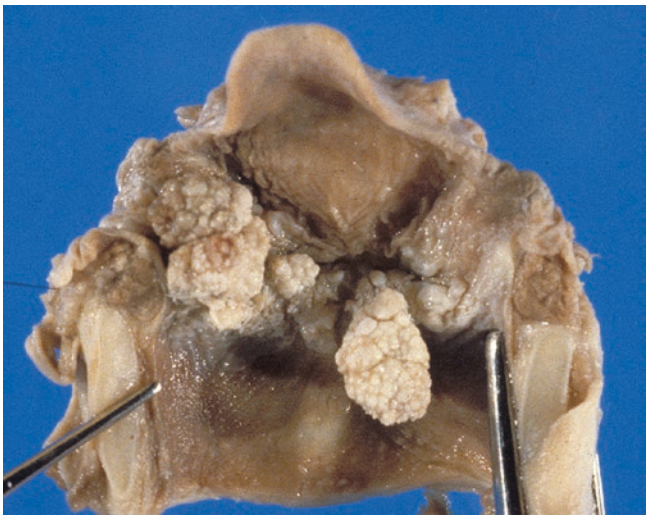

Fig. 1.4 Laryngeal papillomatosis. Numerous clusters of papillomas obliterate the laryngeal lumen

usually extending up to the mid-portion of the epithelial thickness. A thin parakeratotic layer is frequently seen on the surface. Mitotic features can be found, especially in the lower half of the epithelium. Irregularly scattered clusters of koilocytes, the only visible cytopathic effect of HPV infection, are seen in the upper part of the epithelium. Koilocytes have characteristic dark, wrinkled or angulated nuclei surrounded by a clear cytoplasmic area. These cells are always present in the upper third of the epithelium, where HPV replicate (Fig. 1.5b, c). Cytological changes, such as mild to moderate nuclear atypias and hyperchromatism, increased nuclear cytoplasmic ratio, increased mitotic activity with pathological features and prominent surface keratinization, are rarely found in SCPs [34, 44, 47].

Genetics and molecular genetic methods for HPV detection Several groups of genes of cell cycle, apoptosis and inflammatory cytokines have been studied in LSCPs versus normal tissue for a better understanding of the molecular mechanisms of the disease trying to discover more successful novel therapies. Rodman et al. discovered that *MCL-1* gene of the apoptosis pathway is significantly downregulated as well as cytokine genes *IL1-A*, *IL-8*, *IL-18* and *IL-31*. Downregulation of inflammatory cytokine genes *IL1-A*, *IL-18* and *IL-31* may explain why patients infected with HPV are unable to mediate T-cell immune clearance of their disease [48].

In situ hybridisation (ISH) is a frequently used method for HPV detection in SCPs (Fig. 1.5d). It is the only molecular method allowing the identification of a single infected cell in the squamous epithelium [18]. A diffuse nuclear staining pattern of the infected cell is consistent with episomal HPV DNA, while tiny punctate signals are related to a form in which HPV is integrated into host cell chromosome [49]. Negative results of ISH can indicate either a low-copy

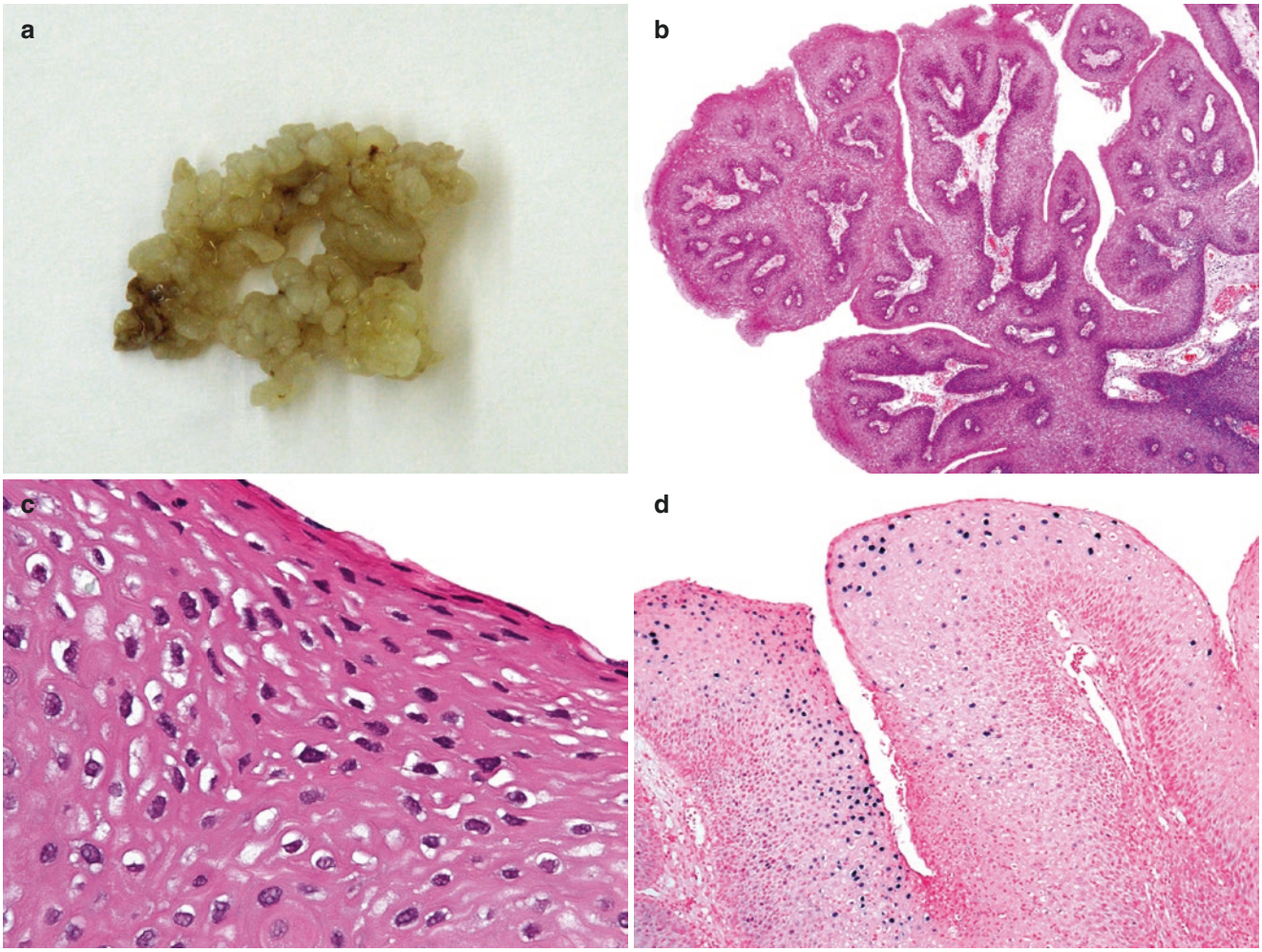

Fig. 1.5 Laryngeal papillomatosis. (**a**) Several pieces of clusters of multilobulated papillomatous lesion. (**b**) Branches of laryngeal papillomatosis is covered with hyperplastic squamous cell epithelium. (**c**) Numerous koilocytes are seen in the upper part of the epithelium. (**d**) Positive signals of the in situ hybridisation for HPV DNA genotypes 6/11 in the upper half of the epithelial thickness

number HPV, below the detection threshold (less than 20–50 viral copies per cell), or the absence of viral infection. Polymerase chain reaction (PCR), probably the most frequently used method for viral detection, is much more sensitive and can detect one infected cell out of 100,000 studied [18, 49, 50]. The detection of HPV DNA by PCR with consensus primers and subsequent restriction mapping or hybridisation methods using probes for each HPV type is available for the specific typing of HPV [46].

Differential diagnosis Distinguishing among various lesions with papillary structures and laryngeal SPs is a demanding task, especially if the biopsy specimen is small and superficial. An adult solitary keratinizing SCP, in contrast to HPV-induced SCPs, usually shows prominent surface keratinization, with keratohyalin granules. There is no evidence of koilocytosis and the hyperplastic epithelium is frequently atypical. VC is covered by a prominent keratotic or parakeratotic layer, which is not a characteristic of SCPs, and koilocytes are usually absent. Stromal fibrovascular projections are not present in VC, which characteristically shows broad epithelial projections with central keratin pearls infiltrating underlying tissue in a pushing manner. An exophytic variant of SCC is composed of broad-based projections of the neoplastic epithelium but without fibrovascular cores, which are characteristically present in SCPs. Papillary squamous carcinoma resembles the architectonic structures of SCPs, but the covering epithelium is clearly neoplastic, with an absence of maturation, loss of nuclear polarity and possible evidence of invasive growth.

Treatment and prognosis There is no specific and definitive therapy for LSCPs. In order to maintain the laryngeal function, adequate, multiple palliative surgical removals are

required. Laser surgery, and especially a microdebrider blade, has become the first choice of surgical therapy for LSCPs [24]. However, macroscopically unaffected mucosa remains an HPV reservoir and source of recurrence of the disease. When surgical procedures are needed more frequently than four times in 12 months, or there is evidence of LSCPs outside the larynx, adjuvant medical therapy should be considered [25]. Adjuvant therapy with antiviral drugs, such as acyclovir, valacyclovir, cidofovir or indole-3-carbinol, requires an individually designed and properly controlled study. More promising results are expected from the use of vaccination with a quadrivalent vaccine [51].

The clinical course of SCPs is unpredictable, characterised by periods of active disease and remissions. HPV present in apparently normal mucosa serves as a virus reservoir responsible for repeated recurrences of papillomas. The presence of LSCPs in the neonatal period is a negative prognostic factor, with a greater need for tracheotomy and likelihood of mortality. HPV 11 positive LSCPs are considered more aggressive than HPV 6 tumors. Although HPV subtype and early onset of LSCPs are well characterised as worse prognostic factors, current study also documents the significance of E6 and E7 oncogene expression as a potential biological marker of clinical behaviour in both HPV 6- and HPV 11-induced LSCPs [52]. Prominent histological atypias of epithelial cells are also reported to be associated with increased severity and recurrences of LSCPs, while others suggest that histological changes are not a good predictor of potential malignant transformation [53].

Malignant transformation of LSCPs is a rare occurrence, with roughly 40 reported cases, and of those in which HPV genotyping was obtained, all demonstrate HPV 11 infection [54–56]. In children, malignant alteration of LSCPs preferentially appears in the bronchopulmonary tree and in adults in the larynx [57]. However, a recent study first reported malignant transformation of juvenile-onset LSCPs in a 24-year-old female patient with HPV 6 infection. Molecular analysis showed viral integration in the *AKR1C3* gene on chromosome 10p14-p15.2, in association with deletion of the chromosomal region 10p14-p15.2, transcription of a virus–human product as well as AKR1C3 protein expression [58]. Mortality of patients with LSCP is mostly causally related to asphyxia, pulmonary complications and cancer development [59, 60].

1.3 Squamous Intraepithelial Lesions

General considerations Head and neck carcinogenesis is an incompletely elucidated, multistep process characterised by the progressive accumulation of genetic changes and followed by increasing architectural and cytological alteration of the squamous epithelium in this region [61–65]. The etiological, genetic, immunological and morphological parameters of the wide spectrum of epithelial changes, also called squamous intraepithelial lesions (SILs), ranging from squamous cell hyperplasia to carcinoma in situ (CIS), have been investigated for decades, in order to discover more reliable predictive values for invasive malignant growth [66–78]. A variety of terminology has been used in the literature for SILs, such as dysplasia, squamous intraepithelial neoplasia and low- and high-risk lesions [68, 72, 77, 78]. Clinically, oral and laryngeal SILs are mainly recognised as leukoplakia, erythroplakia and chronic laryngitis. However, none of these macroscopically recognised lesions carry any microscopic connotation, which must always be determined by histology [72, 79, 80].

Despite extensive research in molecular genetics, reliable marker(s) with diagnostic and prognostic value are still lacking. Traditional light microscopic examination thus remains the mainstay of accurate diagnosis, in spite of subjectivity in interpretation [77]. In their evolution, some cases of SILs are self-limiting and reversible, some persist and some of them progress to SCC in spite of treatment [81]. Particular interest has been focused on potentially malignant or risky or precursor lesions [44, 68, 72, 82, 83]. These lesions have been defined as histomorphological changes of the squamous epithelium from which invasive cancer develops in a higher percentage than from other grades of SILs [44, 66, 72, 84, 85].

Epidemiology Oral SILs, more frequently described as oral dysplasia or potentially malignant disorders, affect approximately 2.5–5 per 1000 of population. Clinically, they are usually detected as leukoplakia or white patches that cannot be rubbed off; 1–2.5 % of the population is affected at any one time [71, 86]. Oral leukoplakia is a clinical diagnosis of exclusion. If any oral white patch can be diagnosed as some other condition, such as candidosis, leukoedema, white sponge nevus, lichen planus, frictional keratosis, nicotine stomatitis, etc., then the lesion should not be considered a case of leukoplakia [87]. The global prevalence of leukoplakia, based on a systematic review of 23 studies published from 1986 to 2002, is 2.6 % (95 % CI 1.72–2.74 %), although there was a high degree of heterogeneity among the included studies [88]. It is important to note that in countries with high daily use of tobacco, whether smoked, chewed or both, the annual incidence rate ranges from 5/1000 to 30/1000, depending on the pattern of use [89, 90]. However, recent studies have reported a tendency towards a lower prevalence of oral leukoplakia compared to the past, which might be the result of the massive public health education against tobacco [91].

Oral SILs, called erythroplakia or red patch, are significantly less common, ranging between 0.02 and 0.83 % in different geographical areas [92].

The reported age, sex and intraoral site distribution of leukoplakia depend on ethnicity, tobacco and alcohol habits and

the selection bias of the samples surveyed; it mainly occurs between the fourth and seventh decades and males are predominantly affected [88, 89]. The location of intraoral leukoplakia is seen in a descending order of occurrence in the following sites: buccal mucosa, tongue, labial mucosa and gingival [93]. Erythroplakia can be found together with leukoplakia and predominantly occurs in the floor of the mouth, the soft palate, the ventral tongue and the tonsillar fauces. Red patch mainly occurs in middle-aged and elderly patients and there is no distinct gender preference [94]. A special type of oral leukoplakia is proliferative verrucous hyperplasia (PVL), with a proven high risk of becoming malignant. Women predominate over men in PVL by 4:1, with a mean age at diagnosis of 62 years. It appears most frequently in the buccal mucosa, followed by gingiva, tongue and floor of the mouth [93, 95, 96].

Laryngeal SILs are mainly limited to the adult population and affect men more often than women. The estimated incidence varies worldwide and depends on the amount, manner and types of exposure to the most frequent carcinogens, tobacco and alcohol abuse. Epidemiological studies of laryngeal SILs are scarce in comparison with similar studies of oral leukoplakia, necessitating the use of hospital-based data or the results of epidemiological studies of smaller populations [72]. Bouqout and Gnepp published in 1991 that the annual incidence of laryngeal SILs in the USA was 10.2 and 2.1 lesions per 100,000 in males and females, respectively [97]. Another series of 1042 patients with laryngeal leukoplakia and/or chronic laryngitis was published in 2009; the patients were followed from 1979 to 2004 in Slovenia [72]. The incidence of patients covering the region with approximately 800,000 inhabitants or 40 % of the population of Slovenia, varied for the low-grade SILs (squamous hyperplasia and basal–parabasal hyperplasia) from 0.84 to 4.62/100,000 inhabitants (mean value 2.61/100,000, SD = 110). The incidence of patients for high-risk SILs (atypical hyperplasia) ranged from 0.25 to 2.62/100,000 inhabitants (mean value 0.86/100,000 inhabitants, SD = 0.49) [72].

SILs appear mainly along the true vocal cords and supraglottis and rarely in other parts of the larynx. The vocal cord lesions are frequently bilateral but, rarely, commissures are involved [44, 98].

Etiology and pathogenesis SILs in the oral cavity and oropharynx are associated with tobacco, whether smoked, chewed or used as snuff, and tobacco seems to be the major carcinogen in this region [89, 93, 99–102]. Smoking 20 or more cigarettes per day, particularly non-filtered, and alcohol drinking, particularly fortified wines and spirits, are important risks for the development of oral dysplasia in the European population. Tobacco is a stronger independent risk factor for oral SILs than alcohol [103]. The use of smokeless tobacco in the western world has a rather lower correlation with oral precancerous and cancerous lesions compared to Southeast Asia where chewing habits, including betel quid, strongly correlate with oral precancer and cancer development [93, 102]. The etiology of PVL does not highlight a particular causal agent and the lesion would appear to be multifactorial [104, 105]. The relatively common absence of well-known risk factors associated with oral cancer and a preponderance of older women patients could indicate a different pathogenesis of PVL related, compared to non-PVL-related cancer [105]. It may occur in both smokers and non-smokers.

Alcohol has been considered the second most important risk factor for oral and pharyngeal cancer development [99], but there is some uncertainty about the role of alcohol in the etiology of oral SILs [101]. In contrast, Maserejian et al. showed that alcohol increased the risk of oral SILs in those who have never used tobacco, as well as in past or current users. The authors reported that alcohol is an independent risk factor for oral SILs, regardless of the beverage type or drinking pattern [106].

The involvement of HPV in the initiation and progression of oral neoplasia is still a matter of debate. Different studies have generated conflicting results concerning the prevalence of HPV, ranging from 0 to 90 % [107, 108]. The observed discrepancy may be related to the varying sensitivity of the methodologies applied for HPV detection and the epidemiologic factors of the studied patient groups. In contrast to the high percentage of HPV-related tumors of the oropharyngeal region, HPV-positive premalignant lesions are extremely rare findings in tonsillectomy specimens [109, 110]. Although there appears to be some link between HPV infection and oral leukoplakia, there is little evidence to support a causal relationship either between HPV infection and oral leukoplakia or between HPV-infected leukoplakic keratinocytes and their malignant transformation [111]. However, Woo et al. recently published a subset of oral epithelial dysplasia, mainly located on the lateral or ventral tongue of 17 men and 3 women, all adult and with transcriptionally active high-risk HPV. In these cases, epithelial hyperplasia with marked karyorrhexis and apoptosis were histologically detected, together with features of conventional dysplasia. The authors propose the use of the term HPV-associated oral intraepithelial neoplasia for such lesions [112]. Another case has been published of an HPV-related lesion, designated non-keratinizing CIS, typical of HPV-related SCC, involving the surface epithelium of the oral cavity, oropharynx and larynx. The lesion was strongly p16 positive and harboured transcriptionally active HPV 16, as demonstrated by E6/E7 RNA in situ hybridisation [113].

Most reports agree that cigarette smoking and alcohol abuse, and especially a combination of these two detrimental factors, are major identifiable risk factors of laryngeal SILs. The role of smoking has been proven both clinically and

experimentally. The risk of SIL development was found to be related to the age of the patient at the start of smoking, duration of smoking and the quality of tobacco [114–117]. Bosatra et al. analysed 97 dysplastic lesions of the head and neck region, including 47 cases of laryngeal dysplasia, and found a direct correlation between the degree of dysplasia, malignant transformation and amount of cigarette smoking and alcohol consumption [117]. Additional aetiological factors are industrial pollution, chronic infections, voice abuse, obstruction of the upper respiratory tract, vitamin deficiency and hormonal disturbance [44, 115, 116, 118]. Whereas tobacco has been established as the principal aetiological factor of SIL development for more than half a century, several authors have recently devoted more attention to the potential role of gastroesophageal reflux disease (GERD). Lewin et al. in 2003 published the first study of laryngeal dysplasia and early cancer in relation to GERD [119]. Similar data from Cianci et al. also showed that of 93 patients with gastric resection, seven (8 %) had current or previous laryngeal malignancies or current precancerous lesions. In the control group, in contrast, only one patient showed mild dysplasia of the vocal cord [120].

The role of HPV infection in the pathogenesis of laryngeal SILs remains uncertain. The prevalence of HPV infection in laryngeal SILs varies widely, between 0 and 56 %, with an overall prevalence of HPV infection of 12.4 % [121–124]. SILs harbour mainly high-risk HPV types, with HPV 16 being the most frequent. In addition to laryngeal SCC and SILs, HPV DNA has also been detected in a substantial proportion, 12–25 %, of individuals with clinically and histologically normal laryngeal mucosa [72]. The absence in viral genomes in laryngeal SILs and cancers additionally suggests that the existence of other aetiological factors plays a more important role in laryngeal carcinogenesis than HPV infection [50]. A final answer on the role of HPV infection in the aetiopathogenesis of laryngeal SILs can thus only be reliably provided by additional studies, in which biological evidence of the existence of truly HPV-driven SILs can be detected [125].

Clinical aspects Patients with oral leukoplakia or erythroplakia usually have no distinctive symptoms, especially in the case of the homogenous type of oral leukoplakia. However, some patients may complain of a sensation of a foreign body or burning and/or soreness [94]. In the case of erythroleukoplakia with palpable induration, malignancy may already exist.

Most patients with laryngeal SILs present a history of a few months or more of symptoms, which depend on the location and severity of the disease. Patients may complain of fluctuating hoarseness, throat irritation, sore throat and/or chronic cough [68]. Hypopharyngeal SILs are rarely found and poorly defined [126].

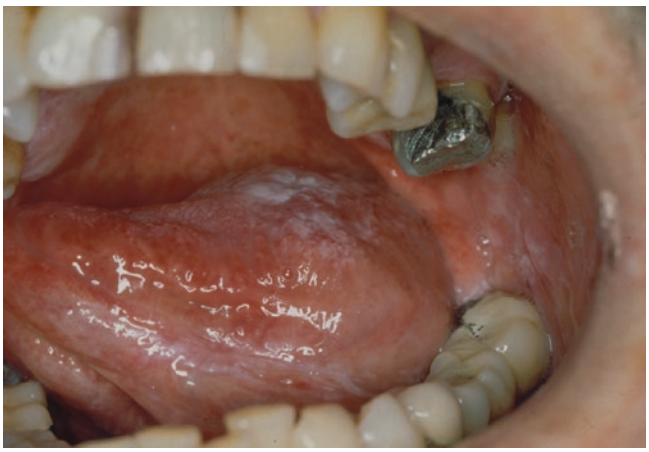

Fig. 1.6 Leukoplakia of the dorsal tongue. The microscopic diagnosis was low-grade squamous intraepithelial lesion with superficial parakeratotic layer (Courtesy of Dr. J. Fischinger, Ljubljana, Slovenia)

Clinical detection of oral and laryngeal SILs can also be supported by autofluorescence, chemiluminescence or vital staining with toluidine blue [44, 90].

Macroscopy Head and neck SILs are most frequently visible as white, red or mixed red-whitish (speckled) patches, known as leukoplakia, erythroplakia or erythroleukoplakia. Leukoplakias can be either sharply circumscribed and exophytic or predominantly flat and diffuse, related in part to the amount of keratin (Fig. 1.6). A speckled appearance of leukoplakias can also be present, caused by an unequal thickness of the surface keratin layer [77].

Oral leukoplakia is also clinically divided into homogenous and nonhomogenous types. The former is characterised as a uniform, flat, thin lesion with a smooth or wrinkled surface showing shallow cracks but a constant texture throughout. The latter type is defined as a predominantly white or white-and-red lesion that may be irregularly flat, nodular or exophytic. Nodular lesions have slightly raised rounded, red and/or whitish excrescences. Exophytic lesions have irregular blunt or sharp projections. The term nonhomogenous is applicable both to the aspect of colour (a mixed white and red lesion) and texture (exophytic, papillary or verrucous) of the lesions [127].

PVL is initially a relatively benign-looking, homogenous solitary patch, which turns gradually into an exophytic, diffuse or multifocal, progressive and irreversible lesion [105]. The diagnosis is made retrospectively after evidence of a progressive clinical course, accompanied by a particular deterioration of histological changes.

Erythroplakias, which are the least frequent lesions, are characterised by a thinner epithelium and dilated subepithelial vessels. All these lesions are associated with different degrees of epithelial changes; in general, leukoplakias are thought to have a low risk of malignant transformation; pure

red lesions have the highest risk of cancer development, especially in high-risk areas, such as the floor of the mouth, lateral borders of the tongue and soft palate/retromolar areas within the oral cavity [87]. Red patch occurs as a red macula or plaque with a soft, velvety texture, quite sharply demarcated and regular in coloration. Oral erythroplakias that are intermixed with white areas are called erythroleukoplakia or speckled mucosa and are believed to behave similarly to pure oral erythroplakia (Fig. 1.7a, b).

Laryngeal SILs do not have a single distinctive or characteristic clinical appearance and are variously described as leukoplakia, chronic hyperplastic laryngitis or, rarely, erythroplakia. A circumscribed thickening of the mucosa covered by whitish patches (Fig. 1.8) or an irregularly growing, well-defined warty plaque may be seen. A speckled appearance of lesions can also be present, caused by an unequal thickness of the keratin layer. However, the lesions are commonly more diffuse, with a thickened appearance, occupying a large part of one or both vocal cords (Fig. 1.9). Leukoplakic lesions, in contrast to erythroplakic ones, tend to be well demarcated. The macroscopic features of hypopharyngeal and laryngeal SILs are not as well defined as their counterparts in the oral cavity, and their relative importance is not generally accepted [44, 72].

Microscopy Traditional light microscopic examination, in spite of a certain subjectivity in interpretation, remains the most reliable method for determining an accurate diagnosis of SILs. The clinical validity of any histological grading system depends on the degree of accord with the biological behaviour of the lesions. Worldwide, neither morphological criteria nor the terminology for a histological grading system in the head and neck region in relation to the severity of SILs and propensity for malignant transformation are generally accepted. This evident disagreement is reflected in the World Health Organization (WHO) Classification of Head and Neck Tumors 2005, in which three different classifications of SILs are presented in the chapters on epithelial precursor lesions of the larynx and oral cavity. The dysplasia system (WHO 2005 classification (WHODC)) is presented alongside the classification of squamous intraepithelial neoplasia (SIN) and the Ljubljana classification (LC) [68]. Other grading systems for oral SILs have also been proposed in the literature, including the Smith and Pindborg system [128], Brothwell system [129], a new classification of the Japanese Society of Pathology – oral intraepithelial neoplasia/carcinoma in situ classification (OIN/CIS) [130] – and the binary system described by Kujon et al. [69].

Several groups of pathologists have assessed the interobserver variability in the classification of laryngeal SILs, using WHODC, SIN and LC [131–134]. None of the authors was able to give precedence to any particular system for classifying laryngeal SILs. For oral SILs, or more widely for the whole head and neck region, the WHO grading systems [135] have been additionally compared with new proposals, such as a binary grading system and OIN/CIS [68, 69, 130, 136, 137]. A reduction in the number of grades has been shown to have merit, with an improvement in kappa values of interobserver agreement in comparison to the dysplasia system [69, 138, 139]. It has also been found that the lack of defined criteria for grading dysplasia is an important source of inconsistent and poorly reproducible results [140].

These discouraging results have led us to submit a proposal for a unified classification of laryngeal SILs, which may also be implemented for oral SILs in the near future.

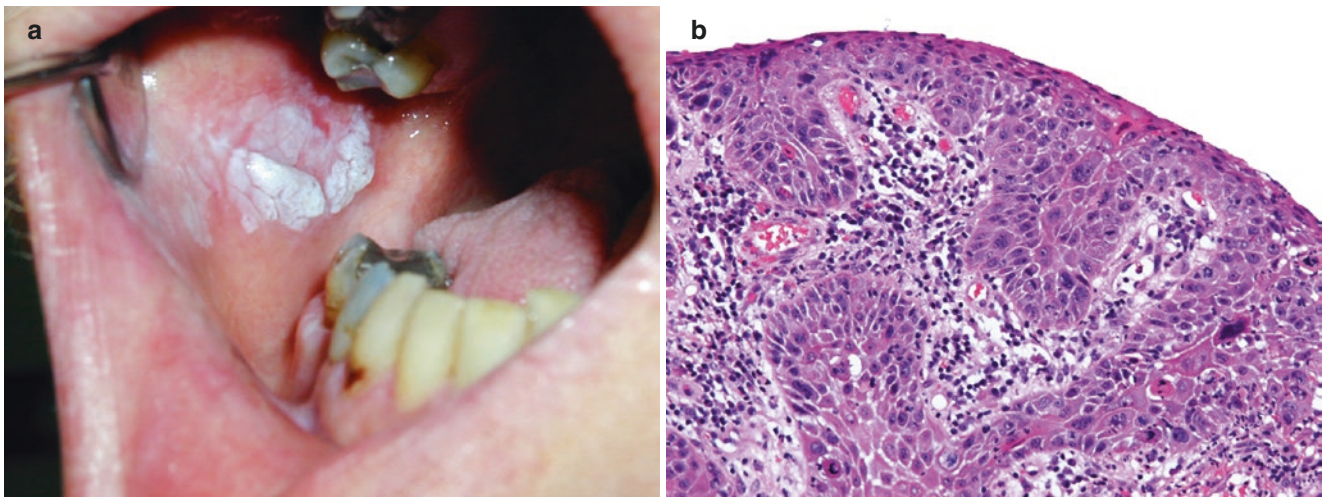

Fig. 1.7 Erythroleukoplakia of the buccal mucosa. (**a**) Thickened whitish plaques with uneven surface and with speckled foci of erythema on the periphery of the lesion (Courtesy of Dr. D. Dovšak, Ljubljana, Slovenia. (**b**) Histologically, architectural and cellular atypias meet the criteria of carcinoma in situ

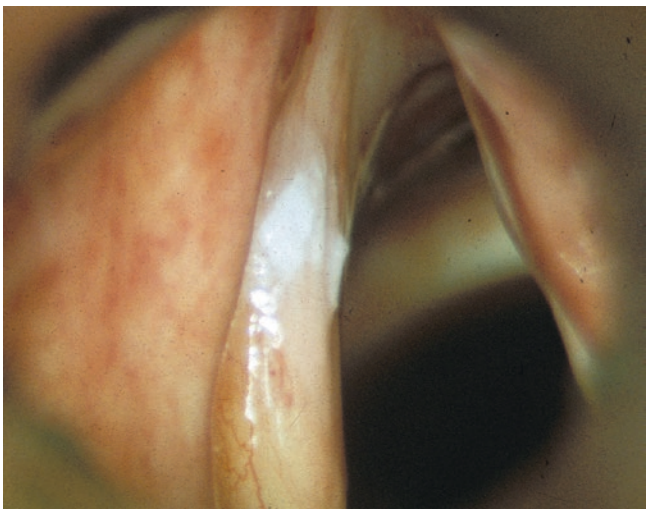

Fig. 1.8 Leukoplakia of the left vocal cord. The middle third of the left vocal cord is covered with a well-circumscribed whitish plaque. The microscopic diagnosis was low-grade squamous intraepithelial lesion with a prominent superficial keratotic layer

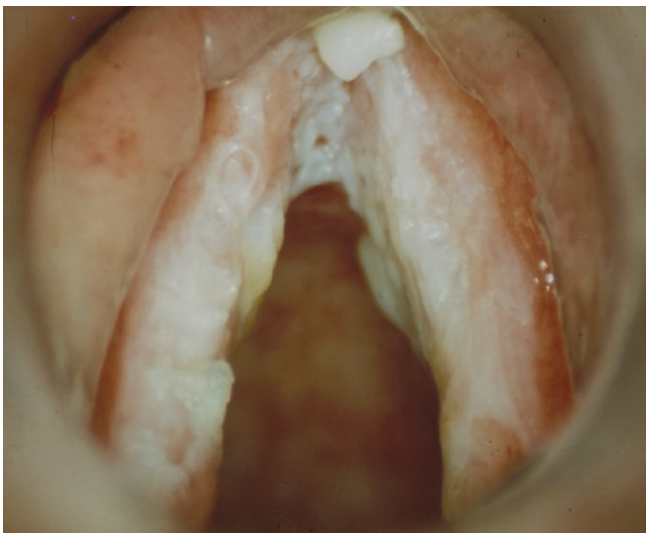

Fig. 1.9 Chronic laryngitis. Both vocal cords are irregularly thickened and covered by whitish plaques. The microscopic diagnosis was high-grade squamous intraepithelial lesion

The new proposal is actually a revision of the LC, based on amended morphological criteria that allow for a reduced number of grades from four to three and accompanied by a more clinically oriented nomenclature [77]. Six internationally recognised experts and three head and neck pathologists from Ljubljana contributed to this study by evaluating a set of laryngeal SILs using the new system: low-grade SIL, high-grade SIL and carcinoma in situ (CIS). The interobserver study of the modified LC showed good overall agreement (unweighted κ-statistic 0.75), which was better than that in previous studies [77, 131, 133, 134].

The general principles of the modified LC for all grades are the following:

- The basement membrane is preserved with no definitive evidence of minimal invasion.
- The presence of a surface keratin layer, which can be present in all grades of SIL, is not considered to be an important prognostic factor.
- Two subtypes of high-grade SIL or CIS can be present, both also in a single specimen.
  (a) Basal cell type, *usually non-keratinizing* and with *no prominent* intercellular prickles (bridges), no cytoplasmic eosinophilia, cells oriented perpendicularly to the basement membrane.
  (b) Spinous cell type, *usually keratinizing* and with prominent intercellular prickles and increased cytoplasmic eosinophilia [77].

The morphological criteria are presented in Tables 1.1, 1.2 and 1.3 (Figs. 1.10a, b, 1.11, 1.12a, b, and 1.13a, b) [77].

The proposed modification to the LC, which has converted the four-grade system of the original LC into a three-grade system, is based on the largest published study of laryngeal SILs, with 1444 patients who were followed for up to 31 years. The results of the follow-up study revealed that it is reasonable to combine the two groups of the old LC, squamous hyperplasia and basal–parabasal hyperplasia, into a single low-grade SILs with 1.6 % of malignant progression over 2–15 years. We have retained the concept of basal–parabasal cell hyperplasia of the LC as the leading morphological criterion for low-grade SILs. Such cells without atypia can be seen in reactive epithelium as part of healing processes and at the edge of erosions or ulceration. The increased number of basal–parabasal cells can occupy half or slightly more of the epithelial thickness [77].

The concept of high-grade SILs eliminates the problem of moderate dysplasia, which is a major source of interobserver variation in the dysplasia system. This grade of the new proposal includes changes with atypical epithelial cells and with still partially preserved stratification and polarity, extending from the mid-portion of the epithelium up to the surface. High-grade SILs, previously called atypical hyperplasia, showed a significantly higher risk of malignant progression (12.6 % over 2–26 years) in comparison with low-grade SILs. The results of the follow-up study thus justify the proposal of the modified LC of a division into two basic groups: low-grade SIL and high-grade SIL and confirms the credibility of the selected morphological criteria for both low- and high-grade SILs. A similar improvement in interobserver agreement has been provided in the grading of oral dysplasia, through introducing a binary system [69, 75]. A distinction between high-grade SILs and CIS is likely to be important for the extent of patient management. In our

Table 1.1 Morphological criteria of the low-grade SILs in the proposed modified Ljubljana classification

Definition	Low-grade SIL is considered to be most often benign, with low malignant potential, and characterised by a spectrum of morphological changes ranging from a simple hyperplastic process with retention of the basal layer and increased prickle cell layer, up to an augmentation of basal and parabasal cells occupying up to the lower half of the epithelium, while the upper part remains unchanged, containing regular prickle cells	
Criteria	Architectural criteria:	Stratification is preserved – smooth transition of basal cells or augmented basal–parabasal cell layer with perpendicular orientation to the basement membrane to prickle cells oriented in the upper part horizontally to the basement membrane
		Hyperplastic variant is predominant and the epithelium is rarely atrophic
		Prickle (spinous) layer – spectrum of changes ranging from increased prickle layer in the whole thickness of the epithelium up to changes in which prickle cells are seen only in the upper epithelial half with normal maturation
		Basal–parabasal layer – spectrum of changes, from unchanged (2–3 layers) layer to augmentation of basal and parabasal cells in the lower half of the epithelium or occasionally slightly more
		Normal maturation
	Cytological criteria:	No cellular atypia
		Parabasal cells – slightly increased cytoplasm compared to basal cells, no intercellular bridges
		Parabasal cells – slightly enlarged nuclei, uniformly distributed chromatin
		Rare regular mitoses in or near basal layer
		Few dyskeratotic cells are present

See Fig. 1.10a, b

Table 1.2 Morphological criteria of high-grade SILs in the proposed modified Ljubljana classification

Definition	High-grade SIL is considered to be a potentially premalignant lesion with 12 % or more patients subsequently developing malignancy. Morphologically it is characterised by a spectrum of changes including augmentation of immature epithelial cells, which occupy the lower half or more of the epithelial thickness	
Criteria	Architectural criteria:	Polarity and perpendicular orientation of augmented atypical epithelial cells
		Stratification may be seen
		Hyperplastic variant is predominant, rarely an atrophic layer of increased immature epithelial cells occupies the lower half or more of the entire epithelial thickness
		Prickle cell layer may be present in the upper part of the epithelium with normal maturation
	Cytological criteria:	Cellular atypia present
		Nuclear pleomorphism – variations in size (enlargement) and shape, irregular contours, marked variations in staining intensity with frequent hyperchromasia, nucleoli increased in number and size
		Nuclear/cytoplasmic ratio increased
		Increased mitoses mainly in lower two thirds of epithelium
		Dyskeratotic and apoptotic cells frequent within entire epithelium

See Figs. 1.11 and 1.12a, b

Table 1.3 Morphological criteria of CIS in the proposed modified Ljubljana classification

Definition	The term "carcinoma in situ" is reserved for lesions showing features of conventional carcinoma, e.g. structural and cellular abnormalities but without invasion (intraepithelial carcinoma)	
Criteria	Architectural criteria:	Loss of stratification and polarity of the whole epithelium
		The surface of the epithelium may be covered by three to five layers of compressed, horizontally oriented cells
		No stromal changes
	Cytological criteria:	Conspicuous cellular atypia
		Marked variation in size and shape of nuclei, marked variations in staining intensity with frequent nuclear hyperchromasia, nucleoli increased in number and size
		Increased mitotic figures in the whole epithelium, abnormal mitoses are frequently seen
		Dyskeratotic cells and apoptotic cells are often numerous

See Figs. 1.13a, b

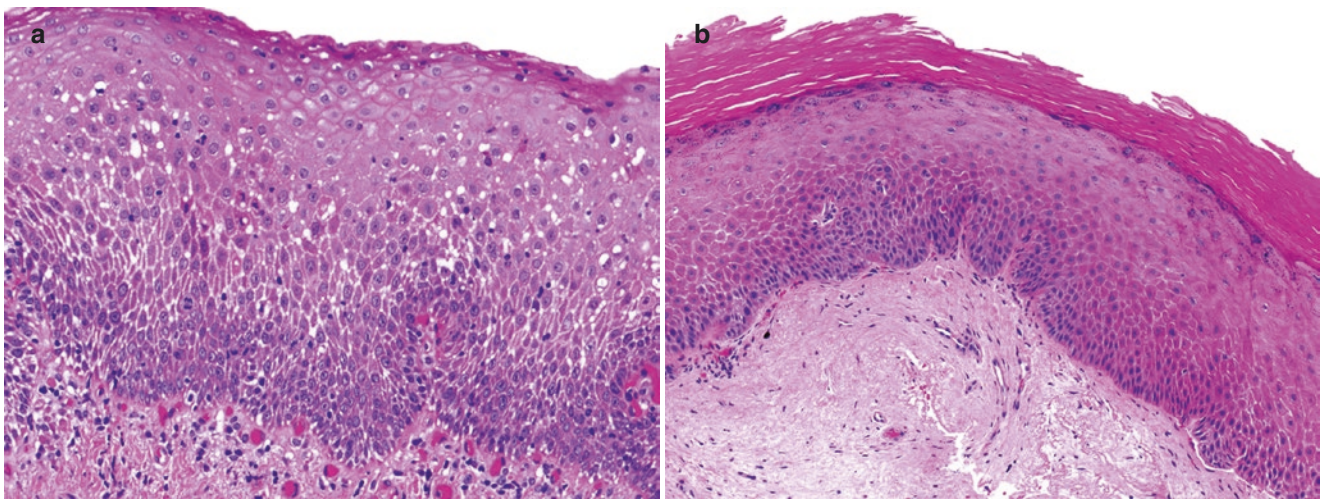

Fig. 1.10 Low-grade squamous intraepithelial lesion. (**a**) Thickened squamous epithelium with increased number of reactive basal–parabasal cells in the lower part of the epithelium. The upper part of the epithelium is unchanged. (**b**) Thickened squamous epithelium with prominent keratotic layer shows transition from squamous hyperplasia to basal–parabasal cell hyperplasia; parabasal cells occupy the low third of the epithelium thickness

experience of the LC over the last three decades, at least two out of three major histological criteria should be fulfilled to diagnose CIS: marked architectural disorder of the epithelium, conspicuous cellular atypia and increased mitotic activity with atypical mitoses. The Ljubljana criteria for CIS do not require the full thickness of the epithelium to be replaced by atypical cells without evidence of maturation, as a prerequisite for a diagnosis of CIS, as is required in cervical lesions [77].

Immunohistochemistry and biomarkers Although the gold standard for prediction of the behaviour of head and neck SILs is histological assessment, it is currently evident that even the best grading system currently available cannot reliably predict the evolution of SILs. It is especially evident when smoking or chewing tobacco and drinking alcohol continue.

One possible method of improving prediction may be the use of biomarkers, i.e. proteins and genes expressed in SILs during the process of possible malignant progression [75]. Nankivell et al. found that only 9 out of 286 studies of laryngeal dysplasia biomarkers met the inclusion criteria to calculate the risk ratio for a single biomarker. Relative risks ranged from 0.60 (95% CI 0.10, 3.75) for mdm2 to 84.55 (95% CI 5.30, 1348.56) for Cortican. They conclude that there is no good evidence of biomarkers predicting the future behaviour of laryngeal SILs [75]. A recent review of evidence of biomarkers related to oral field cancerisation is also not promising,

and the authors conclude that the search for an adequate molecular marker that maps field cancerisation lesions should continue [141]. The ploidy status, as determined by high-resolution flow of oral SILs, shows that it may be of value in predicting biological behaviour in oral potential malignant disorders [142]. Details are given in the Sect. on 1.8.

Differential diagnosis Macroscopically and microscopically, oral leukoplakia needs to be differentiated from white sponge nevus leukoedema, candidosis, discoid lupus erythematosus, hairy cell leukoplakia and lichen planus; differential diagnosis of erythroplakia includes early cancer, local irritation, mucositis, drug reaction, lupus erythematosus and median rhomboid glossitis.

Histologically, reactive, regenerative epithelial changes in response to trauma, ulcerative changes of various etiology, haemorrhages, inflammation and deficiency of iron and vitamin B12 may mimic epithelial abnormalities in different grades of oral and laryngeal SILs. Clinical data are always of considerable help in distinguishing different grades of SILs from regenerative changes, in which epithelial abnormalities are generally less pronounced than in high-grade SILs, and atypical mitoses are almost never present. The epithelium may be thinned or thickened, epithelial maturation is at least partially preserved. In addition, a pronounced inflammatory infiltrate beneath the epithelium can cause the appearance of disruption of the basement membrane. A repeated biopsy after the recovery of inflammation may solve this serious dilemma.

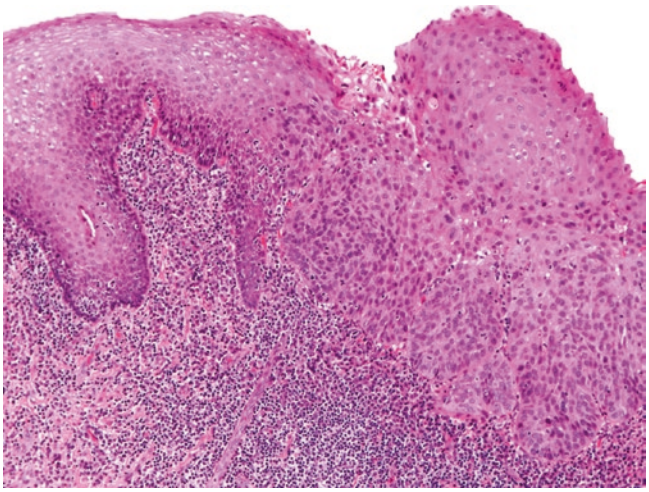

Fig. 1.11 Transition from low-grade to high-grade intraepithelial lesion. Hyperplastic squamous epithelium with a slightly increased number of basal–parabasal cells transfers sharply to the high-grade lesion with atypical epithelial cells occupying partially the whole epithelial thickness

Treatment and prognosis The flowchart for the management of oral leukoplakias is described by van der Waal. In the presence of possible etiological factors, including tobacco habits, an observation of 2–4 weeks is tolerated to detect a possible regression after elimination of aetiological factors. If the lesion persists, a biopsy is always mandatory. The management of oral SILs varies according to the type of lesion. The most common treatment modalities are surgical excision or laser therapy [71]. Additional treatment modalities include chemoprevention, photodynamic therapy and topical chemotherapy [143]. However, recurrences after local excision are common, and it is essential to follow up patients carefully, since patients with oral SILs are prone to field cancerisation effects and increased risk of additional development of oral SIL or even overt malignancy. In a series of 59 patients treated with cold knife, 10% of patients developed recurrence; in

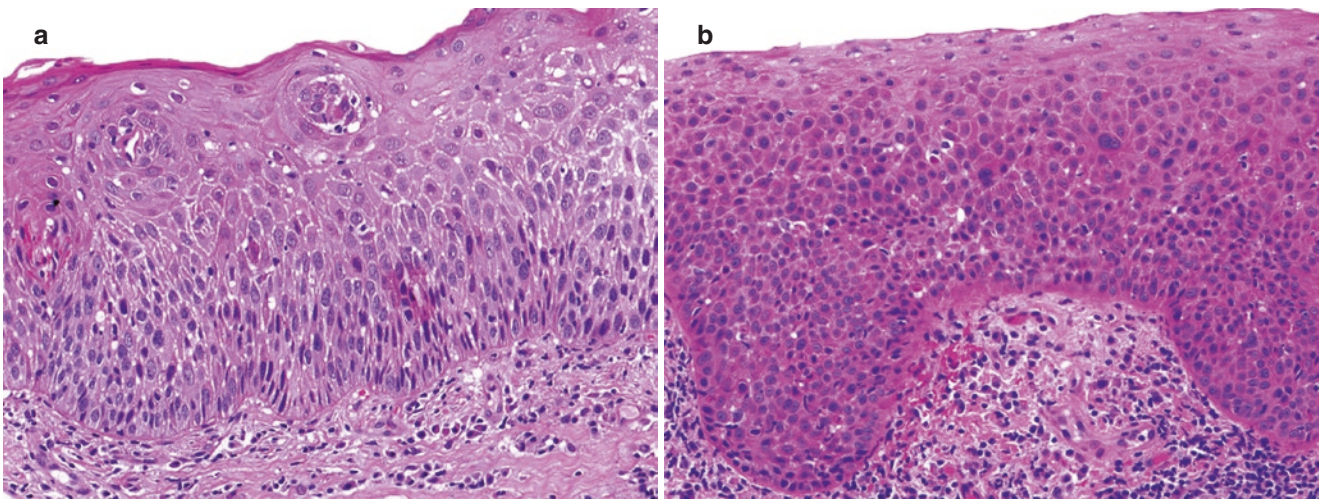

Fig. 1.12 High-grade squamous intraepithelial lesion. (**a**) Augmented epithelial cells showing mild to moderate grades of atypias, the cells are aligned perpendicularly to the basement membrane. (**b**) Atypical epithelial cells occupy almost the whole epithelial thickness with increased nuclear/cytoplasmic ratio and some regular mitoses

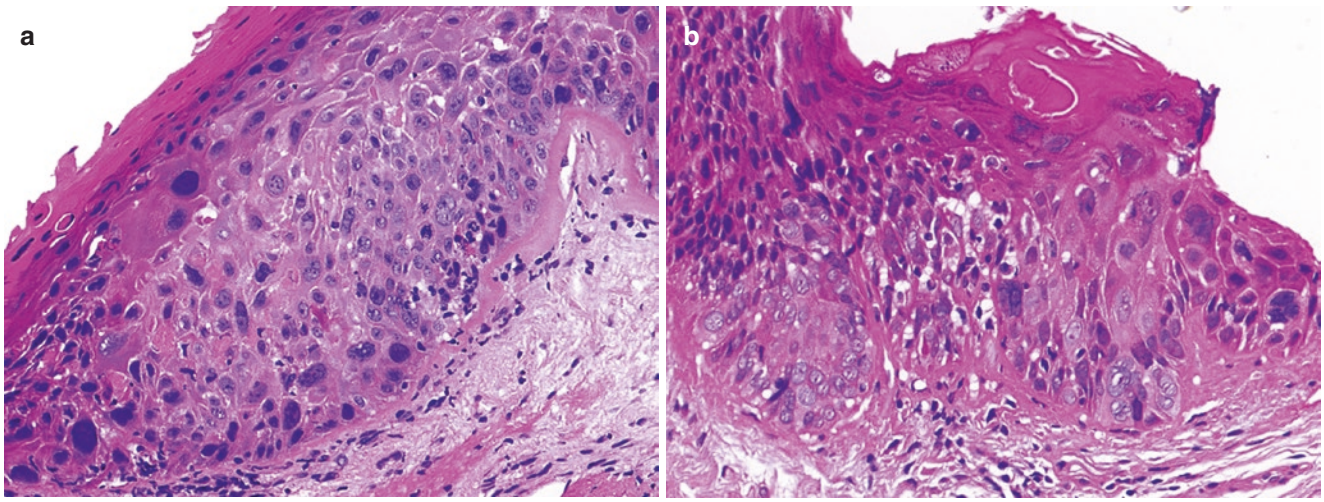

Fig. 1.13 Carcinoma in situ. (**a**, **b**) The epithelium shows loss of stratification and polarity, polymorphic malignant cells replace almost the entire epithelial thickness, mitotic activity is increased; the surface of the epithelium is covered by a few layers of compressed, horizontally oriented cells

another series of 167 patients treated with CO_2 laser excision, the recurrence rate was 18 % [143]. A systematic review of 14-nonrandomised studies compared surgical excision of oral leukoplakia vs. follow-up of patients with no treatment. A considerably higher malignant transformation rate was found among lesions that were not surgically treated than for those that were excised (14.6 % vs. 5.4 %; $p=0.003$); surgical treatment thus considerably but not entirely decreases the risk of malignant progression [86]. For all types of leukoplakia together in Western countries, the annual malignant transformation rate is approximately 1 %. This percentage is much higher for nonhomogenous leukoplakia, including proliferative verrucous leukoplakia, with 49 % of patients with malignant transformation, with a mean follow-up of 7.53 years [71, 144]. However, it has to be emphasised that the degree of severity of all oral SILs is the main risk factor of malignant progression. A follow-up of 32 patients with oral dysplasia over a 15-year period showed that 17 (53 %) developed invasive carcinoma: 1 of 9 patients with mild dysplasia, 8 of 12 with severe dysplasia and 8 of 10 with carcinoma in situ [145]. The commonly recognised factors that statistically carry an increased risk of malignant progression in oral mucosa are the following: epithelial dysplasia, often associated with a clinically nonhomogeneous erythroleukoplakic lesion, is the most important indicator, followed by female gender, long duration of leukoplakia, leukoplakia in nonsmokers (idiopathic leukoplakia), location on the tongue and/or floor of the mouth, size >200 mm^2, nonhomogenous type and the presence of *Candida albicans* [71].

PVL should be considered a possible diagnosis when a specific discrepancy between bland histological features of oral leukoplakia and an aggressive clinical course is established [104]. Whether verrucous hyperplasia forms a separate stage in this series of histological features shown by PVL is debatable, since there seems to be considerable histological overlap between this lesion and VC. There are thus no convincing arguments that verrucous hyperplasia is anything other than a variant of VC [146, 147]. A mean time of 7.7 years was found from the diagnosis of PVL to cancer development in 70.3 % of patients [148]. The treatment of PVL continues to be an unsolved problem, with high rates of recurrence, since total excision is rarely possible because of the widespread growth [105].

Erythroplakia is most frequently associated with high-grade SILs or CIS and should thus be excised without delay with clear margins [90]. A review of ten studies of oral erythroplakia from six different countries revealed a malignant transformation rate of 44.9 % [94]. Recurrence rates of oral erythroplakias seem to be high but reliable data are lacking [92].

For laryngeal SILs, a transoral endoscopic approach with direct microlaryngoscopy enables an accurate examination of the whole laryngeal mucosa with an adequate excisional biopsy, using stripping microflap excision or laser ablation [72]. For suspicious aberrations, the removal of the entire lesions, which must be properly oriented and prepared for serial sections, is required in order to exclude invasive SCC. There is still no consensus in the literature on the treatment of glottic high-risk SILs (severe dysplasia) or CIS [149]. Various European centres have a policy of surgical removal of high-grade SILs and lifelong close follow-up. In cases of CIS, if complete surgical removal is not possible, radiotherapy is an alternative treatment. A similar recommendation has been adopted in Dutch national guidelines and in other centres in which radiotherapy is used in patients for whom complete endoscopic de-epithelisation is not possible [44, 149–153]. Radiotherapy is never used for treatment

of high-grade SILs in Slovenia [44, 77, 78], and it is therefore important to distinguish high-grade SILs from CIS in laryngeal pathology. In the Ljubljana retrospective study, which included 1444 patients, 9 of 49 patients who progressed to cancer were diagnosed as CIS. Eight patients were additionally treated with radiotherapy and one patient with cordectomy. None of these patients progressed to invasive SCC [77]. A 10-year retrospective study of head and neck CIS, including laryngeal (25/55), reported that primary therapy consisted of surgery, radiotherapy or a combination of both. The overall 5-year disease-specific survival was 98 %. The recurrence rate after primary therapy was 20 % [154]. One of the most decisive factors for malignant transformation remains an unchanged lifestyle. Failure to give up smoking and drinking alcohol may be the real factor in malignant progression [155]. In addition, a correctly performed biopsy is an important prerequisite for reliable grading of SILs.

The histopathologic degree of severity of laryngeal SILs are still used as the most reliable predictive factor [72, 77, 156]. This is also confirmed by the results of a systematic review and meta-analysis showing an overall malignant transformation rate for laryngeal dysplasia of 15 % (95 % CI – 8–22 %) in 940 patients. The risk of malignant transformation increases threefold between mild/moderate dysplasia (10.6 %) and severe dysplasia/CIS (30.4 %), which is a statistically significant difference [156].

1.4 Invasive Squamous Cell Carcinoma

1.4.1 Microinvasive Squamous Cell Carcinoma

Microinvasive SCC is SCC with invasion beyond the epithelial basement membrane, extending into the superficial stroma (Fig. 1.14). There is little consensus among pathologists on the maximum depth of invasion in microinvasive SCC, but it generally ranges from 0.5 to 2 mm [60, 157]. The depth of invasion must be measured from the basement membrane of the adjacent (non-neoplastic) surface epithelium, because of the great variations in epithelial thickness.

Microinvasive SCC is a biologically malignant lesion capable of gaining access to lymphatic and blood vessels which may result in metastases. However, metastases are rare in microinvasive SCC and the prognosis is excellent. Studies of SCC of the floor of the mouth have shown that there is little or even no metastatic potential for SCC penetrating less than 2 mm beyond the basement membrane but a substantially higher risk of metastases in more deeply invasive SCC at this site [157, 158]. The prognosis is also excellent in microinvasive SCC of the laryngeal glottis because of the poor lymphatic and vascular network in this location. Some authors have therefore recommended more conservative treatment of these lesions, such as endoscopic removal, with a careful follow-up [159].

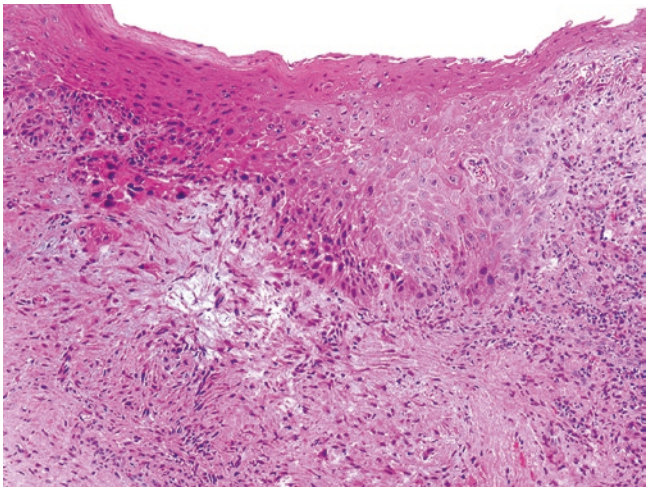

Fig. 1.14 Microinvasive squamous cell carcinoma: invasion of carcinoma into superficial stroma

A reliable diagnosis of microinvasive SCC can only be made with certainty if the whole lesion is examined. It should not be made in small, tangentially cut biopsy specimens.

1.4.2 Conventional Squamous Cell Carcinoma

Definition Squamous cell carcinoma (SCC) is a malignant epithelial tumor with evidence of squamous differentiation, such as intercellular bridges and keratin formation. It originates from the surface squamous epithelium or from ciliated respiratory epithelium that has undergone squamous metaplasia.

Epidemiology SCC of the head and neck is the sixth most prevalent cancer worldwide, accounting for 5 % of all new cancers, with a global annual incidence of 500,000 [160]. The vast majority of SCC is the conventional-type SCC, accounting for more than 90 % of cases. The remaining cases belong to the variants of SCC that will be discussed later in this chapter.

SCC of the head and neck occurs most frequently in the oral cavity and lip, in the oropharynx, larynx and hypopharynx. Less frequently it arises in the nasopharynx, nasal cavities and paranasal sinuses. Predilection sites in the oral cavity are the lateral tongue and floor of the mouth. In the oropharynx, the most commonly involved sites are the base of the tongue and the tonsils. In the larynx, there are geographic differences in the topographic distribution, glottis being the most frequent location in some, and supraglottis in other countries [60, 161].

Etiology Smoking and alcohol abuse are the most important risk factors for the development of SCC of the head and neck. Much attention has been paid to the possible role of viral infection in the pathogenesis of the head and neck carcinoma, particularly with Epstein–Barr virus (EBV) and HPV.

EBV is aetiopathogenetically strongly related to nasopharyngeal carcinoma [162] and to rare cases of lymphoepithelial carcinoma of the salivary glands [163, 164]. It appears that EBV plays little, if any, role in the pathogenesis of SCC in other locations in the head and neck [163, 165–167].

HPV 16/18 have been aetiopathogenetically linked to SCC of the oropharynx, particularly of the palatine and lingual tonsils [168, 169]. HPV-related SCC may also arise in the sinonasal tract and oral cavity, whereas they are very rare in the larynx and hypopharynx [170, 171].

Macroscopy The macroscopic appearance of invasive SCC is variable and includes flat lesions with a well-defined, raised edge, polypoid exophytic and papillary lesions, as well as endophytic infiltrative lesions. The surface of the tumor is frequently ulcerated.

Microscopy Microscopically, SCC is characterised by invasive growth and evidence of squamous differentiation. Invasive growth is manifested by interruption of the basement membrane and the growth of islands, cords or single (dyscohesive) tumor cells in the subepithelial stroma; large tumors may invade deeper structures, i.e. muscle, cartilage or bone. Perineural invasion and invasion of lymphatic and blood vessels may be present, which is a reliable proof of an invasive cancer. Squamous differentiation is evidenced by intercellular bridges and/or keratinization, with keratin pearl formation.

Grading SCC is traditionally graded into well-, moderately, and poorly differentiated SCC. The criteria for grading are the degree of differentiation, nuclear pleomorphism and mitotic activity. Well-differentiated SCC closely resembles closely normal squamous epithelium and contains varying proportions of large, differentiated keratinocyte-like squamous cells and small basal-type cells, which are usually located at the periphery of the tumor islands. There are intercellular bridges and usually full keratinization: mitoses are scanty (Fig. 1.15a). Moderately differentiated SCC exhibits more nuclear pleomorphism and more mitoses, including abnormal mitoses; there is usually less keratinization (Fig. 1.15b). In poorly differentiated SCC, basal-type cells predominate, with a high mitotic rate, including abnormal mitoses, barely discernible intercellular bridges and minimal, if any keratinization (Fig. 1.15c). Although keratinization is more likely to be present in well- or moderately differentiated SCC, it should not be considered an important histological criterion in grading SCC.

Invasive front Tumor growth at the invasive front (tumor–host interface) shows an expansive pattern, an infiltrative pattern or both. An expansive growth pattern is characterised by large tumor islands with well-defined pushing margins, whereas an infiltrative pattern is characterised by scattered small irregular cords or single tumor cells, with poorly defined infiltrating margins. It has been demonstrated that the growth pattern at the invasive front has prognostic implication: an infiltrative pattern is associated with a more aggressive course and poorer prognosis than an expansive pattern [172–174].

Stromal reaction Invasive SCC is almost always associated with a desmoplastic stromal reaction, which consists of a proliferation of myofibroblasts, excessive deposition of extracellular matrix and neovascularisation [175–178]. In our experience, a desmoplastic stromal reaction is present only in invasive SCC and never in SILs, regardless of the grade, and may be considered to be an additional marker of invasion [176, 177]. The desmoplastic stromal reaction tends to be pronounced in well- and moderately differentiated SCC and weak or absent in poorly differentiated SCC, as well as in HPV-positive non-keratinizing SCC and lymphoepithelial carcinoma. The intensity of desmoplasia is inversely related to the density of stromal lymphocytic infiltration [177]. In SCC with marked desmoplasia, lymphocytic infiltration is usually focal and scarce, while intense lymphocytic infiltration is found in SCC with little or no desmoplasia.

Immunohistochemistry Immunohistochemically, SCC expresses epithelial markers, such as cytokeratins and epithelial membrane antigen (EMA). The patterns of expression of cytokeratin subtypes are related to the degree of SCC differentiation and to the degree of keratinization [179].

The pattern of cytokeratin expression in low-grade SCC is similar to that observed in non-neoplastic squamous epithelium and is characterised by medium-high-molecular-weight cytokeratins and a lack of expression of low-molecular-weight cytokeratins. High-grade SCC tends to lose the expression of medium and high-molecular-weight cytokeratins and expresses low-molecular-weight cytokeratins [179].

Among various cytokeratin subtypes, cytokeratin 8 and to a lesser extent cytokeratin 7, recognised by antibody CAM5.2, could be used as indicators of malignant transformation. In a study by Mallofré et al. 40 % of SCC were positive for CAM5.2, but it was never positive in non-neoplastic squamous epithelium [179]. In poorly differentiated SCC, expression of vimentin may occur.

Genetics Molecular carcinogenesis of the head and neck SCC is discussed in the Sect. 1.8.

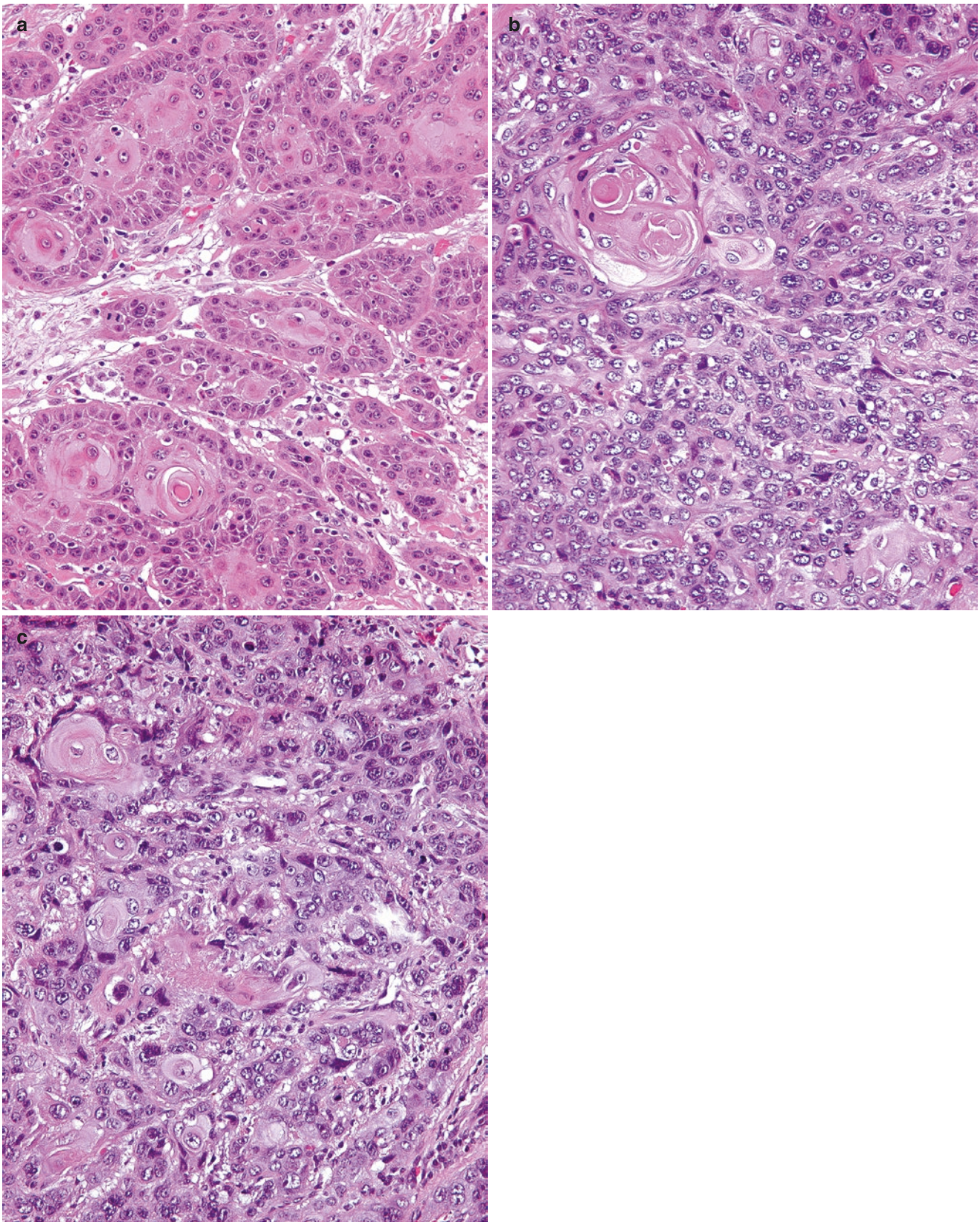

Fig. 1.15 (**a**) Well-differentiated squamous cell carcinoma. (**b**) Moderately differentiated squamous cell carcinoma. (**c**) Poorly differentiated squamous cell carcinoma

Differential diagnosis A diagnosis of SCC must be confirmed by biopsy, which must be taken from the clinically most suspicious area, avoiding the central necrotic area. In well-oriented, adequate biopsy samples, the diagnosis does not usually represent a diagnostic problem, since evidence of invasive growth and of squamous differentiation is easily found.

However, well-differentiated SCC must be distinguished from VC and papillary SCC, as well as from benign conditions, such as pseudoepitheliomatous hyperplasia. VC lacks atypias, which are always present in SCC. Papillary SCC is characterised by papillae formation, which is not the prevailing feature in conventional SCC.

Pseudoepitheliomatous hyperplasia is a benign condition associated with granular cell tumor or mycotic infection or tuberculosis. It consists of deep irregular tongues and rete pegs but there are no atypias and abnormal mitoses as in SCC. Identifying the associated condition (granular cell tumor or infection) may be helpful in establishing a diagnosis of pseudoepitheliomatous hyperplasia.

Poorly differentiated SCC must be differentiated from malignant melanoma, malignant lymphoma, neuroendocrine carcinoma, adenocarcinoma and adenosquamous carcinoma. The correct diagnosis is best achieved by the use of appropriate immunohistochemistry and special stains for demonstration of mucin production.

Malignant melanoma is distinguished from SCC by the expression of S-100, HMB-45 and melan-A. Neuroendocrine carcinoma expresses neuroendocrine markers (synaptophysin, chromogranin) and does not show evidence of squamous differentiation, while SCC does not express neuroendocrine markers. Malignant lymphoma is differentiated from SCC by the presence of leucocyte common antigen and markers of B- or T-cell differentiation. Adenocarcinoma and adenosquamous carcinoma can be distinguished from SCC by the presence of glands and mucin secretion within the tumor cells.

Treatment and prognosis SCC of the head and neck has an overall death risk of 40 % [180]. The most important prognostic factor is the TNM stage, based on the size of the primary tumor, the presence of regional lymph node metastases and distant metastases [181].

Another important prognostic factor is surgical resection margins. The complete excision of tumor is the most important principle of surgical treatment of malignant tumors. Resection margins clear of tumor are associated with a lower recurrence rate and a better survival [180–183]. An adequate margin of resection has not been precisely defined, but margins of 5 mm are generally believed to be adequate for oral carcinoma and margins of 1–2 mm for glottic carcinoma. The adequate resection margins have not been precisely defined for carcinoma of the supraglottis, hypopharynx and oropharynx [183].

Additional important prognostic features are localisation and depth of the tumor [157, 158, 184–186], presence of extracapsular spread in lymph node metastases [187–189] and pattern of tumor growth at the invasive front [172–174].

Recent studies have shown that HPV status has an important prognostic implication. HPV-positive SCC has been demonstrated to respond well to radiotherapy and to have an improved survival, especially oropharyngeal SCC, in comparison to HPV-negative SCC [190–192].

Treatment and prognosis The prognostic value of some other parameters, e.g. differentiation of the tumor [193, 194] and DNA ploidy [195–197], is controversial.

The treatment of choice is complete excision of the tumor. For small tumors at some locations, such as the glottic SCC, the primary treatment is radiation. In large tumors, surgery is usually followed by radiotherapy. Patients with advanced, unresectable tumors, with or without metastases, are treated by concurrent chemotherapy and radiotherapy [161].

1.5 Variants (Subtypes) of Squamous Cell Carcinoma

The majority of cases of SCC of the head and neck are the conventional-type SCC. Several variants (subtypes) of SCC have been described, including VC, spindle cell carcinoma, basaloid SCC, papillary SCC, lymphoepithelial carcinoma, adenoid (acantholytic) SCC and adenosquamous carcinoma. A specially rare variant is SCC with sebaceous differentiation (Fig. 1.16a, b). Their recognition is important because most of them are true clinicopathologic entities, with a different prognostic implication: basaloid SCC, adenosquamous carcinoma and lymphoepithelial carcinoma are more aggressive than conventional SCC, whereas verrucous SCC and papillary SCC have a better prognosis than conventional SCC [198]. Their recognition will also enable to differentiate them from other malignant neoplasms, such as adenocarcinoma, malignant melanoma, neuroendocrine carcinoma, malignant lymphoma and sarcoma.

During the last two decades, it has become clear that a subset of head and neck SCC are related to infection with HPV and that these tumors have a better prognosis than non-HPV-related SCC. They usually arise in the oropharynx and most frequently exhibit non-keratinizing morphology. The term “non-keratinizing SCC” has been proposed for these tumors and should be recognised as a new subtype of head and neck SCC [192].

However, there is merging evidence that HPV-related SCCs can also show other morphologic patterns, and the list of variants of SCC which may be related to HPV is increasing

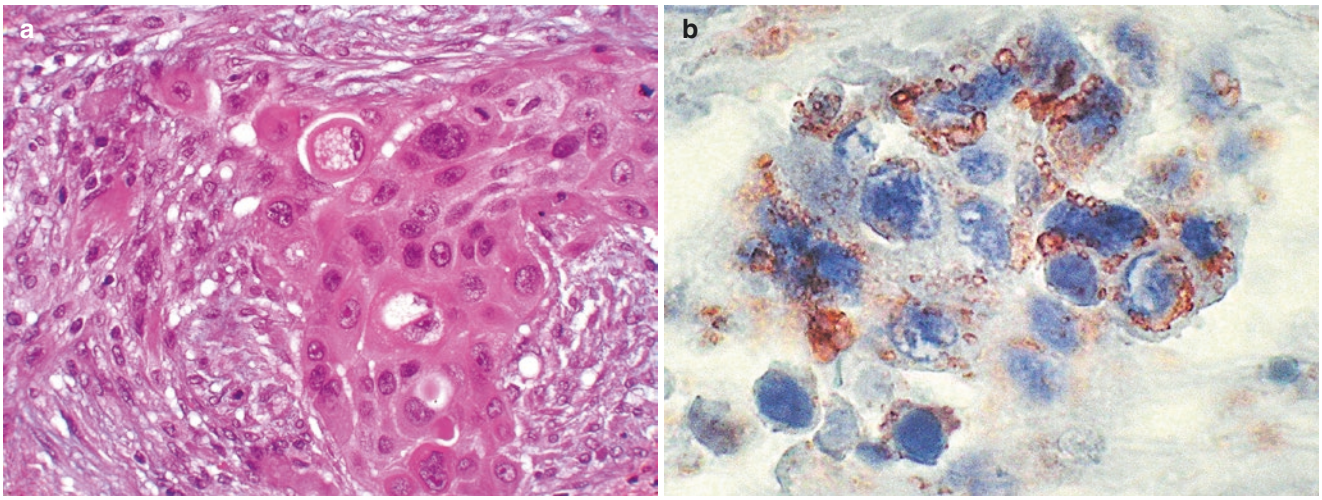

Fig. 1.16 Sebaceous carcinoma. (**a**) The tumor is composed of atypical squamous cells with sebaceous differentiation. (**b**) Oil red O stains lipid droplets in cytoplasm of the tumor cells

[199]. These variants include papillary SCC, adenosquamous SCC and lymphoepithelial carcinoma. It appears that VC and spindle SCC are not related to HPV. In view of these recent findings, it appears that all these variants should be further categorised as HPV-positive or HPV-negative variants. It remains to be determined whether HPV positivity in variants of SCC also implies better prognosis as it has been demonstrated for non-keratinizing HPV-positive SCC.

1.5.1 Spindle Cell Carcinoma

Definition Spindle cell carcinoma (SpCC) is a biphasic tumor composed of conventional SCC and a malignant spindle cell component. Synonyms for SpCC are sarcomatoid carcinoma, carcinosarcoma, collision tumor and pseudosarcoma.

Epidemiology It has been described in various sites of the body, including the upper and lower respiratory tract, breast, skin, urogenital and gastrointestinal tracts and salivary glands [200]. In the head and neck, SpCC occurs most frequently in the larynx [201–203] and oral cavity [204, 205], followed by the skin, tonsils, sinonasal tract and pharynx [67, 204].

Etiology and pathogenesis Similar to conventional SCC, SpCC has been aetiologically related to cigarette smoking and alcohol consumption. It has been suggested that SpCC may develop after radiation exposure; however, some authors believe that this is not a major etiologic factor. The reported incidence of radiation-induced SpCC of the head and neck is between 7.7 % and 9.1 %. It develops after a latent period of 1.2–16 years after radiation exposure [203].

The histogenesis of this tumor is controversial, but there is mounting evidence that SpCC is a monoclonal neoplasm originating from a noncommitted stem cell, giving rise to both epithelial and mesenchymal components [206, 207].

Recent studies suggest that the characteristic spindle cell phenotype of the neoplastic cells in SpCC is the result of epithelial–mesenchymal transition. Epithelial–mesenchymal transition has been postulated as a versatile mechanism that facilitates cellular reposition during embryonal development and can be reactivated in later life, contributing to various pathologic processes, including the progression of malignant tumors and the development of SpCC [208, 209]. There are several features in SpCC that support this hypothesis, e.g. an altered composition of cell-to-cell contacts (adherens junctions, desmosomes), and upregulation of transcription repressors, e.g. Snail, Slug, SIP and Twist [208–210]. These transcription repressors have been demonstrated in experimental models to be potent inducers of epithelial–mesenchymal transition.

Macroscopy Macroscopically, SpCC are usually exophytic polypoid (Fig. 1.17) or pedunculated tumors, with frequent surface ulceration. Less often, SpCC manifest as sessile, endophytic or ulcero-infiltrative tumors.

Microscopy Microscopically, SpCC consist of an SCC component and a spindle cell component (Fig. 1.18a). The former is represented by in situ carcinoma or by an invasive SCC and is often small, requiring multiple sections for demonstration.

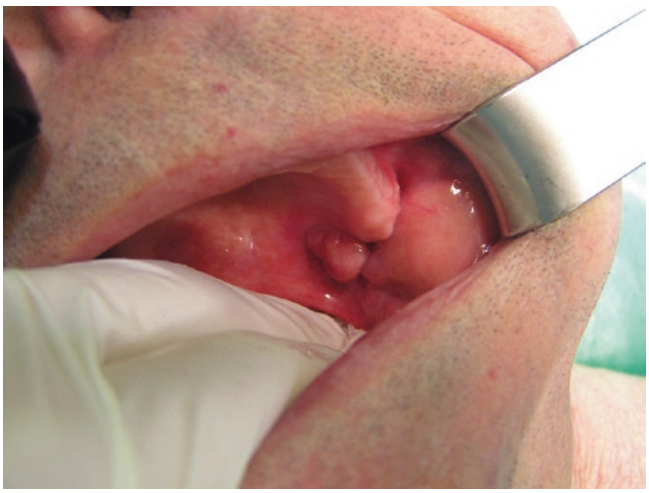

Fig. 1.17 Spindle cell carcinoma of the soft palate: an exophytic, polypoid tumor, covered by intact epithelium

The spindle cell component usually forms the bulk of the tumor. Spindle cells are often pleomorphic, with large hyperchromatic nuclei, prominent nucleoli and numerous mitoses (Fig. 1.18b). They are arranged in fascicles or whorls and can assume many histologic patterns, the most common being that of a malignant fibrous histiocytoma or fibrosarcoma [202, 203]. Foci of osteosarcomatous, chondrosarcomatous or rhabdosarcomatous differentiation may be present, particularly in patients who had previously been treated by radiotherapy [202, 203]. Only spindle cells are sometimes present; in such cases, SpCC can be mistaken for a true sarcoma.

However, occasional cases of SpCC may be less cellullar, closely resembling a reactive fibroblastic proliferation and can thus be mistaken for a pseudosarcomatous reaction in SCC or for a radiation-induced stromal atypia [204].

Metastases usually contain SCC alone or both SCC and spindle cell components and, rarely, only the spindle cell component.

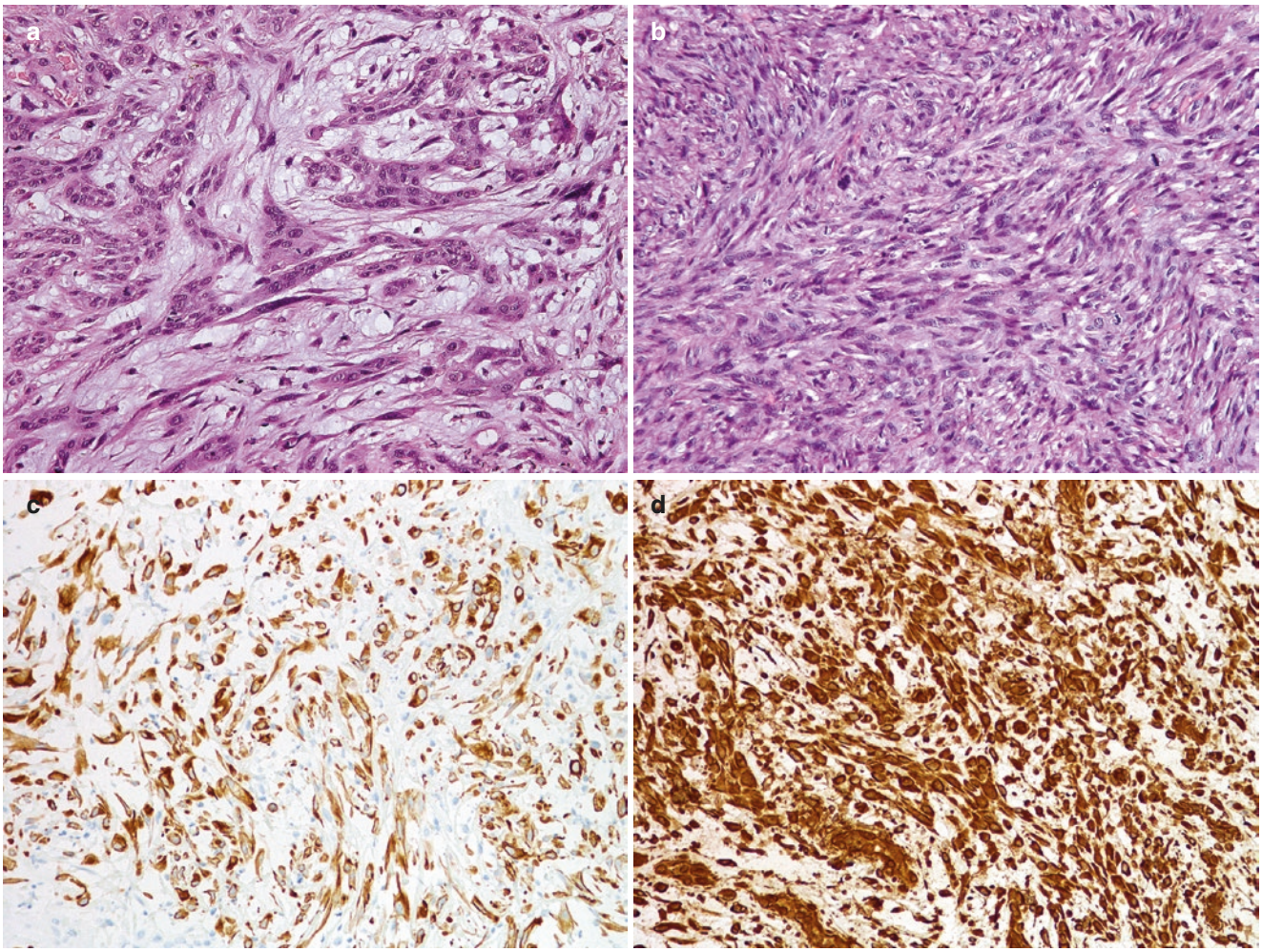

Fig. 1.18 Spindle cell carcinoma. (**a**) Biphasic tumor composed of islands and cords of squamous cell carcinoma and malignant spindle cells. (**b**) Spindle cell component: pleomorphic cells with large hyperchromatic nuclei. (**c**) Positive immunohistochemical staining for cytokeratin in spindle cells. (**d**) Positive immunohistochemical staining for vimentin in spindle cells

Electron microscopy has revealed evidence of epithelial differentiation in spindle cells, such as desmosomes and tonofilaments [211, 212].

Immunohistochemistry Immunohistochemically, tumor cells in SpCC often express epithelial and mesenchymal markers (Fig. 1.18c, d); moreover, keratin and vimentin coexpression has been observed in individual tumor cells [211]. Cytokeratin expression can be demonstrated in spindle cells in 40–85.7 % of cases (Fig. 1.18c), depending on the number of antikeratin antibodies used. The most sensitive/reliable epithelial (keratin) marker for SpCC seems to be cytokeratins AE1/AE3 and CK18 [203]. Spindle cells always express vimentin and often other mesenchymal filaments, such as myogenic markers (smooth muscle actin, muscle-specific actin, desmin). The presence of S-100 protein has been reported in rare cases of SpCC. SpCC does not express glial fibrillary acid protein (GFAP), chromogranin or HMB-45 [203].

Differential diagnosis A diagnosis of a SpCC is based on demonstration of an invasive or in situ SCC and a malignant spindle cell component. However, when a SCC component cannot be demonstrated, the diagnosis is more difficult and SpCC must be distinguished from a number of benign and malignant processes, such as spindle cell sarcomas, nodular fasciitis, inflammatory myofibroblastic tumor and malignant melanoma.

In the head and neck, true sarcomas (with the exception of chondrosarcoma) and benign mesenchymal tumors are very rare; if present, they are usually located in deep structures [203]. It is therefore the general view, that a malignant spindle cell tumor in the mucosa of the upper aerodigestive tract is probably a SpCC and not a sarcoma.

A negative reaction for S-100 protein and HMB-45 helps to distinguish SpCC from malignant melanoma.

Treatment and prognosis Wide surgical excision, alone or with radical neck dissection is the most successful treatment for SpCC. Radiation therapy is generally considered less effective.

The prognosis is similar to that of conventional SCC and depends on the location of the tumor and stage: glottic SpCC has a good prognosis, while SpCC in the oral cavity and paranasal sinuses behaves more aggressively [201]. Prognostic significance has been also suggested for the gross appearance of the tumor, i.e. polypoid lesions have a better prognosis than flat ulcerative tumors [67].

The reported 5-year survival is between 63 and 94 %; the overall lethality of the tumor is 30–34 % [203].

1.5.2 Verrucous Carcinoma

Definition Verrucous carcinoma (Ackerman's tumor) (VC) is a variant of well-differentiated SCC that was originally described by Ackerman in 1948 [213]. It is characterised by an exophytic warty growth, which is slow but locally invasive and can cause extensive local destruction if left untreated. It rarely, if ever, metastasises [214].

Epidemiology The majority of VC (75 %) occurs in the oral cavity and 15 % in the larynx. In the oral cavity, buccal mucosa and gingiva are most frequently involved, and in the larynx, the most frequent site of occurrence is the vocal cords. It occurs rarely in other locations in the head and neck, such as the nasal cavity, sinonasal tract or nasopharynx. It has also been described elsewhere in the body, i.e. the skin, anus, genitalia, urinary bladder and oesophagus [215].

Etiology and pathogenesis VC has been aetiologically related to the use of chewing tobacco or snuff. The habitual chewing of "pan", a mixture of betel leaf, lime, betel nuts and tobacco, has been implicated in the high incidence of VC of the oral cavity in India [216].

Human papillomavirus (HPV) is also a possible etiologic factor. Although this association has been commonly quoted, the reported prevalence of HPV in VC ranges from 0 to 100 % [217]. Three studies have recently investigated the possible role of HPV in VC [217–219]. Using highly sensitive and specific molecular methods, it was shown that HPV of α, γ or μ genera are not associated with the aetiopathogenesis of VC of the head and neck. Furthermore, no evidence of transcriptionally active high-risk α-HPV was found in VC by real-time polymerase chain reaction (RT-PCR) for HPV E6/E7 mRNA. It appears that VC of the head and neck is not associated with infection with HPV.

Macroscopy Macroscopically, VC usually presents as a large, broad-based exophytic tumor with a white keratotic and warty surface (Fig. 1.19a). On the cut surface, it is firm or hard, tan to white and may show keratin-filled surface clefts. It is usually large by the time of diagnosis, measuring up to 10 cm in the largest dimension.

Microscopy Microscopically, VC consists of thickened club-shaped filiform projections lined by thick, well-differentiated squamous epithelium with marked surface keratinization ("church-spire" keratosis). The squamous epithelial cells in VC are large [220] and lack the usual cytologic criteria of malignancy. Mitoses are rare and are only observed in the suprabasal layer; there are no abnormal mitoses. VC invades the subjacent stroma with well-defined pushing rather than infiltrative borders (Fig. 1.19b). A lymphoplasmacytic inflammatory response is common in the stroma.

Hybrid (mixed) tumors also exist, composed of VC and conventional well-differentiated SCC; the reported incidence for oral cavity and the larynx is 20 % and 10 %, respectively (Fig. 1.20a–c) [221–223].

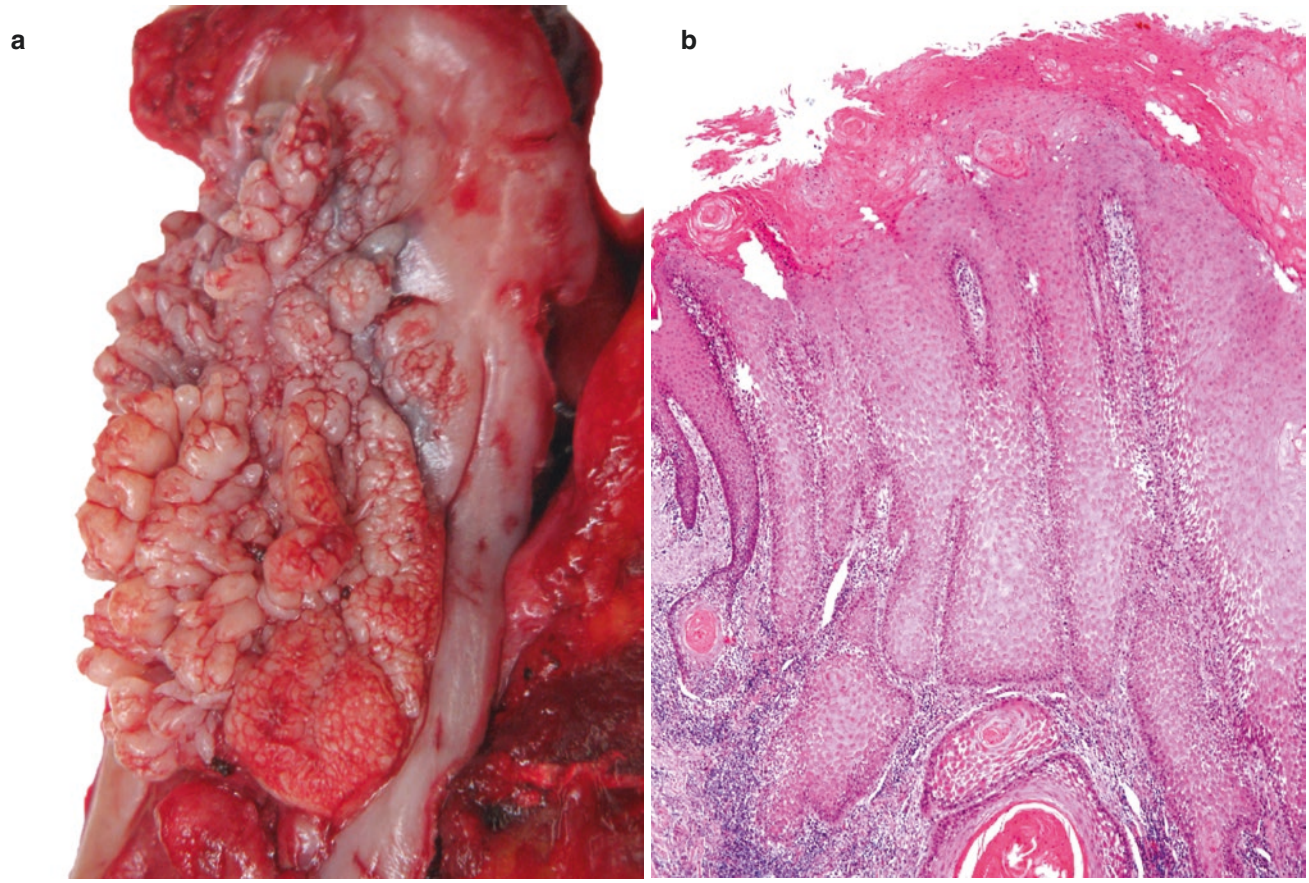

Fig. 1.19 (**a**) Verrucous carcinoma of the oral mucosa: a broad-based exophytic tumor with a warty surface. (**b**) Projections and invaginations lined by thick well-differentiated squamous epithelium with marked surface keratinization, invading the stroma with well-defined pushing margins

Immunohistochemistry Differential expression patterns of desmosomal proteins and p63 has been described in VC of the head and neck in comparison to conventional SCC. Therefore, immunohistochemistry against p63 and some desmosomal proteins, e.g. plakophilin 1, desmoglein 2, desmoglein 3, desmocollin 2 and desmoplakin, labels foci of SCC in VC and might be used as an adjunct in the diagnostics of hybrid carcinoma [224–226].

Differential diagnosis VC is characterised by a high frequency of initial misdiagnosis; Orvidas et al. reported a series of 53 laryngeal VC; 16 of 31 patients (52 %) had received an incorrect diagnosis of a benign lesion [221]. This emphasises the need for close cooperation between the pathologist and the clinician in order to establish a diagnosis of VC. An adequate, full-thickness biopsy specimen must be taken, when a clinician suspects VC; moreover, multiple biopsies may be needed to rule out a conventional SCC component in a VC.

Differential diagnosis includes verrucous hyperplasia, well-differentiated SCC, papillary SCC and squamous papilloma.

Invasion below the level of the basal cell layer of the neighbouring normal squamous epithelium distinguishes VC from verrucous hyperplasia. However, whether this feature adequately discriminates between VC and verrucous hyperplasia is debatable, since verrucous hyperplasia could also be an exophytic form of VC [146].

Lack of atypia helps to rule out conventional SCC and papillary SCC.

VC also lacks the well-formed, wide papillary fronds of a squamous cell papilloma.

An additional feature supporting the diagnosis of VC is enlarged spinous cells by morphometrical analysis [220].

Treatment and prognosis VC may be treated by excision (by laser or surgery) or by radiotherapy. It appears that surgery is more effective treatment for VC [221, 227]. Hagen et al. reported a 92.4 % cure rate for primary surgery in patients with laryngeal VC [228]. In contrast, Ferlito and Recher reported a 29 % cure rate for radiotherapy in laryngeal VC [229]. Some other studies have shown a 46–57 % rate of failure for primary radiation therapy in VC [230, 231].

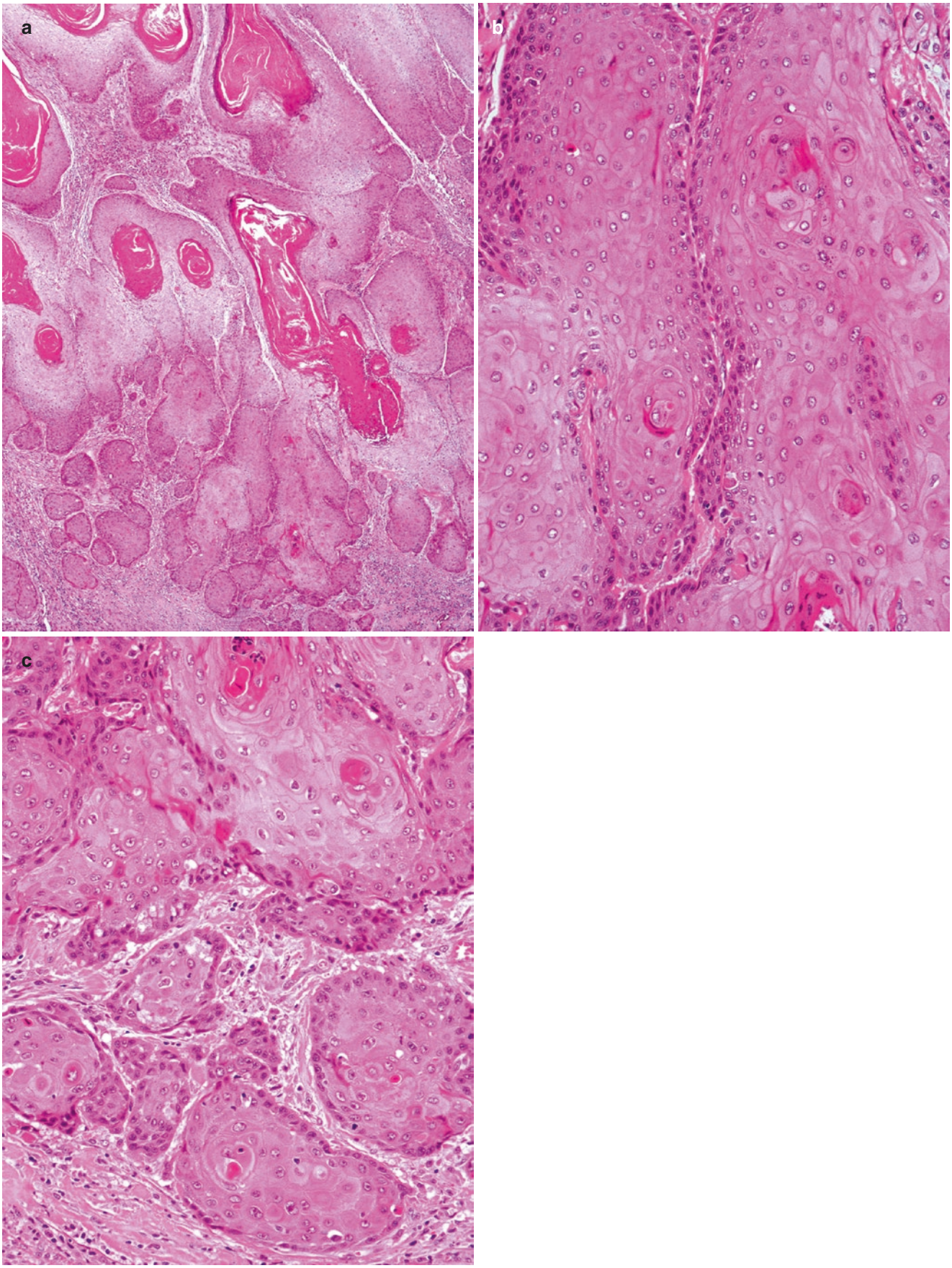

Fig. 1.20 Hybrid carcinoma. (**a**) Verrucous carcinoma in the upper part, with transition to well differentiated conventional squamous cell carcinoma in the lower part. (**b**) Higher magnification of verrucous carcinoma. (**c**) Higher magnification of transition to conventional squamous cell carcinoma

Furthermore, early reports suggested anaplastic transformation following radiotherapy [228, 232, 233]. However, recent studies do not support this notion. It appears that some of the reported cases of transformation of VC to SCC after radiotherapy were mixed (hybrid) tumors. Moreover, a similar transformation can also occur after surgical treatment of VC [231, 234, 235]. Radiotherapy is now believed to be an appropriate mode of treatment for oral VC [236] and laryngeal VC [235].

Patients with hybrid carcinoma must be treated aggressively as if they had conventional SCC [221].

In his original report, Ackerman noted metastasis in the regional lymph node only in 1 of the 31 patients, and no distant spread was observed in his series [213]. Further studies have confirmed his observation that pure VC does not metastasise [221]; cases of VC with metastases were really hybrid carcinoma that were not detected at initial biopsy.

VC has therefore an excellent prognosis; the overall 5-year survival rate is 77 % [237]. It is important to recognise hybrid tumors as foci of conventional SCC in VC indicate a potential for metastasis. Orvidas et al. reported that a patient with hybrid carcinoma of the larynx died of the disease [221].

1.5.3 Papillary Squamous Cell Carcinoma

Definition Papillary squamous cell carcinoma (PSCC) is an uncommon variant of SCC originally described by Crissman et al. in 1988 [238]. Its main characteristics are a papillary growth pattern and a good prognosis.

Epidemiology and etiology In the head and neck, PSCC shows a predilection for the oropharynx, hypopharynx, larynx and the sinonasal tract [238–243]. It also occurs in other parts of the body, such as the skin, uterine cervix and conjunctiva.

According to recent findings, PSCC of the head and neck should be divided into two groups: HPV-negative and HPV-positive group. The former is etiopathogenetically associated with smoking and alcohol abuse, whereas the latter is related to infection with HPV, particularly type 16 [242, 244, 245]. A further similarity to HPV-positive SCC of the upper respiratory tract is the location: the majority of reported HPV-positive papillary SCCs were located in the oropharynx (lingual and palatinal tonsils) and oral cavity, although a few cases of sinonasal and laryngeal PSCC were also HPV positive [244, 245]. HPV-positive cases also overexpressed p16, which is generally accepted as a surrogate marker of HPV infection, though lacking sufficient specificity to replace in situ hybridisation or PCR. The general consensus is that p16 immunohistochemistry and HPV in situ hybridisation are complementary, with p16 being an appropriate screening tool [244].

Macroscopy Macroscopically, PSCCs present as papillary, friable and soft tumors, ranging in size from 2 mm to 4 cm (Fig. 1.21a).

Microscopy The main histologic feature of PSCC is the papillary growth pattern, which comprises the majority of the tumor. Papillae consist of a central fibrovascular core covered by neoplastic squamous epithelium. The covering epithelium may be composed of immature basaloid cells or may be more pleomorphic resembling CIS (Fig. 1.21b); it may be non-keratinizing or keratinizing [245].

Multiple lesions can be found, consisting either of invasive PSCC or mucosal papillary hyperplasia. Stromal invasion is often difficult to demonstrate in biopsy specimens, and additional biopsies are sometimes needed to make a diagnosis of an invasive PSCC. A dense lymphoplasmacellular infiltration is usually present in the stroma at the base of the carcinoma but is scarce within the papillae. If no stromal invasion is found, the lesion is called papillary atypical hyperplasia, or PSCC in situ, or non-invasive PSCC.

Differential diagnosis Differential diagnosis includes squamous papilloma, VC and SCC with an exophytic or fungating pattern. Papilloma and VC share similar architecture with PSCC, but PSCC is differentiated from both VC and papilloma by the presence of atypia of the squamous epithelium covering the papillae. Differentiation between exophytic and PSCC can be more difficult, since the histologic criteria for a diagnosis of exophytic SCC are not clearly defined [241, 246].

Treatment and prognosis Treatment of PSCC is similar to that of conventional SCC. Patients with PSCC are generally believed to have a better prognosis than those with conventional SCC [241, 242]. Patients with HPV-positive tumors have been reported to have a slightly better outcome: the 5-year survival rates for p16-positive and p16-negative cases were 80 % and 70 %, respectively [245].

1.5.4 Basaloid Squamous Cell Carcinoma

Definition Basaloid squamous cell carcinoma (BSCC) is a poorly differentiated SCC composed of basaloid cells and squamous cell carcinoma, characterised by an aggressive clinical course [247]. It was first described by Wain et al. in 1986 [248]. It has a predilection for the upper aerodigestive tract but also occurs in other locations such as the uterine cervix, oesophagus, lung and anus. It is morphologically similar to HPV-positive non-keratinizing SCC. However, the behaviour and prognosis in these two tumor types is different, and determining HPV status is important, enabling to separate basaloid SCC from HPV-positive non-keratinizing SCC.

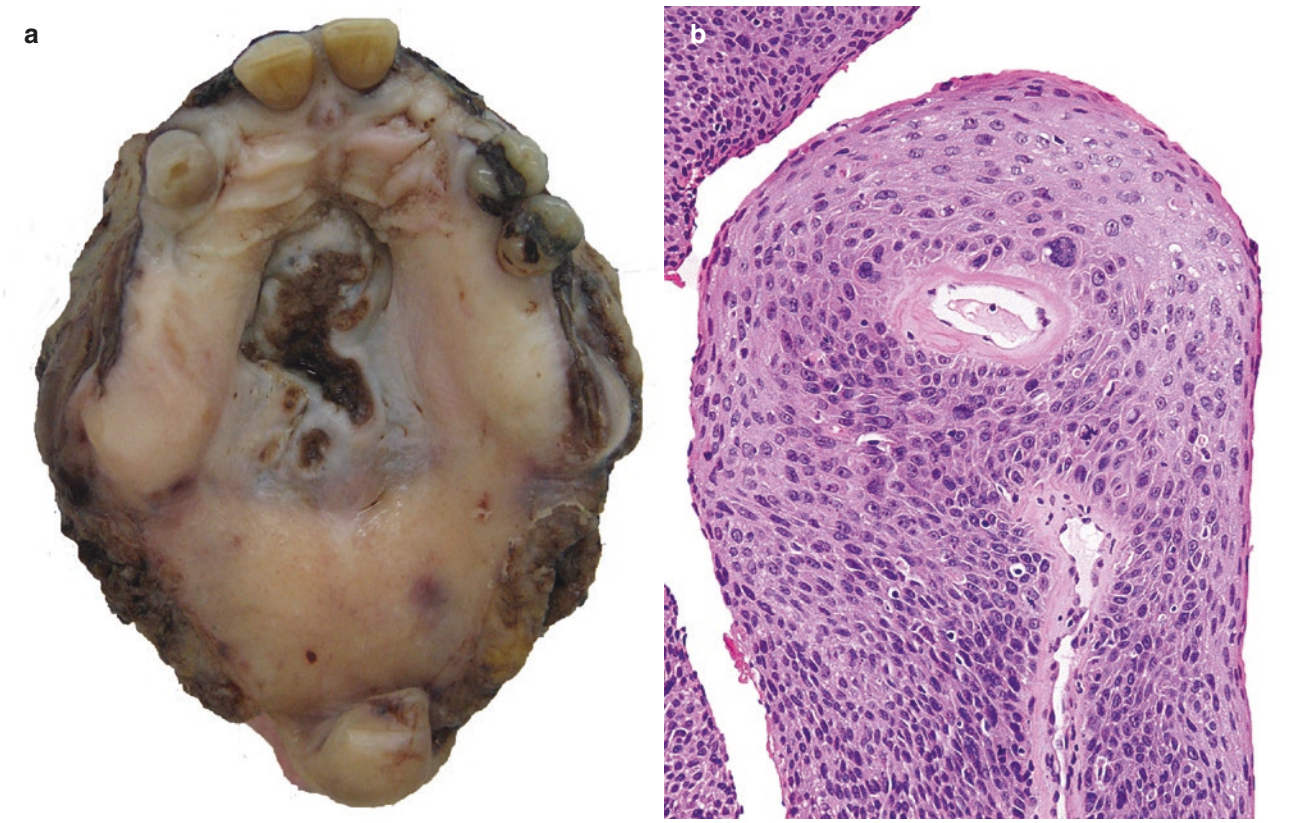

Fig. 1.21 Papillary squamous cell carcinoma of the hard palate. (**a**) An ulcerative lesion on the hard palate, with infiltration and destruction of maxilla. (**b**) Microscopically, tumor consists of papillae with a central fibrovascular core, covered by neoplastic squamous epithelium

Epidemiology and etiology In the upper aerodigestive tract, BSCC shows a predilection for the hypopharynx (piriform sinus), base of the tongue and supraglottic larynx [249, 250]. It has also been described in the oropharynx [249, 250], oral cavity [251, 252] and trachea [253, 254]. The suggested precursor of BSCC is a totipotent primitive cell located in the basal cell layer of the surface epithelium or within the seromucinous glands [248, 250].

Tobacco and alcohol use are strong risk factors for the development of BSCC [255].

Macroscopy Macroscopically, the tumor usually appears as a white, firm, poorly defined, exophytic, polypoid and centrally ulcerated mass with a peripheral submucosal infiltration [256].

Microscopy Microscopically, BSCC is composed of closely packed basaloid cells, which are small, with hyperchromatic nuclei with or without nucleoli and scant cytoplasm (Fig. 1.22a). The tumor grows in a solid pattern with a lobular configuration, with a frequent peripheral palisading of nuclei. Large central necroses of comedo type are frequent. Distinctive features of BSCC, not found in conventional SCC, are small cystic spaces containing periodic acid–Schiff (PAS)- and alcian blue-positive material and focal stromal hyalinisation [248, 255].

BSCC is always associated with a SCC component which can present either as in situ or invasive SCC. Invasive SCC is usually located superficially and is typically well to moderately differentiated. It may also present as focal squamous differentiation within basaloid tumor islands. The transition between the squamous cells and basaloid cells is often abrupt (Fig. 1.22b) or there may be a narrow zone of transition.

If there is extensive ulceration, only dysplastic changes may be identifiable in the intact surface epithelium [255, 256]. Rarely, BSCC exhibits a malignant spindle cell component [256, 257]. Metastases may demonstrate basaloid carcinoma, squamous carcinoma or both [256].

By electron microscopy, desmosomes and tonofilaments have been demonstrated in basaloid cells and in squamous cells. There are no neurosecretory granules, myofilaments or secretory granules [248, 258].

Immunohistochemistry Immunohistochemically, BSCC expresses keratin and epithelial membrane antigen but the percentage of positive cells varies among different reports. It is advised to use a cocktail of keratin antibodies (i.e. CAM 5.2, AE1/AE3) to avoid false-negative results [256]. Some cases

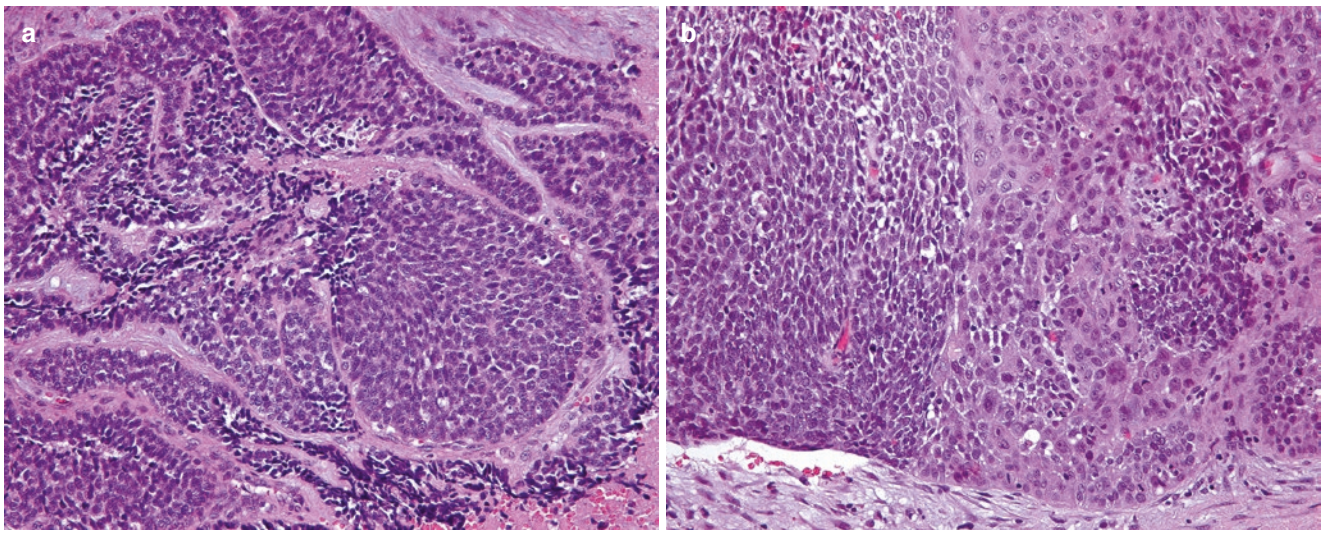

Fig. 1.22 Basaloid squamous cell carcinoma. (**a**) Closely packed basaloid cells with hyperchromatic nuclei and scant cytoplasm, with focal peripheral palisading of nuclei. (**b**) Abrupt transition between squamous and basaloid cells

express carcinoembryonic antigen (CEA) and neuron-specific enolase (NSE) [249, 255, 259], while expression of S-100 protein, vimentin and muscle-specific actin varies among different reports. Vimentin was negative in some studies [249, 251], while Barnes et al. [256] described positive staining in the majority of basaloid cells, with a peculiar pattern of staining, forming a delicate perinuclear rim. Varying results have also been reported for S-100 immunoreactivity. Some authors have described focal immunoreactivity in a few cases [255, 256], while others did not find any S-100-positive tumor cells [249, 251, 260]. However, most cases displayed numerous S-100-positive dendritic cells intermingled with the tumor cells [249, 256, 260, 261]. BSCC does not express chromogranin, synaptophysin or GFAP [249, 255, 256].

Differential diagnosis Differential diagnosis includes HPV-positive non-keratinizing SCC, neuroendocrine carcinoma, adenoid cystic carcinoma, adenocarcinoma and adenosquamous carcinoma.

HPV-positive non-keratinizing SCC is morphologically similar to basaloid SCC. It can be distinguished from basaloid SCC by overexpression of p16 using immunohistochemistry, by the presence of HPV 16/18, which can be proven either by in situ hybridisation, PCR or by identifying oncoproteins E6/E7 by in situ hybridisation.

Neuroendocrine carcinoma expresses various neuroendocrine markers which help to distinguish neuroendocrine carcinoma from BSCC. However, since 60–75 % of cases of BSCC have been reported to express NSE [255, 259, 262], the application of other neuroendocrine markers, including chromogranin, CD56 and synaptophysin, is advised [255, 262].

Adenoid cystic carcinoma, especially the solid variant, may resemble BSCC but adenoid cystic carcinoma rarely shows squamous differentiation (Fig. 1.22b). Immunohistochemistry may also be helpful: tumor cells in adenoid cystic carcinoma express S-100 protein and vimentin, while tumor cells in BSCC do not express either of the two markers [249, 256].

Adenocarcinoma and adenosquamous carcinoma can be distinguished from BSCC by the presence of gland formation and mucin secretion within the tumor cells.

Treatment and prognosis BSCC is an aggressive, rapidly growing tumor characterised by an advanced stage at the time of diagnosis and a poor prognosis. Metastases to the regional lymph nodes have been reported in two thirds of patients [249, 250, 253, 255] and distant metastases involving lungs, bone, skin or brain in 37–50 % of patients [249, 250, 255].

It is generally believed that BSCC is more aggressive than conventional SCC [248, 249, 263]. However, some studies indicate that BSCC exhibits behaviour similar to that of high-grade conventional SCC of the head and neck [255, 264, 265].

The treatment of choice is radical surgical excision and, because of early regional lymph node and distant visceral metastases, radical neck dissection and supplemental radio- and chemotherapy [248, 256].

1.5.5 Non-keratinizing HPV-Positive Squamous Cell Carcinoma

Definition Non-keratinizing HPV-positive SCC is a new category of SCC of the head and neck. It is the most frequent morphologic pattern in HPV-related SCC of the head and

neck [266, 267]. However, it is becoming clear that HPV can be also associated with other well-defined subtypes of SCC [267], as well as with other morphologic patterns, which were initially believed not to be related to HPV infection, for example focally keratinizing SCC [266, 267], pleomorphic or giant cell carcinoma, clear cell carcinoma (personal observation), sinonasal carcinoma with adenoid cystic-like features [268], etc. (Fig. 1.23a–d).

Microscopy Non-keratinizing HPV-positive SCC is composed of large nests of cells with hyperchromatic nuclei with inconspicuous nucleoli, scant cytoplasm and indistinct cell borders (Fig. 1.23a). Mitoses and apoptoses are usually abundant. Comedo necroses are often present [266, 267].

Immunohistochemistry Immunohistochemistry characteristically demonstrates strong and diffuse nuclear and cytoplasmic expression of p16 in at least 75 % of the tumor [267] (Fig. 1.23e). HPV 16/18 can be demonstrated by ISH or PCR. However, it has been shown that the mere presence of high-risk HPV in carcinoma can be the result of an incidental colonisation. Only an integrated, transcriptionally active virus is considered an etiologic agent in cancerogenesis, and it can be identified using ISH for mRNA E6/E7 of high-risk HPV, with the characteristic dot-like reaction [269] (Fig. 1.23f).

Differential diagnosis In the differential diagnosis, BSCC, neuroendocrine carcinoma, malignant melanoma and malignant lymphoma must be considered.

BSCC does not demonstrate overexpression of p16 and does not harbour HPV.

Neuroendocrine carcinoma does not exhibit squamous differentiation. It expresses dot-like cytokeratins as well as various neuroendocrine markers which are not expressed in non-keratinizing HPV-positive SCC.

Non-keratinizing HPV-positive SCC usually exhibits at least focal squamous differentiation, enabling to separate it from other malignant tumors. If not, immunohistochemistry must be used to confirm the squamous cell origin and to exclude malignant melanoma and malignant lymphoma.

Treatment and prognosis Patients with HPV-positive non-keratinizing SCC have substantially improved outcomes (28–80 % reductions in the risk of death) than HPV-negative patients due to a better response to treatment with radio-/chemotherapy [270, 271].

1.5.6 Adenoid (Acantholytic) Squamous Cell Carcinoma

Definition Adenoid squamous cell carcinoma (adenoid SCC) is an uncommon histopathologic type of SCC that was first recognised by Lever in 1947 [272]. It resembles ordinary SCC but, because of acantholysis of malignant squamous cells, pseudolumina are formed, creating the appearance of glandular differentiation. There is no evidence of true glandular differentiation or mucin production [273].

Adenoid SCC has been referred to by a variety of names, such as pseudoglandular SCC, acantholytic SCC, SCC with gland-like features and adenoacanthoma.

Epidemiology In the head and neck, it arises most frequently in the skin (especially of sun-exposed areas) [274, 275] and less frequently in mucosal sites of the upper aerodigestive tract, including the lip, oral cavity, tongue and nasopharynx [276–282].

Microscopy Adenoid SCC is composed of islands and cords of keratinizing SCC; because of acantholysis of neoplastic cells, pseudoglandular (adenoid) structures are formed which have central lumina containing detached acantholytic neoplastic cells, necrotic debris or they may be empty (Fig. 1.24a). The conventional squamous cell carcinoma component is nearly always present.

Acantholysis may lead to the formation of anastomosing spaces and channels thus mimicking an angiosarcoma (Fig. 1.24b). This variant of adenoid SCC is termed pseudovascular adenoid SCC or angiosarcoma-like SCC and has been reported in the skin of the head and neck [275], in oral cavity [282], as well as in other organs, such as breast and lungs [283].

Ultrastructural analysis has revealed hemidesmosomes and attached tonofilaments, and no glandular features, thus supporting the squamous origin of adenoid SCC [278].

Immunohistochemistry Immunohistochemically, adenoid SCC is positive for epithelial markers such as cytokeratins and EMA; it may also express CEA and vimentin [284].

Differential diagnosis Adenoid SCC must be differentiated from adenocarcinoma, particularly adenoid cystic carcinoma, adenosquamous carcinoma and mucoepidermoid carcinoma. This is best achieved by demonstrating that in adenoid SCC, there is no true gland formation and that stains for mucin are negative.

Differential diagnosis also includes angiosarcoma but immunohistochemistry helps to distinguish the two tumors. Angiosarcoma typically expresses vascular antigens (CD31, CD34, von Willebrand factor), which are negative in adenoid SCC. Cytokeratin, however, might also be positive in some angiosarcomas.

Treatment and prognosis Treatment and prognosis are similar in adenoid SCC and conventional SCC. Some authors, however, believe that adenoid SCC has an aggres-

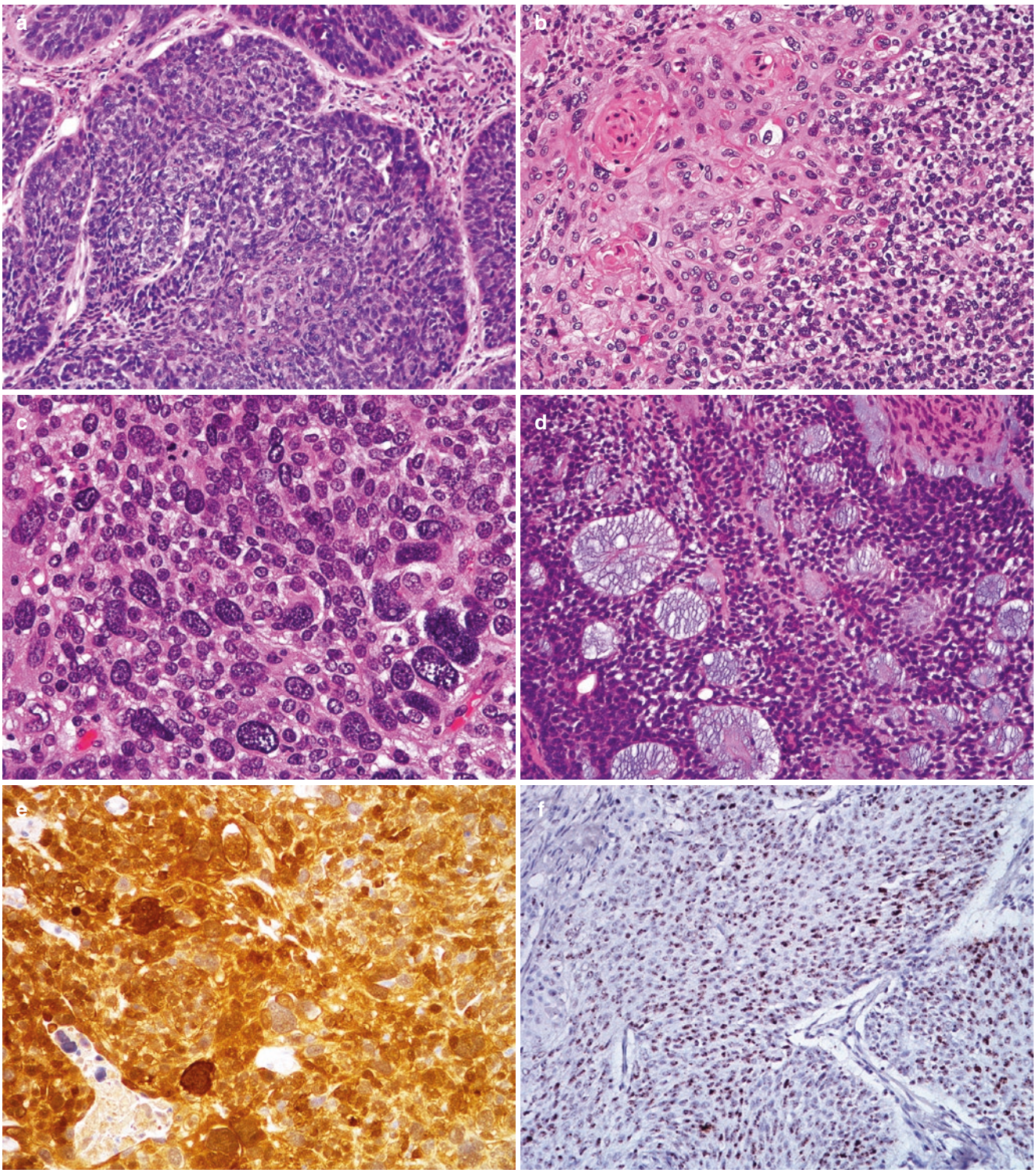

Fig. 1.23 Patterns of HPV-positive squamous cell carcinoma of the head and neck. (**a**) The most common, non-keratinizing pattern. (**b**) Keratinizing squamous cell carcinoma and an area with small, clear tumor cells. (**c**) Poorly differentiated (anaplastic) carcinoma with large, pleomorphic tumor cells. (**d**) Adenoid cystic carcinoma-like features. (**e**) Immunohistochemical staining for p16: diffuse cytoplasmic and nuclear staining. (**f**) In situ hybridisation for mRNA E6/E7 for 7 high-risk HPV: dot-like reaction in tumor cells

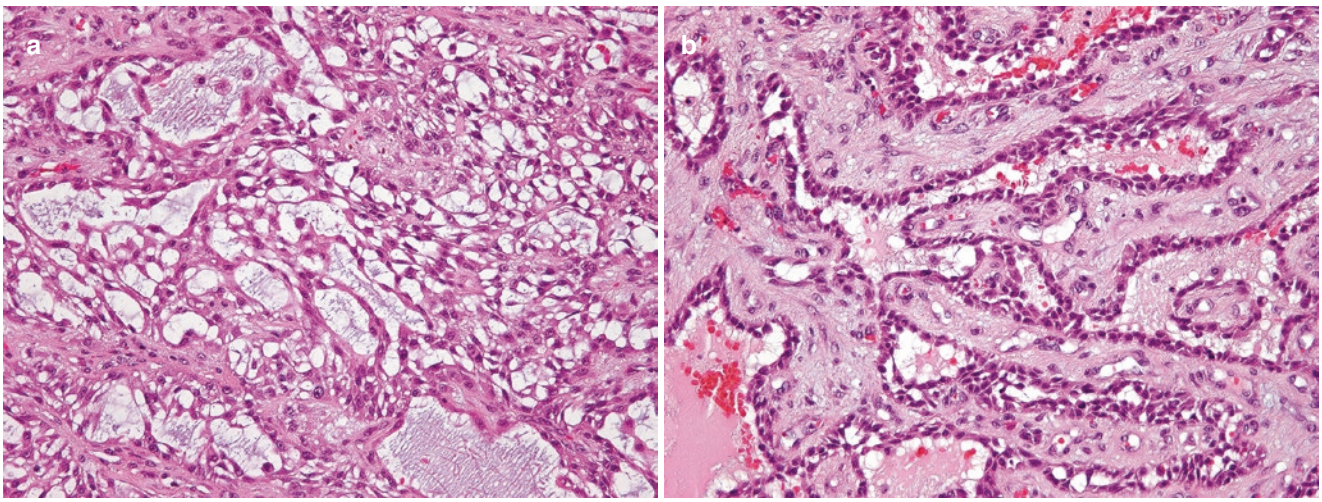

Fig. 1.24 Adenoid squamous cell carcinoma. (**a**) Islands of squamous cell carcinoma with pseudoglandular (adenoid) structures due to acantholysis of neoplastic cells. (**b**) Anastomosing spaces and channels mimicking an angiosarcoma

sive behaviour and a worse prognosis than conventional SCC [277–279, 284], but the number of patients reported so far is too small to draw firm conclusions.

1.5.7 Adenosquamous Carcinoma

Definition Adenosquamous carcinoma (ASC) is a rare malignant epithelial tumor characterised by the presence of both SCC and adenocarcinoma and aggressive behaviour.

Epidemiology It occurs in various sites, such as the pancreas, lung, uterine cervix, prostate, stomach, colon and breast [285]. In the head and neck, it was first reported by Gerughty et al. who described a series of ten patients with nasal, oral and laryngeal ASC [286].

Since then, over 150 cases of ASC of the head and neck have been reported; the most frequent site of occurrence is the larynx [285, 287–289], followed by the oral cavity [285, 288–292], the nose and paranasal sinuses [285, 288, 289], nasopharynx [289, 293], oropharynx [288, 289, 293] and hypopharynx [285, 288, 289, 293].

Etiology and pathogenesis The etiology has not been defined but cigarette smoking and alcohol consumption probably play an important role in the pathogenesis of ASC, similarly to other types of SCC in the upper aerodigestive tract [285, 286, 290].

A few cases of ASC, particularly those occurring at sites with a known high prevalence of HPV, are probably related to HPV infection. Masand et al. described one case of ASC from the nasal mucosa and two cases of ASC from the oropharynx, which have been found to harbour high-risk HPV E6 and E7, suggesting active transcription with detectable E6 and E7 and overexpression of p16 [288].

The histogenesis of ASC has not yet been completely elucidated. Some authors have suggested an origin in the salivary and/or mucoserous glands, while others favour a surface epithelial derivation or a combined glandular and surface epithelial derivation [286]. However, it is becoming increasingly accepted that the basal cells of the surface squamous epithelium, which are capable of divergent differentiation, are the sole origin of ASC [285, 291, 292].

Macroscopy ASC does not differ macroscopically from conventional SCC (Fig. 1.25a).

Microscopy Microscopically, it is characterised by the presence of both adenocarcinoma and SCC. The two components occur in close proximity but are generally distinct and separate and are not closely intermingled as in mucoepidermoid carcinoma. The SCC component can present either as in situ or as invasive SCC, manifesting intercellular bridges, keratin pearl formation or dyskeratosis. The adenocarcinomatous component is usually located in the deeper parts of the tumor; it consists of tubular, alveolar or ductal structures (Fig. 1.25b).

The presence of intracytoplasmic mucin can be demonstrated by special techniques, such as PAS, Alcian blue and Mayer mucicarmine. Necroses and mitoses are common [285, 290]. Perineural invasion is frequently present.

By electron microscopy, features of both squamous and adenocarcinomatous differentiation have been demonstrated [294].

Immunohistochemistry Immunohistochemistry has demonstrated distinctive staining patterns in both components: positive staining for CEA and low-molecular-weight

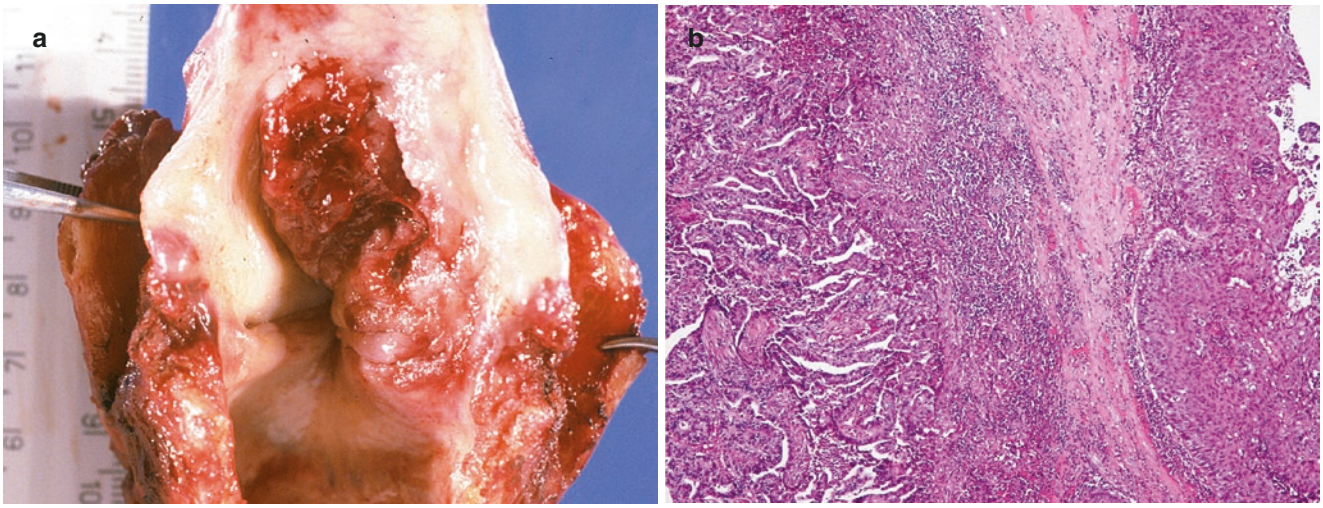

Fig. 1.25 Adenosquamous carcinoma. (**a**) A large transglottic tumor of the right side of the larynx. (**b**) Squamous cell in situ carcinoma and the adenocarcinomatous component in the deeper part of the tumor. The two components are in close proximity, though separate

cytokeratins, such as CK7 and CAM5.2 in the adenocarcinomatous component and positive staining for p63, CK5/6 and CK7 in the SCC component. Both components express high-molecular-weight cytokeratins [285, 293].

Differential diagnosis Differential diagnosis includes mucoepidermoid carcinoma, adenoid SCC, conventional SCC invading the normal salivary glands and necrotising sialometaplasia.

It is important to differentiate ASC from mucoepidermoid carcinoma because ASC has a worse prognosis than mucoepidermoid carcinoma [286, 290]. The histopathologic features favouring a diagnosis of ASC are separate and distinct areas of SCC and adenocarcinomatous components and the involvement of the surface epithelium, exhibiting either high-grade SIL, CIS or invasive SCC.

The presence of mucin in the true glandular spaces helps to distinguish ASC from adenoid SCC.

Conventional SCC invading or entrapping the normal salivary or mucoserous glands can be confused with ASC, especially in small biopsy specimens. In such cases, preservation of the lobular gland architecture and lack of significant atypia are observed, helping to distinguish conventional SCC from ASC.

Finally, ASC must be differentiated from necrotising sialometaplasia, which is a benign condition. The histopathologic features suggesting a diagnosis of necrotising sialometaplasia include surface ulceration, localisation in the minor salivary glands, lobular architecture, partial necrosis of the salivary gland and squamous metaplasia of the salivary ducts.

Treatment and prognosis In general, ASC has a more aggressive course than conventional SCC [286, 291], with a tendency for early lymph node metastases, frequent local recurrences and occasional dissemination [290]. The reported 5-year survival rate is between 13 and 25% [286, 287, 290]. It appears that HPV-positive ASC behaves less aggressively, similarly to other HPV-related SCC, but the number of reported patients is too small to draw any conclusions [199, 288].

The treatment of choice is radical surgical excision. Irradiation alone has had poor results. Some reports indicate that radical surgery combined with irradiation may improve the survival rate [287, 290].

1.5.8 Lymphoepithelial Carcinoma

Definition Lymphoepithelial carcinoma (LEC) is a poorly differentiated SCC or undifferentiated carcinoma, associated with a dense lymphocytic stromal infiltration. It is morphologically indistinguishable from nasopharyngeal carcinoma type 3 of the WHO classification [295]. It was originally described in the nasopharynx in 1921 by Regaud and Reverchon [296] and independently by Schminke [297]. Synonyms for LEC include lymphoepithelioma, nasopharyngeal-type carcinoma, Regaud- and Schminke-type lymphoepithelioma and undifferentiated carcinoma. The specific features of nasopharyngeal carcinoma are extensively discussed in Chap. 6.

Epidemiology It occurs rarely in other locations in the head and neck, such as the oropharynx, salivary glands, tonsils, tongue, soft palate, uvula, floor of the mouth, sinonasal tract, larynx and hypopharynx, as well as elsewhere in the body including the lung, urinary bladder, uterine cervix, breast, skin and stomach [165–167, 298, 299].

Etiology and pathogenesis Nasopharyngeal carcinoma is etiopathogenetically associated with Epstein–Barr virus (EBV)

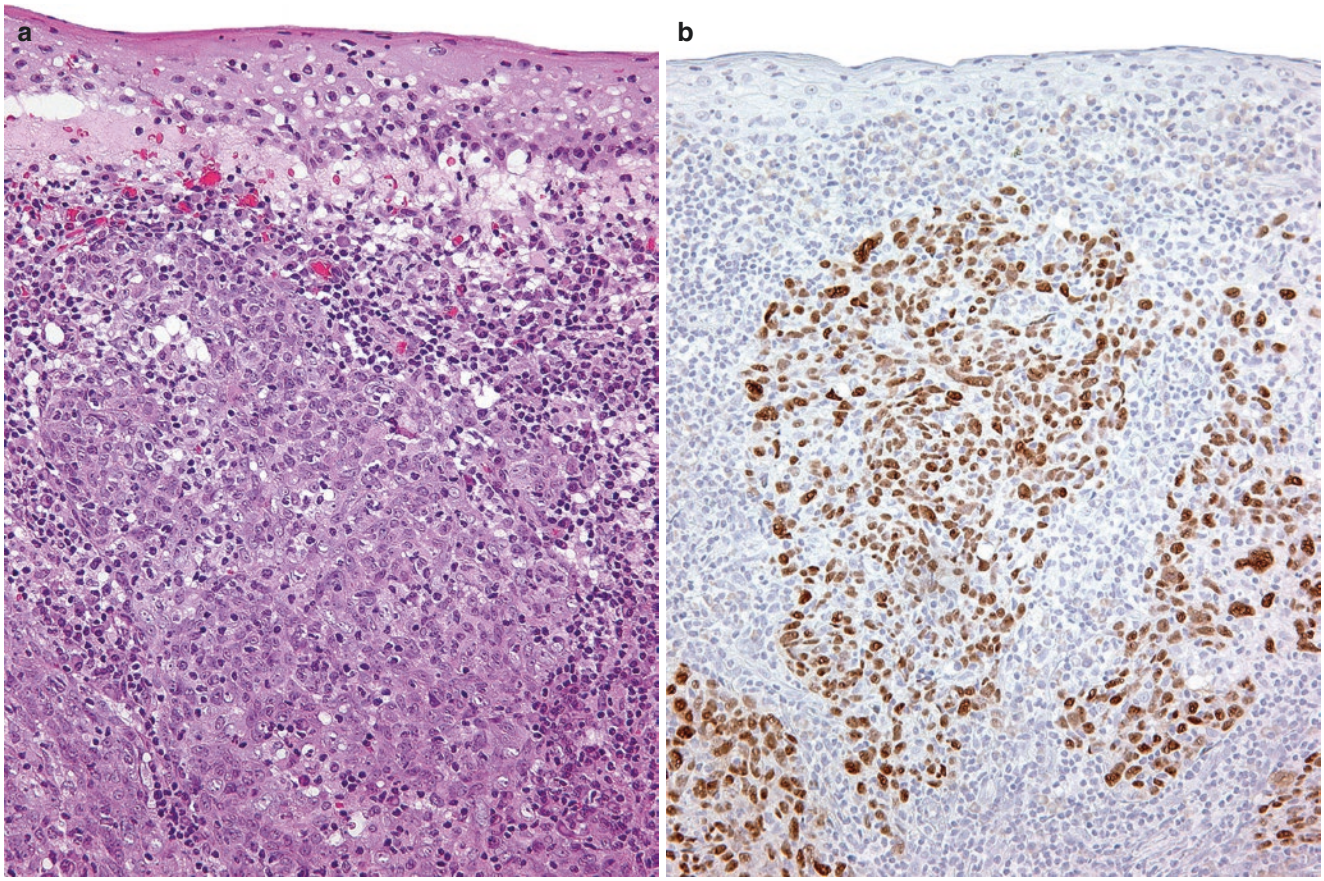

Fig. 1.26 Nasopharyngeal carcinoma. (**a**) Islands of poorly differentiated carcinoma beneath the surface epithelium, with dense lymphocytic infiltration of the stroma. (**b**) In situ hybridisation reveals Epstein–Barr virus RNA transcripts in the nuclei of all tumor cells

(Fig. 1.26b) [162]. In addition to nasopharyngeal carcinoma, EBV has also been implicated in the pathogenesis of LEC of the salivary glands, as well as undifferentiated carcinoma of the stomach, lung and thymus [163, 164]. In contrast, it seems that EBV plays little, if any, role in the pathogenesis of LEC in other locations in the head and neck [163, 165–167].

LEC of the oropharynx has been found to be related to HPV [300].

Microscopy LEC is composed of small clusters or aggregates (Schminke pattern) or large syncytial masses (Regaud pattern) of cells. Tumor cells have oval or round vesicular nuclei and one to three prominent nucleoli (Fig. 1.26a). The cytoplasm is sparse and poorly defined. Normal and abnormal mitoses may be numerous. Necroses might be present.

LEC may exist in two histological forms: as a pure LEC or as a mixed form composed of both LEC and conventional SCC; such a mixture has been observed in both primary and metastatic tumors [166].

The stroma in LEC is densely infiltrated by T lymphocytes; stromal inflammatory infiltration may also contain plasma cells, follicular dendritic cells and eosinophils.

Immunohistochemistry The vast majority of LEC is positive for cytokeratin and negative for leukocyte common antigen (LCA), as well as other lymphocyte antigens. Cytokeratin positivity has been reported in rare lymphomas [301] but LCA positivity in the tumor cells of LEC has not yet been reported. A negative reaction for S-100, HMB-45 and melan-A helps to differentiate LEC from malignant melanoma.

Differential diagnosis Differential diagnosis includes malignant lymphoma, in particular diffuse large B-cell lymphoma, as well as malignant melanoma and rhabdomyosarcoma. Differentiation is achieved by the use of appropriate immunohistochemical staining.

In cases of metastatic LEC of unknown origin, testing for HPV and EBV must be performed. The presence of HPV and EBV strongly suggests an origin in the oropharynx or nasopharynx, respectively [300].

Treatment and prognosis LEC is more aggressive than conventional SCC, with a higher incidence of cervical lymph node metastases and a propensity for distant metastases, mostly to the lung, liver and bones [165, 166]. In a series of 34 patients

with LEC, 76% of patients had lymph node metastases at the time of diagnosis and 36% had distant metastases [165].

LEC is a radiosensitive tumor and radiotherapy is an appropriate initial therapy for patients with LEC. Surgical treatment should be reserved for patients with persistent disease after completing radiotherapy. In those patients adjuvant chemotherapy is also recommended in an attempt to decrease the rate of distant metastases [165].

1.6 Second Primary Tumors

Definition Patients with SCC of the upper aerodigestive tract are at high risk of developing a second primary tumor (SPT) at a separate anatomic site from the index (first) tumor. The SPT is synchronous if it is diagnosed within 6 months after the index tumor or metachronous if it is diagnosed more than 6 months after the index tumor. Synchronous tumors are simultaneous if they are discovered at the same time as the index tumor.

Epidemiology The median prevalence of SPT in patients with an index tumor in the upper aerodigestive tract is 9% [302–304]. The site of the SPT is affected by the site of the index tumor. In patients with index tumor in the oral cavity, pharynx or oesophagus, SPT tends to arise in the same locations. In patients with index tumors in the larynx, SPT tends to be located in the lungs [305].

Etiology and pathogenesis The risk of developing SPT closely correlates with the use of tobacco and alcohol abuse and is more than doubled in patients who smoke and drink, as compared to those who do not smoke and drink [306]. Moreover, there is a direct dose-dependent relationship between tobacco and alcohol exposure and the risk of SPT.

It is now accepted that long-term exposure to tobacco and/or alcohol causes extensive and diffuse DNA changes leading to widespread genetic damage or "field cancerisation" of the whole respiratory tract and the upper digestive tract [307].

Prognosis The prognosis of patients with SPT is poor, being worse for synchronous SPT than for metachronous tumors. They generally present with a more advanced T stage and have a much lower 5-year survival than the index tumor [303].

It is therefore imperative that a panendoscopy is performed at the time of diagnosis of the index tumor not only as a part of the staging procedure but also to look for a SPT [303].

1.7 Tumor Spread and Metastasising

SCC may spread directly to contiguous structures (Fig. 1.27a, b), as well as via lymphatic and blood vessels, giving rise to regional lymph node and distant metastases or along the nerves. The behaviour and spread of SCC is affected by various factors, the most important being the site of the primary tumor. This has been attributed to the rich vascular and lymphatic network in certain areas, such as the base of the tongue, where metastatic rates are significantly higher than for similar-sized tumors on the oral tongue. Similarly, poorly vascularised areas, such as the glottis, are associated with a lower rate of metastases [308].

Other factors important for the spread and behaviour of SCC include the size and the differentiation of the tumor, as well as poorly defined factors of the host, i.e. the immune status and genetic susceptibility [185].

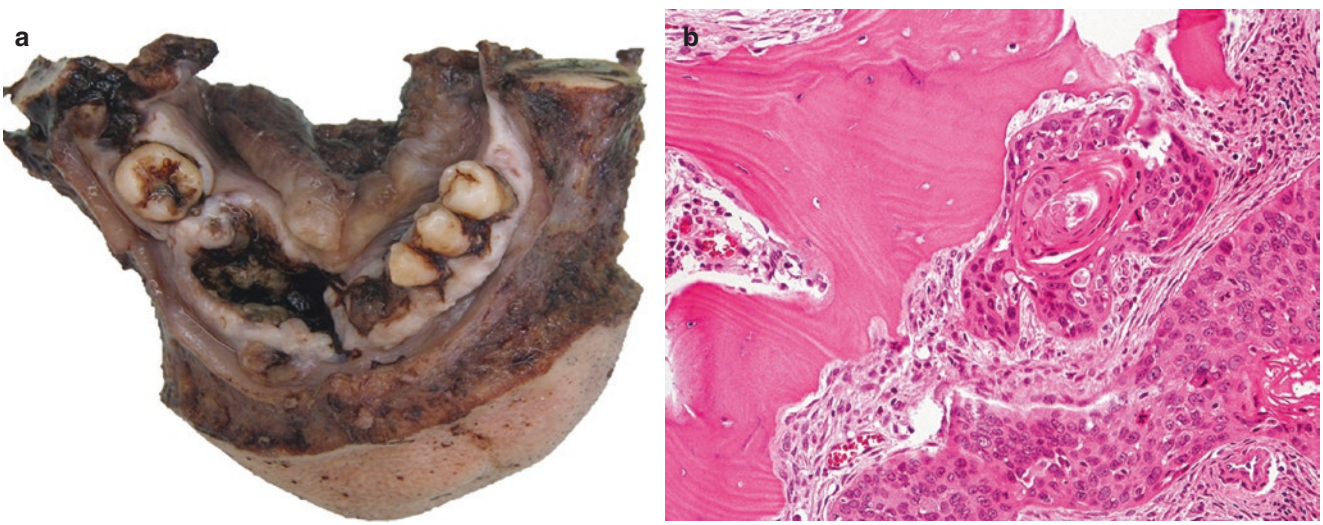

Fig. 1.27 (**a**) Carcinoma of the gingival mucosa, infiltrating mandibula, penetrating to the dermis of the chin. (**b**) Bone invasion by squamous cell carcinoma

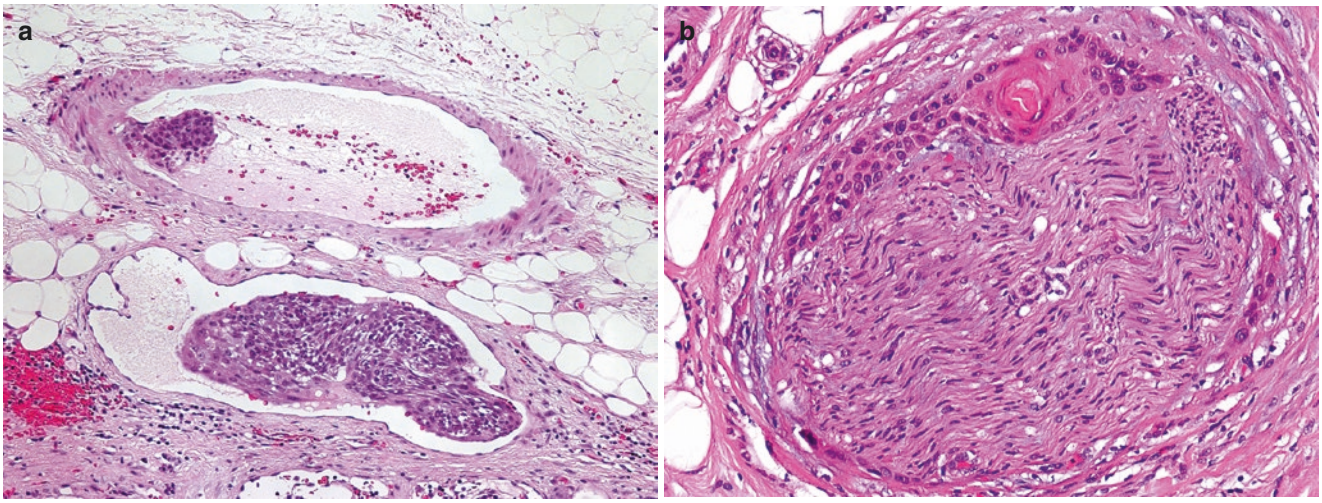

Fig. 1.28 (**a**) Lymphovascular invasion: tumor emboli within a lymphatic vessel and a vein. (**b**) Perineural invasion

1.7.1 Invasion of Lymphatic and Blood Vessels

Experimental studies have shown that metastatic progression is initiated by local invasion: select tumor cells are released from the tumor, where they gain entry to the lymphatic system or circulation, mainly via the production of tumor-derived proteolytic enzymes and angiogenic factors [309].

Cancer cells commonly invade thin-walled lymphatic vessels, capillaries and veins (Fig. 1.28, whereas thicker-walled arterioles and arteries are relatively resistant. The appearance of vascular invasion should not be considered synonymous with metastasis, because most of the tumor cells that enter the lymphatic system and circulation are destroyed [309]. However, the penetration of tumor cells into the lymphatic and blood vessels is associated with a high probability of regional lymph node and distant metastases. Furthermore, it allows the tumor to spread beyond the apparent margins. The presence of vascular invasion is therefore associated with an increased incidence of recurrences and poor survival [310].

1.7.2 Perineural Invasion

In perineural invasion, the tumor cells enter the perineural space (Fig. 1.28b) and spread both proximally and distally along the nerve fibre. Even though perineural spread of more than 2 cm is unusual, cases of tumor cells travelling up to 12 cm away from the primary tumor site along the perineural space have been described [311, 312].

Patients with perineural invasion may be asymptomatic or may experience pain and paresthesias [313]. It appears that perineural invasion is a poor prognostic sign, being associated with an increased risk of local recurrence, regional lymph node metastases and a decreased survival [184, 310, 311, 314].

1.7.3 Regional Lymph Node Metastases

SCC of the head and neck has a high tendency to metastasise to the regional lymph nodes. The localisation and frequency of the lymph node metastases depend on the site and size of the primary tumor. Large metastases can be detected clinically, by examination or using ultrasound or radiographic methods. Smaller metastases evade clinical detection but are detected by light microscopy [314, 315].

Routine analysis of neck dissection specimens is usually limited to examination of a few sections of each node stained by haematoxylin–eosin. During such routine analysis, small metastases can easily be missed. It has been demonstrated that with more sensitive techniques, nodal metastases can be detected in 8–20 % of patients in whom metastases had not been found during routine histologic examination [316–318]. The most commonly used sensitive techniques for detection of small metastases are serial section light microscopy, immunohistochemistry and molecular analysis [185, 319–321].

The prognostic significance of lymph node metastases has been extensively studied. Metastases in the lymph nodes are the most significant adverse prognostic factor in head and neck SCC. The 5-year survival is decreased by approximately 50 % in patients with lymph node metastases as compared to patients without nodal involvement [322–324]. The number and size of positive nodes, their level in the neck and the presence of extracapsular spread are the most important prognostic parameters in the nodal status [187, 322, 325].

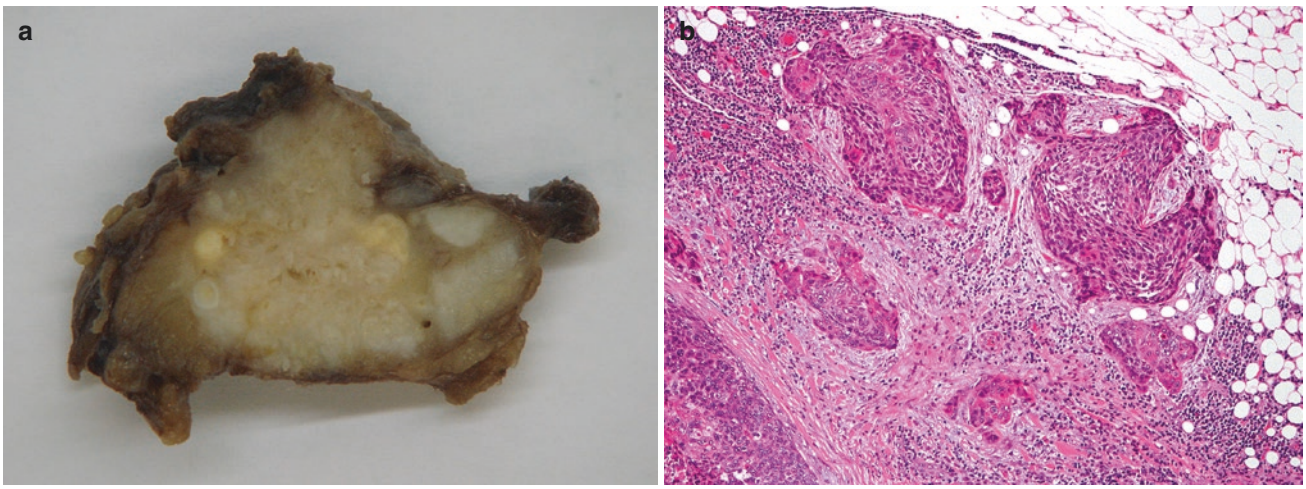

Fig. 1.29 (**a**) Metastasis of squamous cell carcinoma in a lymph node: yellow-white tumor infiltrates the majority of the lymph node, with no macroscopically evident extracapsular spread. (**b**) Microscopic extracapsular spread: squamous cell carcinoma penetrates the capsule of a lymph node and infiltrates perinodal tissue

1.7.4 Extracapsular Spread in Lymph Node Metastases

Cancer cells initially lodge in the marginal sinus and then spread throughout the lymph node. Metastases may be confined to the lymph node or may penetrate the capsule and infiltrate the perinodal tissue (Fig. 1.29a, b); this pattern of growth has been referred to as extracapsular spread (ECS). ECS is further divided into macroscopic and microscopic ECS [326]; macroscopic ECS is evident to the naked eye during laboratory dissection of the surgical specimen and later confirmed by histological assessment; it usually involves not only the perinodal fibro-adipose tissue but also the surrounding structures. Microscopic ECS is only evident on histologic examination and is usually limited to the adjacent perinodal fibro-adipose tissue.

ECS is a significant predictor of both regional recurrence and the development of distant metastases resulting in decreased survival [182, 188, 189, 327, 328]. In some studies, ECS has been shown to be a better predictor than the resection margins. It has therefore been suggested that ECS should be incorporated into the staging system for surgically managed patients [329]. Some studies, in contrast, have not confirmed the independent prognostic significance of ECS [194, 325].

1.7.5 Metastases in the Soft Tissue of the Neck

In some patients, SCC in the soft tissue of the neck is found, with no evidence of lymph node being present. These soft tissue metastases may be the result of either total effacement of a lymph node by SCC or extralymphatic spread of SCC [327].

It has been shown that the presence of soft tissue metastases is associated with an aggressive clinical course and poor survival [327, 330]. In a study of 155 patients, the survival rate was significantly lower for patients with soft tissue metastases than for those without nodal metastases and those with nodal metastases without extracapsular spread; it was similar as for patients with lymph node metastases with extracapsular spread [327].

1.7.6 Distant Metastases

Distant metastases in patients with head and neck cancer are usually defined as metastases below the clavicle and may be the result of lymphogenic or haematogenous spread. Lymphogenic spread results in distant lymph node metastases; the most commonly affected distant nodes are the mediastinal, axillary and inguinal nodes [331]. Haematogenous spread results in distant metastases, most commonly to the lung, liver and bones, followed by the skin and brain [182, 304, 332–338]. Metastases have been also described in the small intestine, spleen and the cavernous sinus [336–340].

Distant metastases in head and neck SCC are infrequent but may occur in late stages of the disease. The reported incidence of distant metastases at initial presentation is mostly between 5 and 17 %, but may increase to 10–40 % in the course of the disease [304, 328, 336, 337]. Postmortem studies have shown a higher incidence, ranging from 24 to 57 % [341–343].

The incidence of distant metastases depends on the site of the primary tumor, as well as the initial size of the tumor and the presence of nodal metastases [328, 332, 334, 344, 345].

The highest incidence of distant metastases has been reported in hypopharyngeal SCC, followed by the SCC of the tongue [334, 336].

Most distant metastases become clinically apparent 2 years after diagnosis of the initial tumor. The average survival once distant metastases have been diagnosed ranges between 4 and 7 months [335].

1.7.7 Micrometastasis

Micrometastasis is defined as a microscopic deposit of malignant cells, smaller than 2–3 mm, that are segregated spatially from the primary tumor [346]. The fate of micrometastases is uncertain; the majority of them are probably being destined for destruction or dormancy, and only a small percentage of circulating tumor cells survive and initiate a metastatic focus [309].

The fundamental characteristic of micrometastasis is the absence of a specific blood supply. Micrometastases are thus dependent on passive diffusion for oxygen and nutrient supply. Experimental studies have shown that without new blood vessel formation (neoangiogenesis), the growth of tumor cells is limited to 2–3 mm, and they may remain dormant for months or even years. During dormancy, the proliferation is balanced by an equivalent rate of cell death by apoptosis. After induction of neoangiogenesis, apoptosis is significantly reduced but the proliferation rate remains unchanged, and the growth of clinically overt metastasis can occur because of the increased survival of the tumor cells [347].

Micrometastases can be detected anywhere in the body but most frequently in the lymph nodes, in the surgical margins, in the blood and in the bone marrow [347]. Their detection can be accomplished by serial sectioning light microscopy, immunohistochemistry and/or molecular analysis [185, 317, 348, 349].

The clinical and prognostic implications of micrometastases are still uncertain. It has been suggested that residual micrometastatic tumor cells may increase the risk of tumor recurrence, thus resulting in failure of the primary treatment. Furthermore, the presence of tumor cells in the blood and/or bone marrow may be an indicator of a generalised disease with possible dissemination to many organs [350]. Several studies have demonstrated that lymph node micrometastases are associated with a high risk of recurrence and poor survival in patients with carcinoma of the breast, oesophagus, stomach, colon, lung [351] and head and neck [321, 348, 351].

It appears that detection of micrometastases is a promising approach that might enable us to identify candidates for adjuvant treatment strategies [350]. However, further studies are needed to define more precisely the clinical implication of micrometastases, as well the most appropriate method for their detection.

1.8 Molecular Carcinogenesis of Head and Neck Squamous Cell Carcinoma

General consideration The recognition of the role of HPV in a significant subset of head and neck SCC (HNSCC) has led to the classification of HNSCC HPV-positive and HPV-negative HNSCCs. These two types show different molecular alterations [352].

1.8.1 Oncogenes

EGFR Epidermal growth factor receptor (*EGFR*) overexpression is frequent in the HNSCC, and amplification is responsible for overexpression in a fraction of the cases [353, 354]. EGFR activation also occurs by binding to EGFR ligands, such as EGF or TGFα, in an autocrine manner [355].

The 3q26-28 amplicon This amplicon includes three putative oncogenes frequently amplified in HNSCC: phosphatidylinositol-4,5-bisphosphate 3-kinase (*PIK3CA*) gene, tumor protein p63 (*TP63*) gene and SRY (sex-determining region Y)-box 2 gene (*SOX2*). *PIK3CA* encodes the p110α catalytic subunit of phosphoinositide-3 kinase (PI3K). *PIK3CA* mutations and amplification occur in HNSCC. Gains and amplifications at 3q26 are important in the transition between preinvasive and invasive lesions [356, 357]. *TP63* produces six different protein products, with ΔNp63 as the predominant form in the head and neck SCC, in which it can have a putative oncogenic function promoting cell proliferation [358, 359]. *SOX2* participates in regulation of self-renewal embryonic and adult tissue stem cells [360] and is amplified and overexpressed in about half of HNSCC [361].

PI3K-AKT-mTOR The phosphatidylinositol 3-kinase/akt/mammalian target of rapamycin (*PI3K-AKT-mTOR*) pathway, located downstream of EGFR, is the target of additional alterations [362]. *AKT* induces antiapoptotic and inhibits proapoptotic factors and can be activated by gene amplification. Active AKT expression increases in dysplastic tissues, suggesting a role in carcinoma progression [363]. *mTOR* (target of AKT) activity, at the end of the pathway, induces protein synthesis related to cell growth and proliferation. Both *AKT*/*mTOR* activation and phosphatase and tensin homolog (*PTEN*) inactivation [364] result in activation of the eukaryotic translation initiating factor 4E (*eIF4E*), facilitating tumor progression [365].

MET *MET* is a receptor tyrosine kinase for which the ligand is hepatocyte growth factor (*HGF*). MET and HGF overexpression are frequent in HNSCC, representing an autocrine activating mechanism. *MET* amplification or mutations are also detected [366].

RAS–RAF–MEK–MAPK Kirsten rat sarcoma viral oncogene homolog (*KRAS*), Harvey rat sarcoma viral oncogene homolog (*HRAS*), B-Raf proto-oncogene and serine–threonine kinase (*BRAF*) mutations are rare in HNSCC [367–370] but in affected cases can contribute to the EGFR activation effect through the RAS–RAF–MEK mitogen-activated protein kinases (MAPK) pathway [371].

CCND1 11q3 gains resulting in *Cyclin D1* (*CCND1*) amplification occur in about one third of cases, irrespective of the subtype [372, 373]. However, co-amplification with other putative targets, such as cortactin, is a confounding factor in the prognostic significance attributed to *CCND1* [374].

1.8.2 Tumor Suppressors

CDKN2A Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) inactivation can be achieved by mutation, promoter hypermethylation and homozygous deletion. *CDKN2A* inactivation is therefore the most frequent alteration in HNSCC, with an estimated rate of up to 80 % of cases based on loss of heterozygosity (LOH) as the marker of *CDKN2A* inactivation [375–377]. 9p LOH is among the more frequent and earliest genomic lesions, indicating that *CDKN2A* inactivation is among the earliest events in neoplastic development in HNSCC [61].

TP53 About 80 % of HNSCC may have tumor protein p53 (*TP53*) gene inactivated [378]. This can be due to inactivating mutations in more than half of cases, whereas in wild-type *TP53* tumor inactivation can be the result of HPV E6 gene expression or MDM2 overexpression, whether by gene amplification (rare) or secondarily to *CDKN2A* deletion resulting in p14ARF loss, a negative regulator of MDM2. Both HPV E6 and *MDM2* gene product proteins bind to and promote degradation by the proteasome pathway of p53 [379]. 17p LOH is frequent in HNSCC, as well as in preneoplastic lesions, pointing to an initial role of *TP53* mutations [61, 380].

Fragile histidine triad A fragile region (FRA3B) lies within the fragile histidine triad (*FHIT*) gene at 3p14, a region frequently lost in HNSCC and its precursor lesions [381]. FHIT protein loss causes defective DNA replication, which places *FHIT* loss among the first steps in the initiation of genomic instability and the facilitation of cancer development [382].

NOTCH *NOTCH1*, *NOTCH2* and *NOTCH3* mutations are mutually exclusive in HNSCC and imply a loss of function, in contrast to the activating mutations found in haematolymphoid malignancies [367, 369]. NOTCH signalling links to self-renewal regulation, cell cycle exit (partly through p21CDKN1A expression induction) and cell survival [383]. *TP63* inhibits *NOTCH1* expression in the basal layers of normal squamous epithelium [384].

1.8.3 Proteinases

Many matrix metalloproteinases are overexpressed in HNSCC and contribute to tumor progression [385, 386]. *MMP-1* and *MMP-9* appear involved in the progression from oral dysplasia to cancer [385], whereas *MMP-13* accumulates in advanced HNSCC tumors [368, 387], and *MMP-2* is upregulated in the normal epithelium to dysplasia step but not in the dysplasia to carcinoma change in the larynx [368, 388].

1.8.4 MicroRNAs

MicroRNAs (miRNAs) are small non-coding RNA species, about 22 nucleotides long, implicated in gene expression regulation through mRNA-enhanced degradation. miR-21, miR-155 and miR-31 are consistently upregulated in the oral cavity and oropharyngeal tumors, whereas miR-1, miR-133a, miR-133b and miR-206 are downregulated [389, 390]. In particular, miR-21 is involved in neoplastic progression based on its differential overexpression in progressing and non-progressing preneoplastic lesions [391] downregulating PTEN, among other tumor suppressors [392]. Epithelial–mesenchymal transition is associated with miR-200 family and miR-205 downregulation [209, 393].

1.8.5 Microsatellite Instability

Microsatellite instability (MSI) plays a role in HNSCC development, although marginal [394, 395]. Occasional cases with exceedingly high mutational rates could be examples of MSI due to inactivation of multiple mismatch repair (MMR) genes [369].

1.8.6 HPV

HPV is the hallmark of conventional non-keratinizing SCC [352]. These tumors are molecularly different from keratinizing SCC. The virus relieves the need for *CDKN2A* and *TP53* inactivation, through the expression of viral oncogenic proteins E6, which binds to and promotes degradation of p53, and E7, which inactivates the target of functional $p16^{INK4a}$, pRb [396]. HPV-positive tumors have mutational and chromosomal alteration rates lower than HPV-negative tumors, harbour wild-type *TP53* and *CDKN2A* but show *PIK3CA* mutations in a rate similar to HPV-negative tumors, suggesting that these mutations efficiently collaborate in neoplastic progression [367, 369, 397].

1.8.7 Gene Expression Profile

Gene expression profiling analyses further classify HNSCC into four molecular subtypes (so-called classical, atypical, mesenchymal and basal), without an actual relationship with histologic characteristics. Although not specific, they show particular site preference. Laryngeal and hypopharyngeal tumors are mostly of the classical subtype, oropharyngeal tumors are of the atypical subtype and oral tumors are of the basal and, less frequently, mesenchymal subtypes [398, 399].

The classical subtype is characterised by high chromosomal instability, accumulating the highest rate of chromosomal copy number alterations, matching the alterations produced by carcinogen exposure, such as to tobacco smoke, with high expression of xenobiotic metabolism genes. The basal subtype is characterised by activation of the *EGFR* pathway, with TGFα and TP63 high expression. The atypical subtype is characterised by low *EGFR* and high *CDKN2A* levels, with the strongest (although not restricted to this particular subtype) HPV signature. The mesenchymal subtype shows high mesenchymal gene expression levels, including vimentin, and might include those with epithelial–mesenchymal transition features and those with a strong stromal (desmoplastic) reaction [399].

1.8.8 Progression Model

Allelic imbalances identified through loss of heterozygosity (LOH) occur in benign-looking hyperplastic lesions. 9p and/or 3p LOHs are the most frequent and earliest genomic lesions, suggesting genomic instability (enhanced through FHIT loss) and cell proliferation (due to *CDKN2A* inactivation) as the first required hits in neoplastic progression. The next hit points to *TP53* inactivation (17p LOH), allowing the cell to survive genome integrity loss. LOH at other chromosome regions leading to oncogenic gain of function (*CCND1* at 11q, *TP63*, *PIK3CA* at 3q, etc.) combined with invasiveness acquisition (extracellular matrix proteases) are thought to complete malignant transformation in conventional keratinizing SCC. The progression model for non-keratinizing SCC still needs to be elucidated.

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Nasal Cavity and Paranasal Sinuses

2

Antonio Cardesa, Llucia Alos, Alfons Nadal,
and Alessandro Franchi

Contents

A. Cardesa, MD, PhD • L. Alos, MD, PhD
A. Nadal, MD, PhD
Department Anatomia Patológica, Hospital Clínic,
University of Barcelona, Villarroel 170, Barcelona 08036, Spain
e-mail: acardesa@clinic.ub.es; lalos@clinic.ub.es;
anadal@clinic.ub.es

A. Franchi, MD
Section of Anatomic Pathology, Department of Surgery and
Translational Medicine, University of Florence,
Largo Brambilla 3, 50134 Florence, Italy
e-mail: franchi@unifi.it

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2.1 Introduction

The nasal cavity and paranasal sinuses occupy the top of the upper respiratory tract and form pneumatic spaces connected with the atmosphere. They are located immediately beneath the base of the cranium, where vital structures are harbored. From this region, very much exposed to airborne agents, arise some of the more complex and rare benign and malignant lesions seen in humans, whose difficulties in interpretation make this remarkable territory one of the most challenging in the practice of surgical pathology. Knowledge of the embryology, anatomy, and histology of the nasal cavity and paranasal sinuses is therefore an essential prerequisite for the precise understanding of the pathology of the lesions that develop in this unique region.

2.1.1 Embryology

The midface, or area between the upper lip and forehead, develops between 4 and 8 weeks of gestation [1]. The frontal prominence forms during the 4th postovulatory week and gives rise to the superior and middle portions of the face. The maxillary and nasal swellings form beneath the frontal prominence. At the end of the 4th week, two surface thickenings of the nasal swellings form the nasal placodes, which are of ectodermal origin and give rise to the epithelial lining of the nasal cavity and paranasal sinuses. The placodes invaginate, producing the nasal pits that become the anterior nares (nostrils) and, more deeply, the primitive posterior choanae. The medial nasal and frontal processes give rise to the nasal septum, frontal bones, nasal bones, ethmoid sinus complexes, and upper incisors. The lateral nasal and maxillary processes fuse to form the philtrum and columella. The cartilaginous nasal capsule forms deep to the nasal and frontal bones from the chondrocranium (skull base) during the 7th and 8th postovulatory weeks. The paranasal sinuses develop from the lateral nasal walls at the sixth fetal week, and their growth continues after birth, throughout childhood and adolescence. The maxillary sinus is the first to develop, starting approximately at the 70th day of gestation from the lateral wall of the nasal cavities. The frontal sinuses derive from the region of the frontal recess of the nose, and the ethmoid sinuses originate as multiple separate evaginations from the nasal cavities, while the sphenoid sinuses take origin as evaginations from the posterior nasal capsule reaching the sphenoid bone.

2.1.2 Anatomy

The nasal cavities are separated by the nasal septum and limited by a roof which is centrally formed by the cribriform plate of the ethmoid (horizontal part), anteriorly by the frontal and nasal bones, and posteriorly by the body of the sphenoid. The floor is formed by the hard palate, which comprises the palatine process of the maxillary bone and the horizontal plate of the palatine bone [2]. The lateral walls have three turbinates or conchae and three horizontal spaces, or meatii, on each side. The nasolacrimal duct opens in the inferior meatus, whereas the middle meatus receives drainage from the frontal, anterior ethmoid, and maxillary sinuses. Below the superior turbinate is the sphenoethmoid recess, with the openings of the sphenoid and posterior ethmoid sinuses. Each nasal cavity communicates posteriorly with the nasopharynx through the choanae and anteriorly with the nostril. The dilatation formed inside the aperture of each nostril is known as the vestibule. The columella separates medially both vestibules. The paranasal sinuses are a group of cavities within the corresponding craniofacial bones (maxilla, sphenoid, ethmoid, and frontal) which communicate with the nasal cavities through an ostium.

2.1.3 Histology

The nasal vestibule shares similar histology with the skin. At the level of the limen nasi, the boundary between the osseous and cartilaginous walls of the nasal cavity, the keratinizing squamous epithelium gradually changes first to cuboidal or columnar epithelium and then to ciliated respiratory-type epithelium, which lines most of the nasal cavity and all the paranasal sinuses, with the exception of the roof [2]. Numerous goblet cells are interspersed in the respiratory-type epithelium. The lamina propria contains several seromucous glands, lymphocytes, monocytes, and a well-developed vascular network, particularly evident in the inferior and middle turbinate. The olfactory mucosa lines the horizontal part of the roof of the nasal cavity. The olfactory epithelium is predominantly made of columnar non-ciliated sustentacular cells, intermingled with scattered bipolar sensory neurons and basal cells; the olfactory serous glands of Bowman are located in the lamina propria.

2.2 Acute and Chronic Rhinosinusitis

2.2.1 Acute Rhinosinusitis

Definition Rhinosinusitis is an inflammatory condition of the nasal and paranasal sinus mucosa. Acute rhinosinusitis (ARS) is usually infectious and can be clinically characterized by purulent (not clear) nasal drainage (anterior, posterior, or both) lasting up to 4 weeks, accompanied by nasal obstruction, facial pain-pressure-fullness, or both [3]. In the

immunocompetent patient, the etiology is predominantly viral or bacterial and less often fungal, whereas in immunocompromised patients, acute fungal sinusitis may occur.

Synonyms Acute sinusitis and acute rhinitis

Epidemiology The true incidence and prevalence of ARS are unknown, because a significant number of cases do not come usually to medical attention. However, the prevalence of rhinosinusitis in the general population is considered to be high, and it is estimated that more than 24 million cases of acute bacterial rhinosinusitis occur annually in the United States [4]. ARS is more common in children than adults. The prevalence of this disease is increased in women. It is generally thought that the process starts in the nasal mucosa and spreads through the ethmoidal prechambers to the frontal and maxillary sinuses.

Etiology and pathogenesis Infectious rhinitis is typically viral and is often referred to as "common cold." It is more common in children than in adults, and the most frequently identified agents are rhinovirus, myxovirus, coronavirus, and adenovirus [3]. Swelling of the mucosa may cause obstruction of a sinus ostium, with subsequent secondary bacterial infection (acute bacterial sinusitis). The most commonly involved agents are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* [5, 6]. Allergic rhinitis (hay fever) is part of an inherited syndrome which also may manifest as atopic eczema and asthma. In allergic rhinitis, airborne particles, such as grass pollens, molds, and animal allergens, are deposited on the nasal mucosa giving rise to acute and chronic reactions. Allergens combine with the IgE antibodies produced by the plasma cells of the nasal mucosa which are avidly bound to the Fc-epsilon receptors on mast cells. This triggers degranulation of mast cells and releases the inflammatory mediators of the type I hypersensitivity reaction, causing rhinorrhea and nasal obstruction.

A further type of rhinitis is the non-allergic form (non-allergic rhinitis, NAR), which is defined by exclusion as a chronic nasal inflammation which is not caused by systemic IgE-dependent mechanisms [7]. Nasal cytology has allowed the distinction of different NAR types on the basis of the inflammatory infiltrate, which include the non-allergic rhinitis with eosinophils (NARES), the non-allergic rhinitis with neutrophils (NARNE), the non-allergic rhinitis with mast cells (NARMA), and the non-allergic rhinitis with eosinophils and mast cells (NARESMA). Their recognition is important in order to choose the appropriate treatment.

Macroscopy The mucosa is thickened and edematous, and there is a prominent exudate, which is purulent in bacterial forms. Necrotic tissue is obtained from debridement procedures in case of acute fungal sinusitis.

Microscopy In ARS, histopathologic examination is rarely requested. The sinonasal mucosa demonstrates extensive inflammation, with neutrophil-rich infiltrate. In some cases, hemorrhage and necrosis may also be noted. In acute fungal sinusitis, fungal hyphae can be recognized with appropriate staining methods. The fungus has a tendency to invade blood vessels causing thrombosis and may spread through the perineural spaces [8]. The affected tissues exhibit coagulative necrosis and hemorrhage, while the inflammatory reaction is scant [9]. In allergic rhinitis, the nasal mucosa shows numerous eosinophils, abundant plasma cells, and in some cases increased number of mast cells. There is goblet cell hyperplasia of the respiratory epithelium, and the basement membrane, which is destroyed in the acute phase, appears considerably thickened in the chronic phase.

Differential diagnosis Clinical data are usually sufficient to separate ARS from other inflammatory conditions. Histochemical stainings for fungi are helpful to recognize acute fungal sinusitis.

Treatment and prognosis The treatment of ARS is medical and depends upon the viral or bacterial etiology. Acute bacterial rhinosinusitis usually resolves with antibiotic therapy. Complications are rare and include contiguous infectious involvement of the orbit or central nervous system and can be potentially life-threatening. They include epidural abscess, subdural empyema, and cerebral abscess. The incidence of these complications seems to peak in early adolescence. Acute fungal sinusitis is lethal in most cases.

2.2.2 Chronic Rhinosinusitis

Definition Chronic rhinosinusitis (CRS) comprises a group of disorders characterized by inflammation of the mucosa of the nose and paranasal sinuses of at least 12 consecutive weeks' duration [10].

Synonyms Chronic sinusitis and chronic rhinitis

Epidemiology CRS is a common disease, but the true incidence is difficult to ascertain, mainly due to the lack of uniformly accepted criteria for the diagnosis. However, it is estimated that in the United States, the prevalence of CRS is 14 % of the global population [11, 12].

Children are more prone to suffer of CRS than adults [12]. The prevalence of the disease is higher in women than in men [13].

Incidence of atrophic rhinitis has markedly decreased in the last century, and nowadays most cases are secondary to trauma, surgery, granulomatous diseases, infection, and radiation exposure [14].

Pathogenesis Local predisposing factors include sinus ostia blockage, repeated episodes of common cold or acute sinusitis determining obstruction of sinus ostia, reduction of ciliary activity (immotile cilia syndrome), and cystic fibrosis. Multiple factors may be involved in the pathogenesis of atrophic rhinitis, including chronic bacterial infections and nutritional deficiencies.

Some patients with predisposing conditions, such as allergy, asthma, transplant, or AIDS, develop CRS more often [12].

Sinonasal infections are frequently observed in HIV patients; they are often asymptomatic and tend to be recurrent or refractory [15]. They are due to various pathogens including cytomegalovirus [16], *Staphylococcus aureus*, fungi (*Aspergillus* sp.) [17], and parasites (*Microsporidia, Cryptosporidium*) [18].

Variants of CRS Atrophic rhinitis is a chronic inflammation of the nasal mucosa of unknown etiology characterized by progressive nasal mucosal atrophy and by a thick, dense secretion, with fetid smelling and crusting [14]. Hypertrophic rhinitis is characterized by thickening of the sinonasal mucosa resulting from chronic inflammatory diseases [10, 19]. Frequently these patients have undergone several sinus operations, each time with limited success and subsequent recurrence. Recurrent nasal polyposis is often associated.

Macroscopy The mucosa is thickened, edematous, and gray white in color. In atrophic rhinitis, the mucosa becomes atrophic.

Microscopy The mucosal changes observed are variable and include basement membrane thickening, goblet cell hyperplasia, mucous gland hyperplasia, edema of varying extent, inflammation (mostly lymphocytes, plasma cells, and eosinophils), and polypoid change of the mucosa. The histopathological patterns do not always correlate with the clinical features although in atrophic rhinitis the nonspecific chronic inflammatory infiltrate goes with squamous metaplasia of the surface epithelium and of glandular excretory ducts and atrophy of seromucous glands [20, 21].

Differential diagnosis The differential diagnosis is with chronic inflammatory processes, including granulomatous infections, granulomatosis with polyangiitis (Wegener's), Churg-Strauss disease, and other noninfectious midline granulomas.

Treatment and prognosis Medical treatment (decongestants, antihistamines, topical steroids) is recommended for most forms of CRS. Surgery is indicated in case of persistence of symptoms despite medical therapy, for correction of anatomic deformities believed to be contributing to persistence of disease and for debulking of advanced nasal polyposis. CRS may relapse and eventually complicate in sinonasal inflammatory polyposis and mucocele formation.

2.3 Sinonasal Polyps

Definition Sinonasal polyps are nonneoplastic pedunculated swellings of the sinonasal mucosa. When multiple, they are referred as polyposis. The majority of them are inflammatory allergic. Other polyps are of infective, chemical, or familial etiology. The histological appearances of nasal polyps do not always correlate well with their etiology.

2.3.1 Inflammatory Allergic Polyps

Definition Inflammatory allergic polyps (IAPs) are those inflammatory swellings of the sinonasal mucosa of allergic origin.

Synonym Inflammatory polyp

Epidemiology IAPs develop in patients of all ages, being most commonly seen over 20 years of age. They arise most frequently from the upper part of the lateral nasal wall and from the ethmoidal region. Nasal cavities and paranasal sinuses may be simultaneously involved, either unilateral or bilateral.

Etiology and pathogenesis IAPs are due to allergens that trigger the path of the hypersensitivity reaction type I (see Sect. 2.2.1).

Macroscopy IAPs are grapelike formations of soft consistency and glassy appearance, measuring from a few millimeters to several centimeters.

Microscopy IAPs are made up largely of myxoid edematous tissue with pseudocysts containing eosinophilic proteinaceous fluid and infiltrates of inflammatory cells usually exhibiting heavy infiltration by eosinophils (Figs. 2.1 and 2.2), being accompanied by variable number of plasma cells and some mast cells [22]. They are covered by respiratory epithelium with goblet cell hyperplasia, squamous metaplasia, and thickening of the basement membrane. Seromucous glands with mucin-containing cysts may also occur (Fig. 2.3). Epithelial dysplasia may be present in rare cases. Granulomas may be seen in polyps treated with intranasal injection, application of steroids, or other oily medications. Atypical fibroblasts with abundant cytoplasm, poorly defined cell borders, and large pleomorphic nuclei are present in a small proportion of cases [23]. These atypical cells occur individually and are more frequently found close to blood vessels

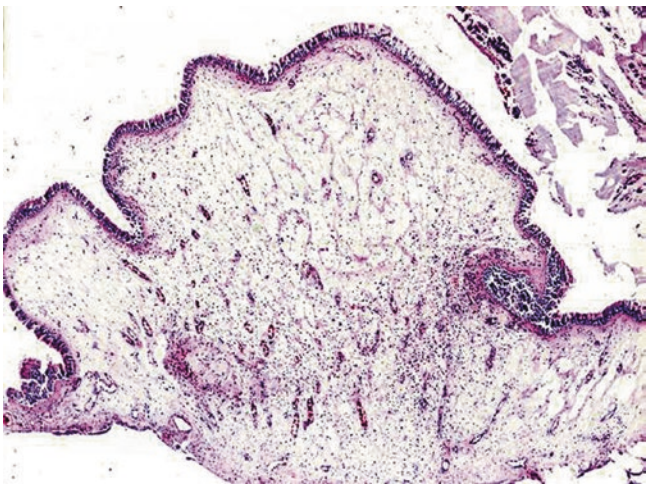

Fig. 2.1 Allergic polyp made up largely of edematous stroma containing proteinaceous fluid and infiltrate of inflammatory cells

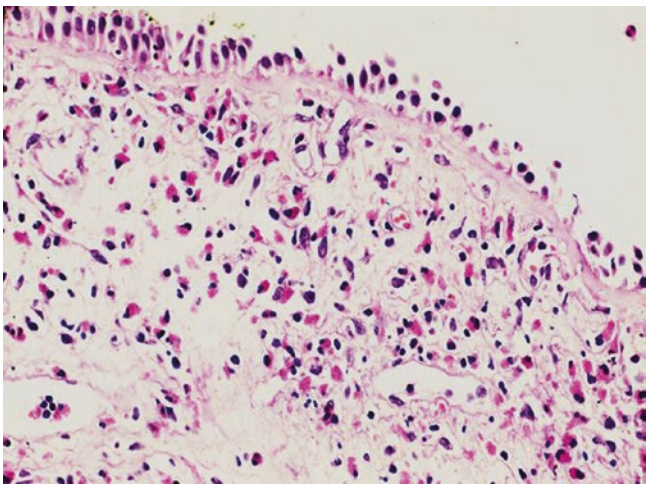

Fig. 2.2 Allergic polyp with marked stromal edema and heavy infiltration by eosinophils

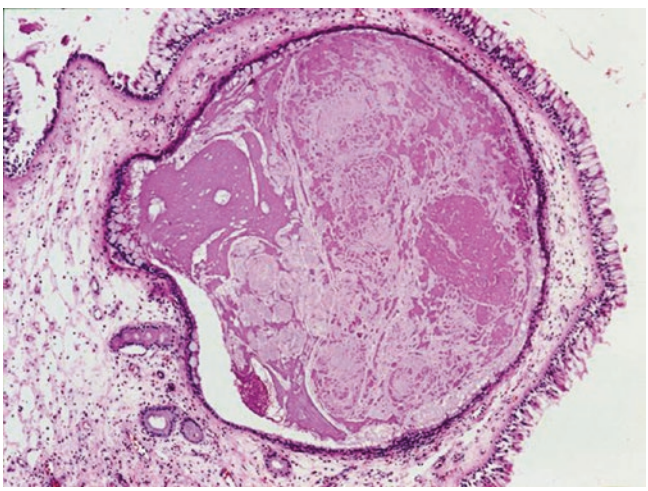

Fig. 2.3 Allergic polyp containing a cystic seromucinous gland

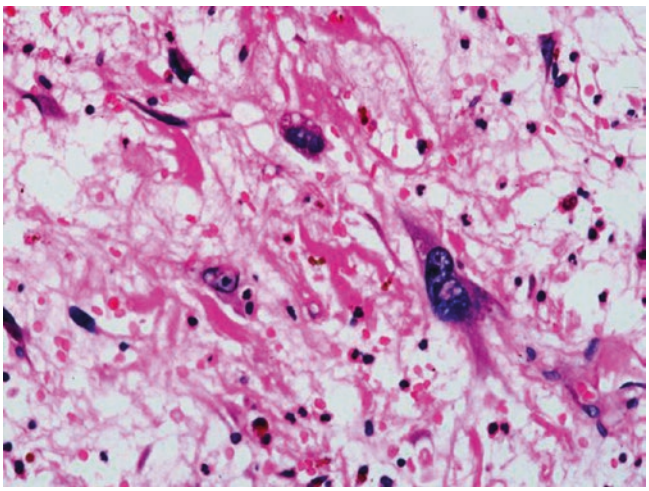

Fig. 2.4 Atypical fibroblasts in an inflammatory allergic polyp: enlarged fibroblasts with bizarre nuclei and occasional prominent nucleoli appear interspersed in granulation tissue

(Fig. 2.4) or near the epithelial surface. Such stromal atypia is a reactive phenomenon that should not be mistaken for sarcoma.

Treatment and prognosis Complete excision is curative, although recurrences may occur if exposure to allergens persists.

2.3.2 Other Polyps

2.3.2.1 Antrochoanal Polyp

Definition Antrochoanal polyps (ACPs) are single polyps that arise in the maxillary sinus, also known as antrum, and extend into the middle meatus projecting posteriorly through the ipsilateral choana [24]. Those polyps that arise in the maxillary antrum and extend into the middle meatus projecting anteriorly are known as antronasal polyps. Killian polyp is a synonym of ACP commonly used by rhinologists.

Epidemiology ACPs account for about 5 % of all sinonasal polyps. Patients with ACP are younger than those with IAPs.

Macroscopy ACP is characterized by a long and thin stalk which originates in the maxillary mucosa.

Microscopy Typically ACPs are devoid of the marked eosinophilic infiltrate of IAPs and have sparser content in mucous glands than the latter. ACPs usually have a prominent fibrous stroma which surrounds thick-walled blood vessels (Fig. 2.5) [25]. In addition, scattered, enlarged, stromal cells with hyperchromatic nuclei are not an

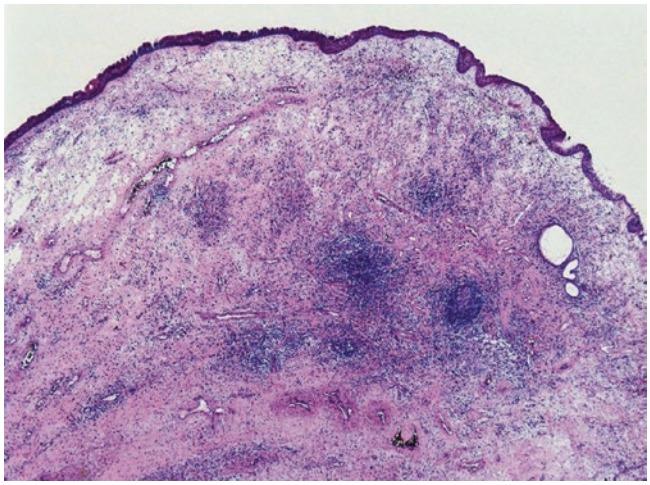

Fig. 2.5 Antrochoanal polyp with conspicuous fibrous stroma surrounding the wall of blood vessels and lack of significant eosinophilic infiltrate

uncommon finding in ACPs that should not be confused with sarcoma [26].

Differential diagnosis ACP must be differentiated from juvenile angiofibroma, as well as from low-grade sarcoma.

Treatment and prognosis Complete resection, stalk included, is curative.

2.3.2.2 Polyposis in Cystic Fibrosis

Definition Sinonasal polyps occurring in the context of cystic fibrosis (CF).

Synonym Polyposis in mucoviscidosis

Pathogenesis CF is an autosomal disorder affecting children with the presence of thick mucus, which obstructs, among other ducts, the lumina of the airways and impairs mucociliary function. CF is due to mutations of the *CFTR* gene located at 7q31.2 [27].

Microscopy Nasal polyps in mucoviscidosis show cystic glands filled with inspissated mucoid material and thickening of the basement membranes that surround the glands [28, 29].

2.3.2.3 Polyposis in Immotile Cilia Syndrome and Kartagener Syndrome

Immotile cilia syndrome (or primary ciliary dyskinesia) is a genetic disease affecting ciliary movement and resulting in respiratory infections and male infertility. Situs inversus may be associated (Kartagener syndrome). About 15 % of patients develop nasal polyps histologically indistinguishable from other nasal polyps. Ultrastructural analysis of nasal biopsies is needed to identify the alterations in the architecture of the cilium in immotile cilia syndrome [30]. Mutations have been detected in the following genes: *DNAI1*(7p21), *DNAH5* (5p14-5p15), and *DNAH11*(7p21) [31].

2.3.2.4 Angiomatoid Sinonasal Polyp

Definition Angiomatoid sinonasal polyps (ASNPs) are characterized by the conspicuous proliferation of small blood vessels, mostly capillaries, occurring within the myxoid background of conventional sinonasal polyps [32].

Epidemiology and etiology ASNP is a rare complication of IAPs and ACPs that may be due to trauma or be iatrogenic.

Microscopy The angiomatoid changes seen in ASNPs are characterized by the proliferation of numerous small blood vessels within a myxoid background. Thrombotic phenomena with heavy fibrin deposition are seen in nearly 50 % of cases. Necrosis is always present if thoroughly searched. Cellular atypia can be prominent but mitosis are rare and atypical mitosis are absent [32].

Differential diagnosis ASNP must be differentiated from angiosarcoma. Although cellular atypia can be prominent in ASNP, malignancy is ruled out by the scarce number of mitotic figures and the absence of atypical mitoses [33].

Treatment and prognosis Complete excision is curative.

2.4 Sinonasal Heterotopias and Hamartomas

2.4.1 Heterotopic Neuroglial Tissue and Encephalocele

2.4.1.1 Heterotopic Neuroglial Tissue

Definition Heterotopic neuroglial tissue (HNGT) is a mass of displaced mature neuroglial tissue presenting intranasally, in the adjacent nasal subcutaneous tissue or in both.

Synonyms Glial heterotopia and nasal glioma, although the latter is a misnomer

Epidemiology HNGT mostly occurs in young children.

Etiology and pathogenesis Usually, HNGT is the result of a congenital abnormality related to a variant of meningoencephalocele in which connection with the intracraneal central nervous system is lost [34, 35]. The lesion mainly arises at the base of the nose or in the upper part of the nasal cavity.

Macroscopy HNGT may be polypoid and rarely measures more than 2 cm.

Microscopy Histologically, HNGT is mostly composed of a mixture of astrocytes, glial fibers, and fibrous connective tissue. Multinucleated glial cells are not infrequently found (Fig. 2.6). Some glial cells can have large nuclei resembling nerve cells. A few true nerve cells or even ependymal elements can rarely be identified. Mitoses are not found. Bona fide gliomas may occur in association with HNGT [36].

Immunohistochemistry Staining for S-100 protein and glial fibrillary acidic protein is positive, the latter being a helpful diagnostic adjunct.

Treatment and prognosis Complete surgical excision is curative. Recurrence may follow incomplete resection.

2.4.1.2 Encephalocele

Definition Nasal encephalocele (EC) is the result of the herniation of brain tissue and its leptomeningeal covering through an osseous defect of the nasal roof.

Synonym Meningoencephalocele

Epidemiology EC mainly occurs in older children and also in adults.

Etiology and pathogenesis An osseous defect at the base of the skull, usually due to trauma, surgery, or infections, facilitates the herniation of the brain.

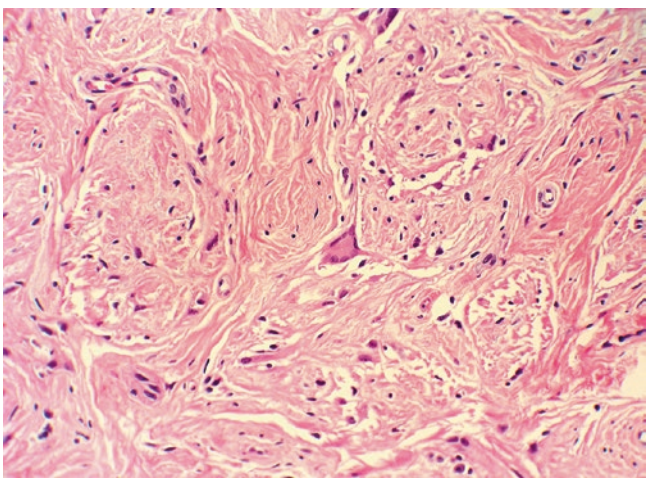

Fig. 2.6 Heterotopic glial tissue composed of a mixture of astrocytes, glial fibers, and fibrous connective tissue. A multinucleated glial cell is seen at the center

Microscopy EC displays a mixture of neural, glial, and leptomeningeal elements.

Differential diagnosis In contrast to heterotopias, encephaloceles are communicated with the central nervous system, and tissues are fairly organized although they can show dysplastic changes [37]. In addition EC has to be distinguished from glioma and teratoma.

Treatment and prognosis Complete resection of EC with repair of the osseous defect at the base of the skull is mandatory to achieve cure.

2.4.2 Hamartomas

Definition Benign polypoid overgrowths in which well-developed epithelial and mesenchymal sinonasal tissues are present with variable participation [38]. Three types are recognized: respiratory epithelial adenomatoid hamartoma, chondro-osseous and respiratory epithelial hamartoma, and nasal chondromesenchymal hamartoma.

2.4.2.1 Respiratory Epithelial Adenomatoid Hamartoma

Definition Respiratory epithelial adenomatoid hamartoma (REAH) is a benign polypoid lesion with well-developed branching glands covered with ciliated respiratory epithelium [38].

Epidemiology REAH occurs in adults and is equally frequent in men and women [39].

Etiology and pathogenesis The cause is unknown. REAH may be the result from an exuberant hyperplastic reaction within an inflammatory context, as most of cases develop in association to nasal polyposis [39].

Molecular genetics The molecular profile of REAH shows tumor suppressor gene alterations with a mean fractional allelic loss of 31 %, an unusually high percentage for a non-neoplastic entity, suggesting the possibility that may be a benign neoplasm rather than a hamartoma [40].

Macroscopy Polypoid formations of soft consistency measuring up to several centimeters.

Microscopy The seromucous epithelium of the deep mucosal glands in REAHs is characteristically replaced by ciliated respiratory epithelium admixed with goblet cells accompanied by thick bands of fibrous stroma in the underlying supportive tissue (Figs. 2.7 and 2.8). When the deep glandular component

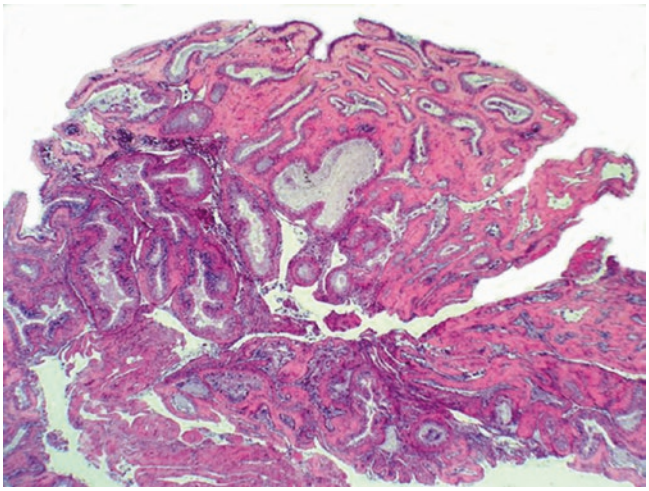

Fig 2.7 Respiratory epithelial adenomatoid hamartoma: polypoid formation with glandular-like spaces lined by respiratory epithelium and supported by fibrous stroma

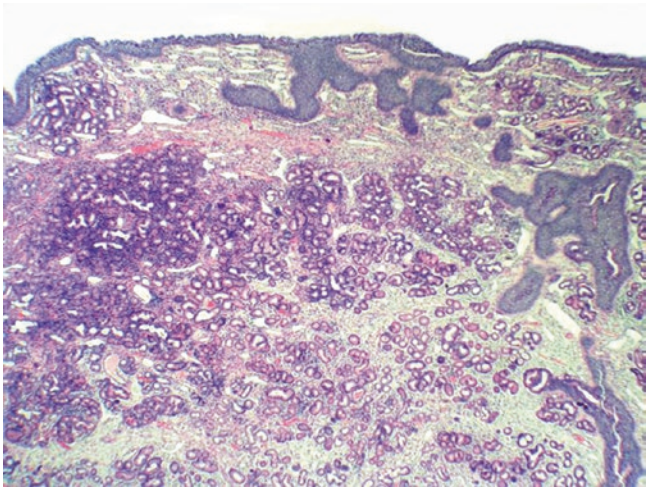

Fig 2.9 Seromucinous glandular hamartoma: abundant lobular aggregates of modified seromucous glands supported by slightly edematous stroma

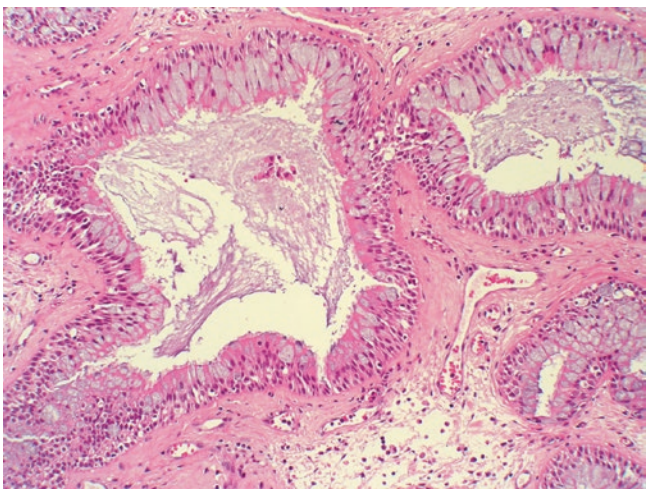

Fig 2.8 Respiratory epithelial adenomatoid hamartoma: glandular-like spaces lined by respiratory epithelium surrounded by fibrous stroma

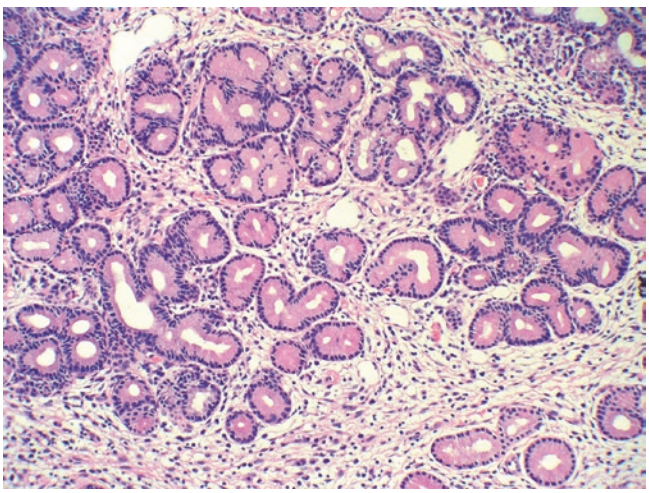

Fig 2.10 Seromucinous glandular hamartoma: disorderly placed seromucous glands, devoid of surrounding myoepithelial cells

is maintained and the overgrowth mainly consists of disorderly placed seromucinous glands, lacking myoepithelial cells, the term "seromucinous glandular hamartoma" (SMGH) is used [41, 42] (Figs. 2.9 and 2.10).

Differential diagnosis REAH and SMGH must be differentiated from sinonasal polyps, inverted papilloma, and the various types of low-grade adenocarcinomas [43]. The latter may show CK20 or CDX2 immunohistochemical expression, not reported in hamartomatous lesions [44].

Treatment and prognosis Conservative complete excision is curative [45].

2.4.2.2 Chondro-osseous and Respiratory Epithelial Hamartoma

Definition Chondro-osseous and respiratory epithelial hamartoma (COREH) combines the features of REAH with juxtaposed cartilaginous and osseous structures.

Epidemiology etiology, and pathogenesis: Similar to REAH

Macroscopy COREH is similar in shape to REAH but has harder consistency and abundant cystic formations on the cut section (Fig. 2.11).

Microscopy Immature to mature benign chondral and osseous trabeculae appear juxtaposed with gland-like formations

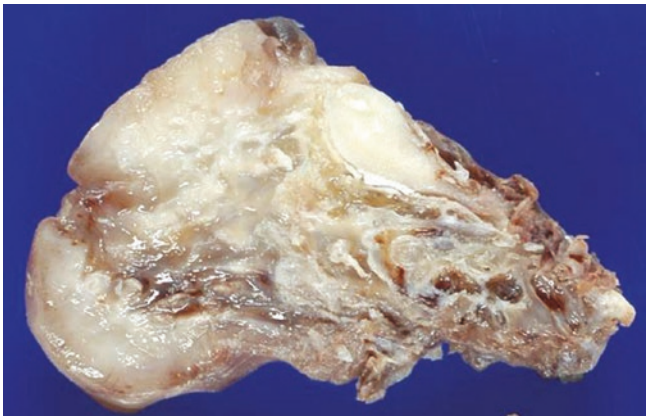

Fig 2.11 Chondro-osseous and respiratory epithelial hamartoma: chondro-osseous septation involving cystic spaces

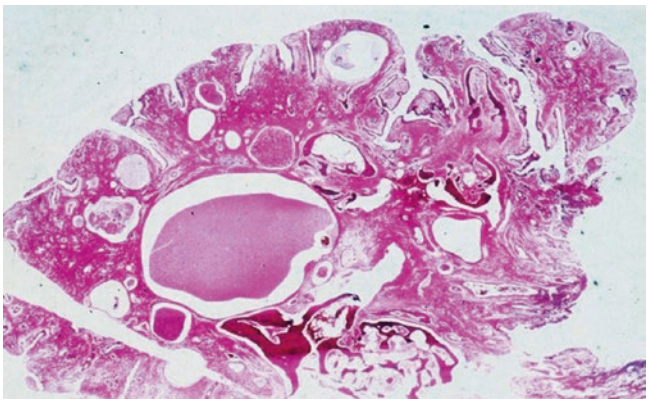

Fig 2.12 Chondro-osseous and respiratory epithelial hamartoma: osseous septa partially divide wide areas containing ducts and cysts lined by respiratory epithelium (Courtesy of Prof. M. Pfalz, Zurich, Switzerland)

covered with respiratory epithelium and supportive fibrous stroma (Fig. 2.12) [46].

Differential diagnosis COREH must be mainly distinguished from nasal chondromesenchymal hamartoma. The latter mostly occurs in children and the former in adults.

Treatment and prognosis As in REAH, conservative complete resection is curative.

2.4.2.3 Nasal Chondromesenchymal Hamartoma

Definition Nasal chondromesenchymal hamartoma (NCMH) is a benign pseudotumoral overgrowth composed of an admixture of chondroid, stromal, and cystic spaces.

Epidemiology and pathogenesis NCMH is a rare lesion mostly occurring in male newborns under 3 months of age [47, 48]. Very rare examples occur later in life. Although NCMHs are more frequent in nasal cavities, they can also arise from the nasopharynx and paranasal sinuses [41]. The cause is unknown. The occurrence of NCMH as initial lesion in children with pleuropulmonary blastoma predisposition syndrome has been reported [49]. In this disorder, NCMH arises secondary to germline and somatic mutations of the gene *DICER1*.

Macroscopy NCMH can reach up to 8 cm in size [48].

Microscopy NCMH consists of irregular nodules of mature hyaline cartilage in lobular arrangement. The chondroid nodules are surrounded by stroma that may be loose and myxoid or dense and collagenous. Blood-filled cystic spaces with features similar to aneurysmal bone cyst, as well as microcysts, within the myxoid areas can be seen. Ossicles, trabeculae of immature bone, osteoclast-like giant cells, and foci of mature adipose tissue can be found occasionally. The stromal spindle cells usually are positive for smooth muscle actin and the chondroid cells for S-100 protein.

Differential diagnosis NCMH needs to be distinguished from COREH, chondrosarcoma, mesenchymal chondrosarcoma, and chondroblastoma, entities that are exceedingly rare in newborns. The ectomesenchymal chondromyxoid tumor, although sharing certain similarities with NCMH, only occurs in the oral cavity.

Treatment and prognosis Combined intranasal and neurosurgical approach may be required to achieve complete resection, which is curative [48].

2.5 Pseudotumors

2.5.1 Mucocele

Definition Mucocele is a cyst filled with mucous that develops within a sinus cavity as the result of occlusion of the ostium.

Epidemiology The most common sites of occurrence are the frontal and the sphenoidal sinuses.

Etiology and pathogenesis Most commonly is due to infection but also may result from trauma or be congenital [50]. Retained secretions cause expansion of the sinus and bone erosion.

Microscopy The cyst is lined by respiratory epithelium that shows prominent goblet cell hyperplasia [51, 52]. Expansion of the cyst may cause atrophy and metaplasia of the epithelium.

Treatment and prognosis Surgical evacuation of the involved sinus by removal of the occlusion achieves excellent results.

2.5.2 Necrotizing Sialometaplasia

Definition Necrotizing sialometaplasia (NSM) is a reactive change of seromucous glands that undergo squamous metaplasia.

Etiology and pathogenesis Etiology relates to an ischemic event. Trauma has been claimed also as a cause of these lesions.

Clinical aspects It presents as a localized swelling that becomes ulcerated.

Microscopy Glandular lobular architecture is preserved, with squamous metaplasia of ducts and acini and glandular infarction. Mucin spillage elicits inflammation. The overlying epithelium can show pseudoepitheliomatous hyperplasia.

Differential diagnosis The most important entities to be considered are squamous cell carcinoma (SCC) and mucoepidermoid carcinoma. Proliferation in NSM is usually low, a feature that can be helpful in the distinction.

Treatment and prognosis Healing occurs spontaneously; therefore, surgical treatment is not necessary [53].

2.5.3 Organizing Hematoma

Definition Sinonasal organizing hematoma (OH) is a mass of hemorrhage in the nose and paranasal sinuses.

Synonyms "Cholesterol granuloma" and "rhinitis caseosa"

Etiology and pathogenesis OH is in most cases the result of occult submucosal hemorrhage in the maxillary sinus due to external trauma or tooth extraction. Resolution of the hematoma produces the formation of cholesterol granulomas [54].

Macroscopy A sessile mass is seen, consisting of dark-red hemorrhagic areas admixed with pale "cheesy" zones composed of cholesterol.

Microcopy In OH, large areas of degenerated blood and deposits of fibrin predominate, which are being organized by granulation tissue. Resolution of the hematoma produces the formation of cholesterol granulomas and fibrosis, simulating a foreign body reaction. Often, there are areas of irregular blood vessels, occasionally lined by bizarre endothelial cells, which may be mistaken for a malignant vascular tumor [55].

Differential diagnosis OH must be mainly differentiated from angiosarcoma.

Treatment and prognosis Surgical removal of the mass is curative.

2.5.4 Amyloidosis

Epidemiology Isolated amyloid deposition in the sinonasal mucosa is a rare event, with about 20 cases reported in the English literature [56, 57].

Macroscopy Grossly, the lesion appears as a friable to hard tumorlike mass, with frequent hemorrhage.

Microscopy Histologically, there is a deposition of intensely eosinophilic material in the stroma, around blood vessels and around ducts of seromucous glands, which is often associated with diffuse chronic inflammation and foreign body granulomatous reaction. Amyloid stains orange with Congo red and is apple-green birefringent at polarized light examination. Immunohistochemistry may help to identify the type of amyloid deposition. In the head and neck, most cases are of the primary (AL) type; therefore, they show immunoreactivity with AL (kappa or lambda light chain amyloid) [58].

Treatment and prognosis Surgical removal has a palliative purpose.

2.5.5 Myospherulosis

Definition Myospherulosis is characterized by the presence of cyst-like spaces lined by flattened histiocytes and containing clusters of brownish spherules resembling fungi [59–61].

Epidemiology Myospherulosis is a rare entity, with less than 200 cases reported [62].

Etiology and pathogenesis The lesion is usually found in patients who have had previous operations [63]. It is now recognized that the spherules are extravasated red cells that have been altered by interaction with traumatized fat or petrolatum-based ointments and gauzes used in surgical procedures.

Microscopy The spherules lie loosely or within sacs formed by thin refractile membranes. The brownish spherules do not

stain with PAS or Gomori methenamine silver, and their morphology does not correspond with any known fungus [64]. They are found within fibrous granulation tissue which may show a foreign body reaction.

Treatment and prognosis Surgical removal produces excellent results.

2.5.6 Eosinophilic Angiocentric Fibrosis

Definition Eosinophilic angiocentric fibrosis (EAF) is a disorder that compromises the airways due to progressive obstruction.

Etiology and pathogenesis Recent findings support that EAF is part of the spectrum of IgG4-related systemic diseases [65, 66].

Epidemiology EAF is a rare, chronic, benign, condition of the upper respiratory tract occurring predominantly in adult women [67, 68].

Microscopy Initially, the histologic picture is characterized by non-necrotizing eosinophilic vasculitis involving capillaries and venules of the sinonasal mucosa, accompanied by an inflammatory infiltrate with lymphocytes, plasma cells, histiocytes, and occasional neutrophils (Fig. 2.13). In late lesions, there is a characteristic obliterative perivascular onionskin fibrosis, while the inflammatory infiltrate is less dense (Fig. 2.14) [67].

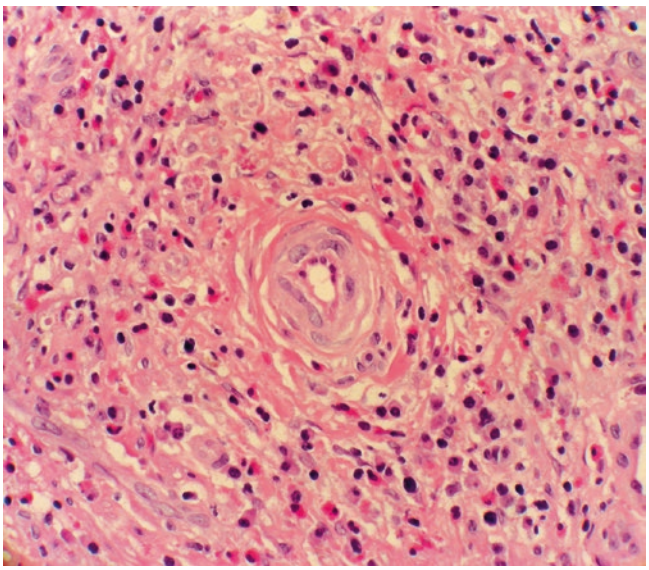

Fig. 2.13 Eosinophilic angiocentric fibrosis: initial lesion with non-necrotizing eosinophilic vasculitis involving capillaries and venules (Courtesy of Dr. F. Garcia-Bragado, Pamplona, Spain)

The differential diagnosis includes reactive processes of the sinonasal mucosa, like granulomatosis with polyangiitis (Wegener's), Churg-Strauss disease, Kimura disease, angiolymphoid hyperplasia with eosinophilia, and IgG4-associated disease.

Treatment and prognosis Surgery offers palliation of the nasal obstruction.

2.5.7 Surgical Ciliated Cyst of the Maxilla

Definition Surgical ciliated cyst of the maxilla is a locally aggressive lesion that develops mainly as a complication of surgery in the maxillary sinus region [69, 70].

Synonyms Postoperative maxillary cyst and paranasal cyst

Epidemiology and pathogenesis The incidence is variable. It represents 19.5 % of all oromaxillary cystic lesions in the Japanese population [69], while it is rare in Europe and the United States. It occurs in adult subjects, with a mean age of 52 years [71]. There is no significant gender predilection [70, 71]. This cyst usually arises in the lateral wall of the maxilla and expands toward the canine fossa or toward the nasal wall or sphenopalatine wall of the sinus. Some lesions may be more aggressive and occupy the orbit floor or ethmoidal air cells (Fig. 2.15). There are also reports of mandibular localization. The lesion is likely to be caused by sinus or nasal mucosa entrapment in the bone healing process after an osteotomy in these sites.

Macroscopy Surgical ciliated cyst is usually unilocular, but multilocular lesions have also been observed. The wall

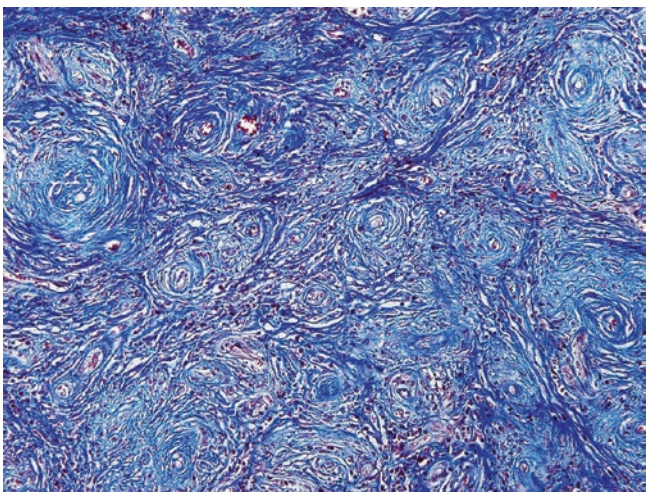

Fig. 2.14 Eosinophilic angiocentric fibrosis: late lesion with obliterative perivascular onionskin fibrosis. Masson's trichrome

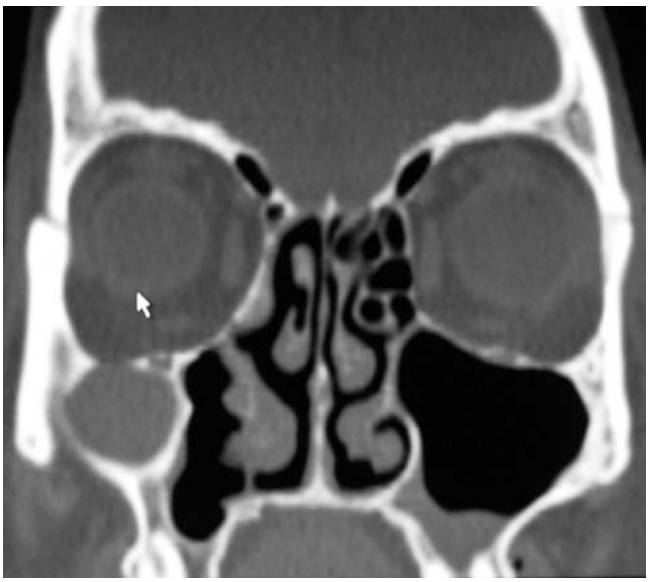

Fig 2.15 Surgical ciliated cyst of the maxilla: CT scan depicts a cyst in the lateral wall of the maxilla that expands to contact with the floor of the orbit (Courtesy of Dr. P. Claros, Barcelona, Spain)

shows variable thickness, and the content is brown mucinous, more rarely serous. Purulent fluid and cholesterol crystals are frequently seen [70].

Microscopy The wall of the cyst characteristically displays a fibrous connective tissue band, often with mild-to-moderate inflammatory infiltrate, which appears entrapped between the osseous wall and the overlying mucosa (Fig. 2.16). The mucosa is lined by an epithelium, which is pseudostratified ciliated in two-thirds of the cases, transitional in 28 % and squamous in 6 %. Goblet cells are also present, and their number increases with local infiltration of inflammatory cells into the cyst wall [71]. Epithelial dysplasia has been rarely observed [70].

Differential diagnosis Surgical ciliated cyst should be differentiated from mucocele of the maxillary sinus, which presents as a cyst containing mucoid or gelatinous material, lined by pseudostratified ciliated epithelium, sometimes with areas of squamous metaplasia. However, the main difference with surgical ciliated cyst is that paranasal sinus mucocele is not found in intraosseous location. Odontogenic cysts, including radicular cysts and keratocyst, may also occasionally present areas of ciliated epithelium. The clinical history of previous surgery of the maxillary sinus (Caldwell-Luc procedure) and the radiological aspect of the lesion are helpful in the distinction [72].

Treatment and prognosis Treatment consists of surgical removal of the lesion. Removal of the lesion is curative.

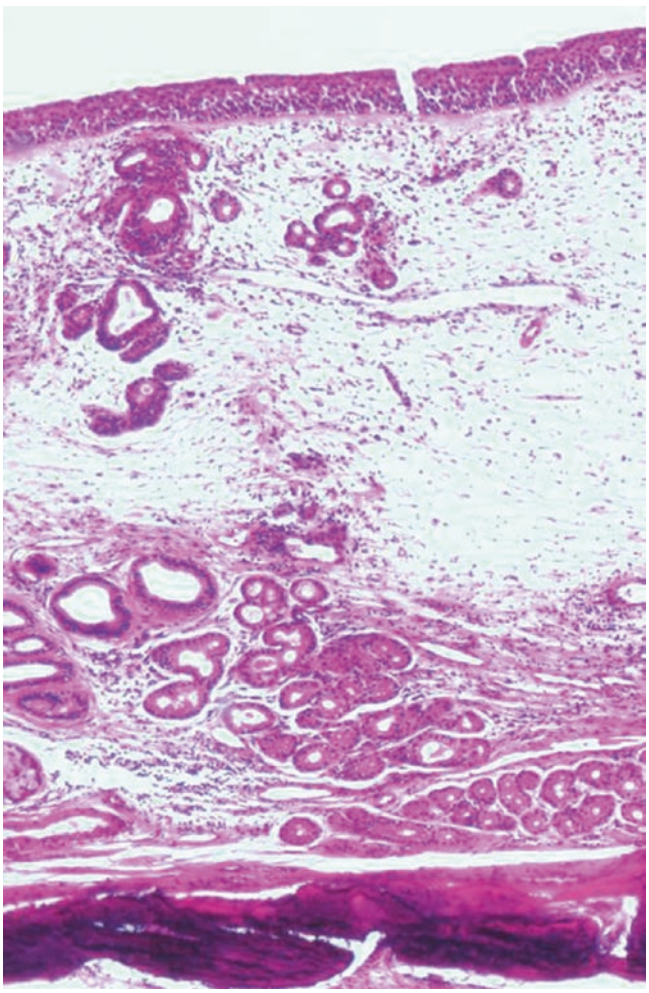

Fig 2.16 Surgical ciliated cyst of the maxilla: notice at the bottom the characteristic band of periosteal fibrous thickening between the calcified osseous wall and the lower part of the lamina propria of the respiratory mucosa

2.6 Fungal Diseases

Sinonasal fungal diseases are clinically classified as non-invasive allergic, non-invasive fungus ball or mycetoma, invasive chronic indolent, and invasive acute fulminant or angioinvasive (Table 2.1). The correct distinction between these four entities sometimes requires histologic, clinical, and radiologic correlations.

2.6.1 Allergic Fungal Rhinosinusitis

Definition The allergic form of fungal rhinosinusitis (FRS) is a non-invasive form of fungal infection, due to a localized hypersensitivity response to fungal growth that arises in areas of compromised mucus drainage.

Synonym Eosinophilic fungal sinusitis

Table 2.1 Fungal diseases of sinonasal tract

Non-invasive rhinosinusitis	Tissue-invasive rhinosinusitis
Allergic fungal sinusitis	Invasive indolent
Fungus ball	Invasive fulminant

Epidemiology Allergic FRS is the most common of all fungal sinusitis and accounts for between 5 and 10% of all chronic rhinosinusitis cases [73]. It most commonly affects adolescents and young adults (mean age at diagnosis 21.9 years) [74]. There is no significant gender predilection. The maxillary, ethmoid, and sphenoid sinuses are most commonly involved. Unilateral involvement may occur in some cases [74]. It is associated with nasal polyps, atopy, asthma, and elevated serum IgE [75].

Etiology In the first description of this disease, *Aspergillus* sp. was recognized as the primary causative fungus [76], but subsequent reports have evidenced that fungi of the dematiaceous family (*Alternaria* sp., *Bipolaris* sp., *Curvularia* sp., and others) are implicated in the majority of the cases [77].

Macroscopy At the time of surgery, allergic fungal mucin is recognized as thick and highly viscous in consistency, varying in color from light tan to brown, black, or dark green.

Microscopy The histological features necessary for the diagnosis of allergic FRS are detected in the mucin, rather than in paranasal sinus mucosa, which shows the changes of a nonspecific inflammatory condition, without involvement by fungi. The hallmark of the disease is the production of allergic mucin in which mucous material alternates with cell debris conferring a wavy appearance (Fig. 2.17). With hematoxylin and eosin stain, the mucin has a basophilic background and contains a mixed inflammatory cell infiltrate with a predominance of eosinophils, necrotic cell debris, and Charcot-Leyden crystals (Fig. 2.18). Fungal hyphae are rare, scattered, and fragmented and can be identified within the mucin with histochemical stainings (Fig. 2.19), including Grocott and Gomori methenamine silver [76, 78]. When fungal hyphae are not identified, the term sinonasal allergic mucinosis is applied.

Differential diagnosis Allergic FRS must be differentiated from other sinonasal fungal diseases, particularly from invasive forms, including indolent, granulomatous, and fulminant variants [9]. These are rare diseases in which fungi are found in the mucosa, soft tissues, and bone [76]. Chronic non-invasive fungal sinusitis, also known as fungus balls or mycetomas, is recognized as self-limited collections of matted fungal hyphae confined most commonly to the maxillary sinus.

Treatment and prognosis To prevent recurrences, a combination of conservative surgery and adjunctive medical

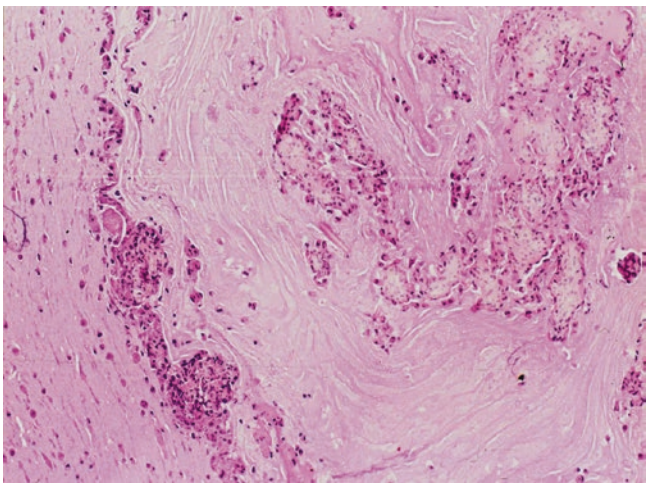

Fig. 2.17 Allergic mucinosis: basophilic pools of mucin alternate with dense aggregates of eosinophilic leukocytes conferring a wavy appearance

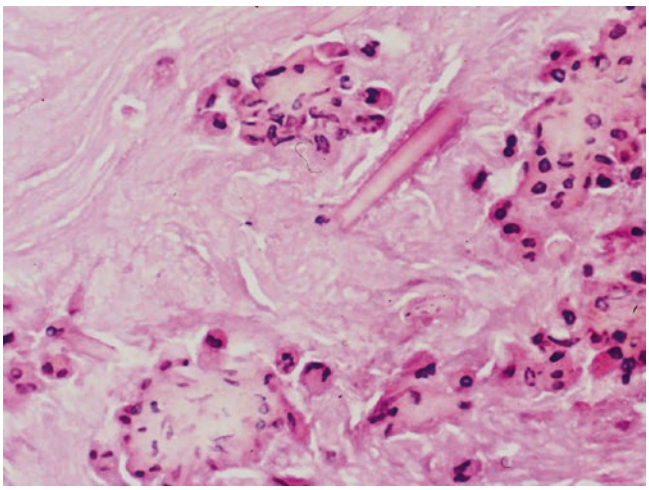

Fig. 2.18 Allergic mucinosis: a Charcot-Leyden crystal between lakes of mucin and aggregates of eosinophilic leukocytes

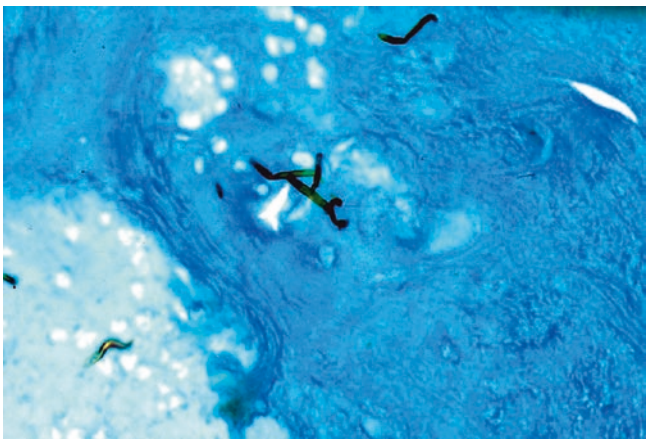

Fig. 2.19 Allergic fungal sinusitis, scarce fungal hyphae in a lake of mucin. Gomori methenamine silver

treatments, including systemic and/or topical corticosteroids, and immunotherapy to pertinent fungal and nonfungal antigens is recommended [75, 79].

2.6.2 Non-invasive Fungal Rhinosinusitis

Definition Non-invasive FRS is a mycotic infection characterized by the presence of a fungus ball in the sinus lumen, without involving the adjacent tissues.

Synonyms Mycetoma, fungus ball, and extramucosal fungal sinusitis

Epidemiology The maxillary sinus is the most commonly involved. Ethmoid, frontal, and sphenoid sinuses are affected less often.

Etiology and pathogenesis Non-invasive FRS occurs in immunocompetent patients, being mainly caused by *Aspergillus* sp. Other fungi are less common.

Macroscopy The consistency of the fungus ball may vary from soft to hard with focal central calcification.

Microscopy As a non-invasive disease, the fungal mass is present in the lumen of the sinus. Usually, the neighboring mucosa shows mild chronic inflammation. In sections stained with PAS-diastase or Gomori methenamine silver, the *Aspergillus* sp. hyphae appear as dichotomously, acute-angle branching septate hyphae 6–8 μm wide (Fig. 2.20).

Differential diagnosis Allergic FRS and invasive forms of fungal sinusitis must be ruled out.

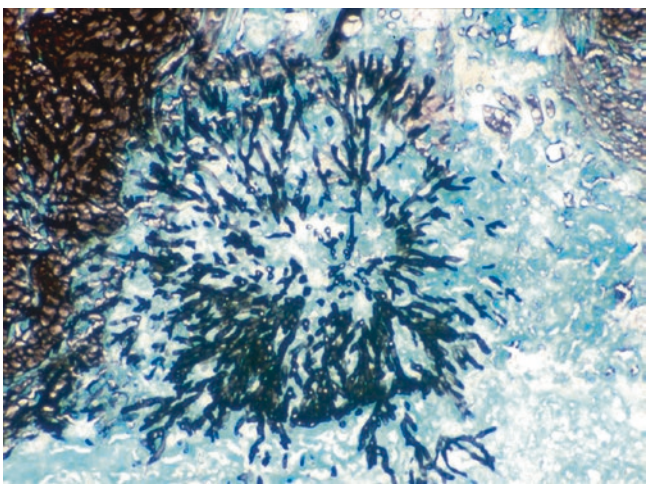

Fig. 2.20 Non-invasive fungal sinusitis: densely packed branching hyphae of *Aspergillus* forming a fungus ball. Gomori methenamine silver

Treatment and prognosis Evacuation of the sinus with removal of the fungus ball by endoscopic surgery is the recommended option. Antifungal medication is not required.

2.6.3 Invasive Fungal Rhinosinusitis

The spectrum of invasive FRS covers the chronic indolent invasive and the acute fulminant forms [9, 75]. The first form is found in immunologically competent patients and the latter is restricted to immunocompromised patients.

2.6.3.1 Chronic Invasive Fungal Rhinosinusitis

Definition Chronic invasive FRS is an uncommon form of sinusitis characterized by a protracted clinical course despite the finding of fungal tissular invasion. Chronic indolent invasive fungal sinusitis is a synonymous.

Epidemiology Two forms of chronic invasive FRS are recognized: the nonspecific chronic invasive fungal sinusitis and the granulomatous chronic invasive fungal sinusitis [80].

Etiology and pathogenesis Both of these conditions are thought to be due to *Aspergillus* sp. and both occur in immunologically competent patients.

Microscopy Fungi are found in the mucosa, soft tissues, and bone. The presence of a granulomatous reaction, the recognition of which is strictly a function of histopathology, is currently the sole means of identifying this category [75]. At present, there is no histopathologic hallmark diagnostic of nonspecific chronic invasive FRS, which may be better labeled as "nongranulomatous chronic invasive" FRS.

Treatment and prognosis Surgical debridement and drainage are required. Systemic antifungal drugs may not be necessary to achieve favorable response to treatment.

2.6.3.2 Fulminant Invasive Fungal Rhinosinusitis

Definition Invasive fulminant FRS is an acute, rapidly progressive, and life-threatening fungal infection characterized by destructive tissue invasion with or without obvious vascular invasion.

Epidemiology Invasive fulminant FRS is most commonly seen in adult immunocompromised patients.

Etiology and pathogenesis Invasive fulminant FRS has been traditionally associated with *Mucor* sp. and poorly controlled diabetics [81], but currently, invasive fulminant FRS also encompasses *Aspergillus* sp., as well as dematiaceous and non-dematiaceous fungi, with a strong association with immunodeficiencies [75].

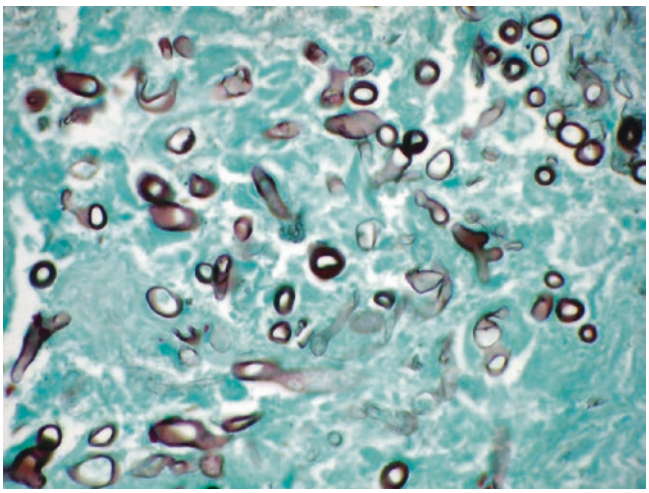

Fig. 2.21 Invasive fulminant fungal sinusitis: necrotic background with wide nonseptated hyphae of *Mucor* species. Gomori methenamine silver (Courtesy of Prof. J. Ramirez, Barcelona, Spain)

Microscopy Invasive fulminant FRS causes destructive inflammation of the sinonasal tissues featured by a combination of necrotic debris and tissue invasion with or without obvious vascular invasion [75, 82]. Fungi are found in the mucosa, soft tissues, and bone [76]. The fungus invasion of the blood vessels causes thrombosis, and the surrounding affected tissues may exhibit coagulative necrosis and hemorrhage, while the inflammatory reaction is scant. Although the architecture of the surrounding tissues may fade away, the fungi can often be recognized (Fig. 2.21). In tissue sections stained with PAS-diastase or Gomori methanamine silver, the *Mucor* sp. fungi are seen as 10- to 20-μm-wide nonseptate hyphae, usually branching at right angles, whereas *Aspergillus* sp. hyphae appear as dichotomously, acute-angle branching septate hyphae 6–8 μm wide.

Differential diagnosis Invasive fulminant FRS must be differentiated from other types of fungal sinusitis, as well as from other midfacial destructive and granulomatous lesions.

Treatment and prognosis The therapy for patients with acute fungal sinusitis is multimodal and involves surgery and antibiotic therapy. Aggressive surgical debridement and drainage and systemic antifungal drugs are mandatory. A quick histological recognition of the fungi is of paramount importance in the proper management of invasive fulminant FRS. A frozen section may be required from the pathologist, as fungal cultures are often negative and an early diagnosis and treatment improves survival rates and lowers morbidity [8].

2.6.4 Rhinosporidiosis

Definition Rhinosporidiosis (RSP) is a special form of chronic invasive granulomatous fungal disease that follows a protracted course, growing in the form of polyps involving the upper respiratory tract, principally the nasal cavity [83, 84].

Epidemiology Most cases of RSP occur in India and Sri Lanka and less frequently in Brazil. Although very rarely, RSP may be seen in any country.

Etiology and pathogenesis RSP is caused by the endosporulating fungus *Rhinosporidium seeberi*. It affects immunocompetent patients through endospores contaminating water or soil.

Macroscopy RSP lesions may look alike allergic sinonasal polyps.

Microscopy In RSP the mucosal and submucosal involvement is characterized by the presence of thick-walled sporangia measuring 50–350 μm in diameter and containing numerous mucicarminophilic spores. They are associated with a heavy chronic inflammatory reaction with occasional foci of suppuration and foreign body giant cell reaction. Sporangia also stain with PAS-diastase and Gomori methenamine silver.

Differential diagnosis Sinonasal polyps and papillomas, as well as coccidiomycosis

Treatment and prognosis Surgical removal of the lesions. Recurrence rate is low.

2.7 Midfacial Destructive Granulomatous Lesions

2.7.1 Granulomatosis with Polyangiitis

Definition Granulomatosis with polyangiitis (GPA) is an immunologically mediated inflammatory disease characterized by granulomatous vasculitis of the upper and lower respiratory tracts together with glomerulonephritis. Variable degrees of disseminated vasculitis involving both small arteries and veins may also occur.

Synonym Wegener's granulomatosis

Epidemiology GPA lesions in the upper respiratory tract are ulcerative and destructive and occur mainly in the nasal cavity and paranasal sinuses. At the time of initial presentation, the full clinical picture of the disease is rarely seen.

Clinical aspects A high percentage of patients develop elevated c-ANCA as well as elevated proteinase 3 (PR3).

Microscopy The hallmarks of GPA are the presence of geographic necrosis surrounded by palisaded histiocytes, granulomas and scattered giant cells, vasculitis with fibrinoid necrosis or infiltration of vessel walls by inflammatory cells, neutrophilic microabscesses, and a mixed inflammatory infiltrate with variable fibrosis (Figs. 2.22 and 2.23) [85–90].

Differential diagnosis The classic histological features of GPA are not present in many biopsy specimens. Repeat biopsies and clinical correlations are often essential for early diagnosis. In the early stages, when GPA is restricted to the upper respiratory tract and ear, the diagnosis can be quite difficult [90]. Stains for acid-fast bacilli and fungi are negative. GPA must be differentiated from allergic granulomatosis and vasculitis (AGV) also known as Churg-Strauss disease, in which, besides vasculitis and poorly formed granulomas, eosinophils predominate with formation of eosinophilic microabscesses [89]. In AGV, c-ANCA may be occasionally elevated but PR3 is absent. NK-/T-cell lymphoma and diffuse large B-cell lymphoma are other differential diagnoses. Increased IgG4-positive cells can be seen in sinonasal, orbital, and periorbital biopsies of GPA that could induce a wrong diagnosis of IgG4-related disease [66].

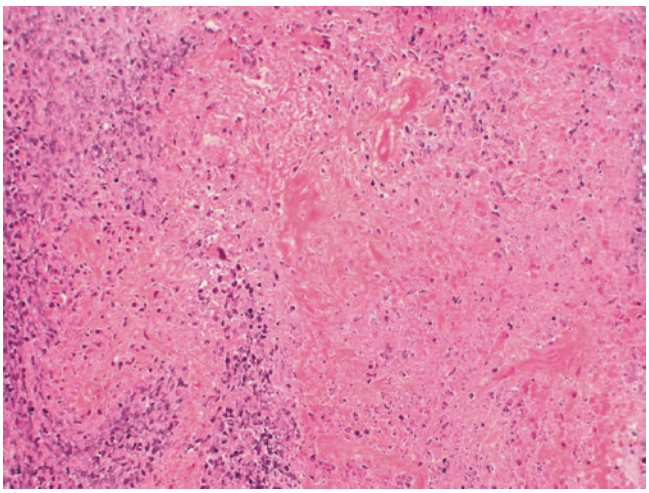

Fig. 2.22 Granulomatosis with polyangiitis: presence of geographic necrosis surrounded by granulomas and scattered giant cells

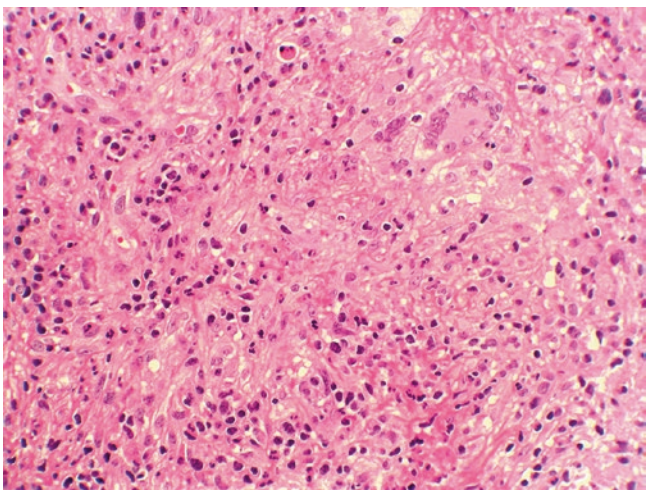

Fig. 2.23 Granulomatosis with polyangiitis: vasculitis with fibrinoid necrosis, infiltration of vessel walls by inflammatory cells, giant cells, and occasional epithelioid cells

Treatment and prognosis Concomitant administration of cyclophosphamide and prednisone is recommended [90].

2.7.2 Leprosy

Definition Leprosy is a chronic infection caused by *Mycobacterium leprae* that depending on the immunoreactivity of the patients presents three clinical forms: lepromatous, tuberculoid, and indeterminate.

Epidemiology and pathogenesis *Mycobacterium leprae* affects principally the cooler parts of the body as the upper respiratory tract and especially the sinonasal region [91, 92].

Microscopy Lepromatous leprosy is the most frequent form of this disease involving the nasal cavity [93]. It is characterized by nodular masses of foamy macrophages (Virchow lepra cells) in which large numbers of acid-fast bacilli (*Mycobacterium leprae*) are demonstrable by the Fite-Faraco stain, a modified Ziehl-Neelsen method. Tuberculoid leprosy is characterized by non-caseating granulomas and the indeterminate variant by a nonspecific chronic inflammatory reaction; acid-fast bacilli are seldom demonstrable in these types.

Treatment Combination of rifampicin, dapsone, and clofazimine

2.7.3 Tuberculosis

Definition Tuberculosis (TBC) is a chronic granulomatous infection caused by *Mycobacterium tuberculosis*.

Epidemiology Tuberculosis (TBC) of the head and neck occurs infrequently, and involvement of the nose is rare, representing in most cases a secondary event to pulmonary involvement [94].

Macroscopy In most cases, there is a polyp of the nasal septum or an ulcerated granular lesion.

Microscopy TBC is characterized by caseating and confluent granulomas with surrounding epithelioid cells palisading

and Langhans-type giant cells. Lack of caseation is uncommon. Histologically, acid-fast bacilli may be occasionally identified by the Ziehl-Neelsen stain. The definitive diagnosis is made by isolating *Mycobacterium tuberculosis* by culture and/or PCR from tissue removed during biopsy.

Differential diagnosis It includes all other granulomatous diseases, mainly those with caseating type of necrosis. The presence of intracranial extension may lead to a clinical diagnosis of malignancy [95].

Treatment and prognosis Administration of tuberculostatic drugs is usually curative.

2.7.4 Sarcoidosis

Definition Sarcoidosis is a chronic multisystem, non-caseating granulomatous disorder of unknown etiology. The upper aerodigestive tract is occasionally involved.

Epidemiology Besides the lung, hilar and mediastinal lymph nodes, skin, liver, and other systems and organs, several head and neck territories may be affected. The sinonasal mucosa is rarely involved, and most patients have generalized disease [96–99].

Microscopy Discrete non-caseating and non-confluent granulomas are a distinguishing feature. Sarcoid granulomas are composed predominantly of epithelioid histiocytes with multinucleated giant cells and a peripheral rim of lymphocytes. Asteroid bodies and Schaumann's conchoid calcium concretions may be found in the cytoplasm of the giant cells. Stains for acid-fast bacilli and for other infectious agents are negative. Although no microorganisms are found in sarcoid granulomas, cell wall-deficient forms of mycobacteria have been detected by PCR [100].

Differential diagnosis Includes other granulomatous disorders, like tuberculosis, leprosy, granulomatosis with polyangiitis, inhalant granulomatous processes, and cholesterol granuloma [85].

Treatment and prognosis Corticosteroids are recommended for treatment of clinically active disease. Outcome is usually favorable. Low-dose corticosteroid treatment may be required to maintain remission and prevent fibrosis.

2.7.5 Rhinoscleroma

Definition Rhinoscleroma (RNS) is a chronic bacterial infection caused by *Klebsiella rhinoscleromatis*.

Epidemiology RNS is most prevalent in Russia, Belarus, Poland, and central European countries. Central and upper South American countries are also endemic areas [101].

Etiology *Klebsiella rhinoscleromatis* is a capsulated gram-negative bacillus [83, 101].

Macroscopy Large nodular tumorlike masses are found in the nasal cavity (Hebra nose). Less often, RNS nodules are found in other parts of the upper respiratory tract.

Microscopy RNS nodules contain large macrophages with abundant clear or vacuolated cytoplasm, known as Mikulicz cells (Fig. 2.24). The causative organism may be seen within these cells by the H&E stain; however, they are better identified by the Warthin-Starry staining method or by immunostaining for the *Klebsiella* capsular antigen. In addition, there is fibrosis and heavy infiltration by chronic inflammatory cells, mainly plasma cells showing numerous Russell bodies. The mucosal epithelium may show squamous metaplasia and occasionally prominent pseudoepitheliomatous hyperplasia. Exceptional examples of squamous cell carcinoma have been reported, in association with RNS [83, 101–103].

Differential diagnosis RNS must be ruled out from leprosy, syphilis, yaws, TBC, leishmaniasis, rhinosporidiosis, and paracoccidioidomycosis [101]. Another entity to be distinguished from RNS is Rosai-Dorfman disease.

Treatment and prognosis Prolonged treatment by tetracycline and ciprofloxacin is recommended. Surgery may be used for debulking the obstruction.

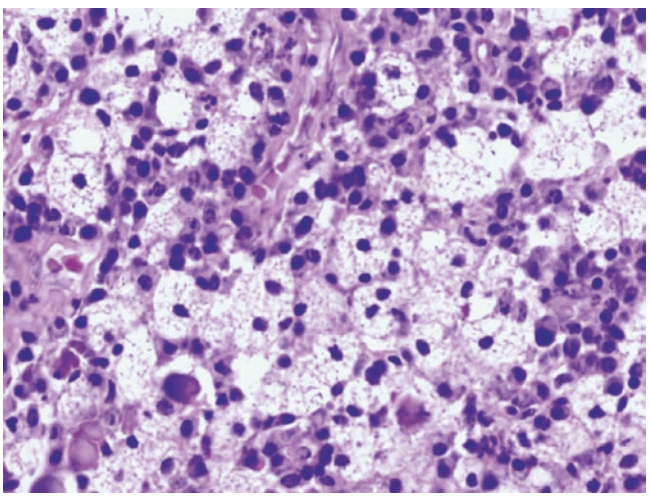

Fig. 2.24 Rhinoscleroma: large macrophages with abundant clear or vacuolated cytoplasm (Mikulicz cells) and heavy infiltration by chronic inflammatory cells (Courtesy of Prof. Y. Rogov, Minsk, Belarus)

2.7.6 Leishmaniasis

Definition Cutaneous leishmaniasis is an infection of the skin by a protozoan of the genus *Leishmania*. It comprises three different entities: localized cutaneous leishmaniasis, also known as "oriental sore" or "tropical sore," mucocutaneous leishmaniasis, and disseminated anergic cutaneous leishmaniasis [104].

Epidemiology Leishmaniasis of the nasal region when seen in Mediterranean and Oriental countries is mostly in the form of "oriental sore" caused by *Leishmania tropica*. In Central and South America, leishmaniasis is mostly seen in the form of mucocutaneous leishmaniasis caused by *Leishmania braziliensis* [105, 106].

Disseminated anergic cutaneous leishmaniasis develops in hosts lacking specific cell-mediated immune responses to the distinct species of *Leishmania*. The parasites are transmitted through the bites of blood-sucking female sand flies of the genus *Phlebotomus* [104].

Microscopy The protozoan parasite (amastigote) is seen in the cytoplasm of histiocytes or, extracellularly, measures 1.5–3.0 μm in maximum dimension and has a nucleus and a rod-shaped kinetoplast which stains positively with Giemsa. The kinetoplast is more readily identified in Giemsa-stained smears of exudates or scrapings than in paraffin sections. The lesions, commonly found in the nasal mucosa and facial skin, are associated with chronic inflammatory reaction and granuloma formation. They are in general circumscribed and self-involutive in the case of the "oriental sore" and disfiguring with marked destruction of the nasal septum in mucocutaneous leishmaniasis. In anergic cutaneous leishmaniasis, the nodules show enormous amounts of histiocytes repleted with leishmania [104].

Differential diagnosis Nasal leishmaniasis must be differentiated from other granulomatous diseases such as rhinoscleroma, paracoccidioidomycosis, yaws, leprosy, syphilis, TBC, and histoplasmosis.

Treatment and prognosis Antimonial compounds remain the treatment of choice. Prognosis is good in oriental sore, resistant to healing in the mucocutaneous form, and unfavorable in anergic leishmaniasis.

2.7.7 Cocaine Abuse

Cocaine abuse (snorting) may be associated with severe nasal necrotizing inflammation [107]. Endoscopically, there is atrophy of the inferior and middle turbinates and ulceration of the nasal septum. Histologically, areas of necrosis are admixed with acute and chronic inflammation; giant cells embracing birefringent foreign body particles are often present; however, vasculitis is minimal or absent. The lesion may be confused with granulomatosis with polyangiitis (Wegener's).

2.7.8 Local Steroid Injections

A granulomatous lesion of the nasal mucous membranes occurs in patients treated with injections of steroid preparations [108]. There is a central deposition of amorphous material bordered by histiocytes and foreign body giant cells. Occasional particles of birefringent crystalline material may be present. Special stains should be performed to exclude the presence of microorganisms.

2.8 Benign Epithelial Neoplasms

2.8.1 Sinonasal Papillomas

Sinonasal papillomas are usually divided into squamous cell papilloma of the nasal vestibule and Schneiderian papillomas of the nasal cavity and paranasal sinuses (Table 2.2). The first are covered by the epithelium of the skin surface. The latter are lined by the respiratory mucosa of the nasal cavity and paranasal sinuses (referred to as the Schneiderian membrane) and comprise three histopathological types: exophytic, inverted,

Table 2.2 Sinonasal papillomas

	Squamous	Everted	Inverted	Oncocytic
Location	Vestibule	Nasal septum	Lateral wall and sinuses	Lateral wall and sinuses
Growth	Exophytic	Exophytic	Endophytic-exophytic	Exophytic-endophytic
Epithelium	Keratinizing squamous	Non-keratinizing squamous and ciliated	Non-keratinizing squamous and ciliated	Oncocytic columnar
Intraepithelial mucin cysts	–	+	+	+++
Recurrence	Unusual	Usual[a]	Usual[a]	Usual[a]
Malignancy	Unusual	Unusual	10 %	<10 %

Modified from Wenig [567]
[a]If incomplete excision

and oncocytic. The histopathologic features that clearly differentiate between the three types of Schneiderian papillomas have been well documented [109]. Human papillomavirus (HPV) types 6 and 11 are involved in the pathogenesis of exophytic papillomas but not so consistently in the other two variants of Schneiderian papillomas [110–112]. All oncocytic papillomas examined have been HPV negative [110, 112, 113].

2.8.1.1 Squamous Cell Papilloma of the Nasal Vestibule

Definition A benign proliferative lesion composed of delicate stromal papillae covered by squamous epithelium. Squamous cell papillomas (SCPs) located in the nasal vestibule are formed by keratinizing stratified squamous epithelium of the skin surface [114].

Microscopy SCPs are exophytic and consist of a thickened layer of differentiated squamous epithelium without evidence of atypia or mitoses which is supported by arborescent stalks of fibrovascular stroma. Varying degrees of keratinization are present and hyperkeratosis, parakeratosis, or both may be seen (Fig. 2.25).

Differential diagnosis SCP of the nasal vestibule must be distinguished from exophytic papilloma of the Schneiderian mucosa. The keratinizing nature of the squamous epithelium in the former and the presence of mucous epithelial cells in the latter are the key differentiating features.

Treatment and prognosis SCPs of the nasal vestibule are benign, rarely recur after simple excision, and in general are not associated with HPV.

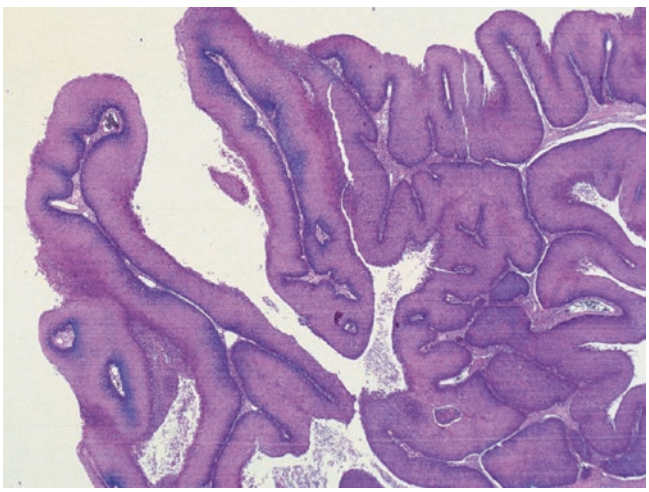

Fig. 2.25 Squamous cell papilloma of nasal vestibule: thickened layer of benign squamous epithelium is supported by arborescent stalks of fibrovascular stroma

2.8.1.2 Everted (Schneiderian) Papilloma

Definition Everted (Schneiderian) papilloma (ESP) is composed of papillary fronds with delicate fibrovascular cores covered by multiple layers of epithelial cells.

Synonyms ESP is also known as exophytic, fungiform, septal, and transitional cell papilloma among other terms [115]

Epidemiology ESPs arise most frequently at the nasal septum and only very rarely in the lateral nasal walls or in paranasal sinuses [115]. Males are predominantly affected. Patients tend to be younger than with other types of Schneiderian papillomas. ESPs are almost always unilateral [116]. No side is preferred and bilaterality is exceptional.

Macroscopy ESP is a single, warty tumor measuring up to 1.5 cm in diameter.

Microscopy ESP is composed of branching papillary structures, with papillae covered by stratified non-keratinizing squamous epithelium, admixed with intermediate or transitional cells and with ciliated respiratory epithelium that contains interspersed mucin-secreting cells (Fig. 2.26). The supporting stroma is fibrovascular.

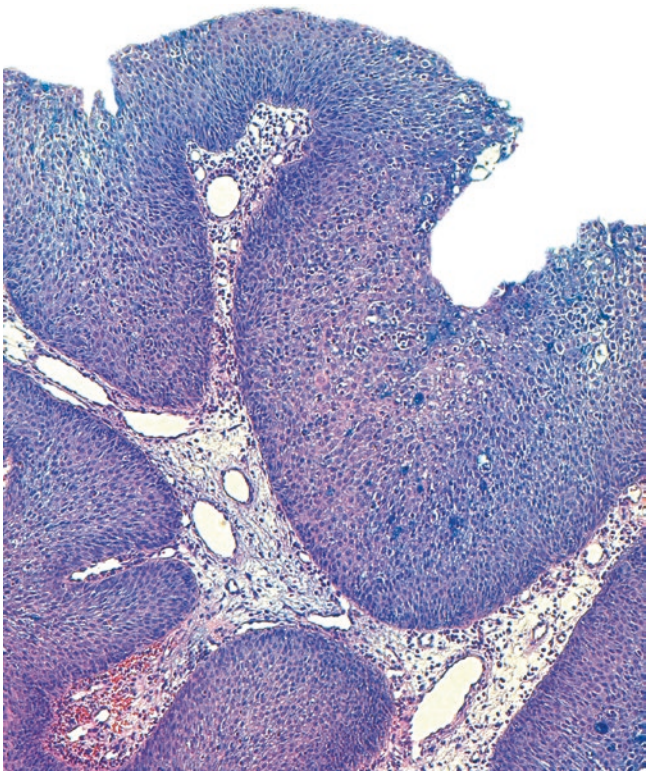

Fig. 2.26 Exophytic papilloma: branching papillary structures mainly covered by stratified non-keratinizing squamous epithelium that contains interspersed mucin-secreting cells. Alcian blue stain

Differential diagnosis The two main differential diagnoses of ESP are inverted papilloma and oncocytic papilloma. Neither the invaginated pattern of growth of inverted papillomas nor the oncocytic columnar epithelium of oncocytic papilloma is found in exophytic papilloma [109]. Non-keratinizing squamous cell carcinoma can be easily ruled out by the lack of atypia and invasion.

Treatment and prognosis Wide surgical excision is the best choice of treatment to avoid recurrences. Recurrences occur in about 20–40 % of cases, which is less than in inverted papillomas. Malignant transformation almost never occurs in ESP.

2.8.1.3 Inverted (Schneiderian) Papilloma

Definition Inverted (Schneiderian) papilloma (ISP) is a papilloma in which the epithelium invaginates and proliferates inward the underlying stroma.

Synonyms Inverting papilloma [115]

Epidemiology and pathogenesis ISP is the most common type of Schneiderian papilloma and accounts for about 60 % of them [117]. This lesion occurs almost exclusively in the lateral wall of the nasal cavity and in the paranasal sinuses, although on rare occasions, it may also arise on the nasal septum [115]. Patient's age ranges between 30 and 50 years and the male to female ratio is 3:1 [118]. Molecular studies show supportive evidence of clonality in ISPs [119].

Macroscopy ISPs frequently have a polypoid appearance and may be grossly indistinguishable from nasal polyps of the common type.

Microscopy ISPs are characteristically composed of invaginating crypts, cords, and nests covered by non-keratinizing squamous epithelium, which alternates with columnar ciliated respiratory epithelium and with intermediate or transitional epithelium. This newly formed duct system is similar to the embryonic development of the nasal mucosa [120]. The multilayered epithelium typically contains mucous cells and mucin-filled microcysts. The invagination of the mucosa may result in the presence of apparently discontinuous cell masses lying deep to the epithelial surface, but the basement membrane is intact and may be shown in continuity with that of the surface epithelium [121]. An inverted growth is the hallmark of inverted papilloma, but varying degrees of papillary growth may be seen at the surface (Fig. 2.27) [116]. The surface is characteristically lined by respiratory type of epithelium; nevertheless, foci of surface keratinization are occasionally present [114]. A few regular mitoses may be found in the basal and parabasal layers. Although the nuclei may show mild nuclear irregularities and hyperchromatism, no disturbances of the cellular polarity are found. An abundant and edematous connective tissue stroma is a common feature of inverted papillomas. It usually contains macrophages and neutrophils, but eosinophils may also be present. This inflammatory infiltrate may also be present between the epithelial cells, within the dilated lumens of invaginated crypts and within the numerous microcysts that usually occur in the respiratory epithelium (Fig. 2.28). Seromucinous glands are absent, but branching gland ducts are often present. The tumor grows by extension to involve the contiguous sinonasal epithelium [122].

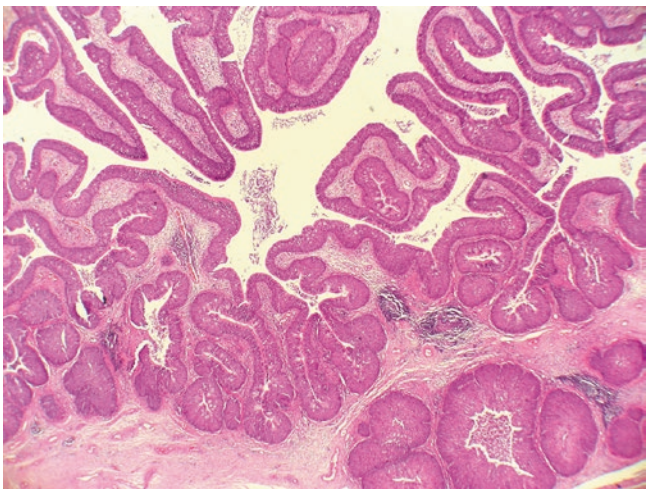

Fig. 2.27 Inverted papilloma: inverted growth is the hallmark of inverted papilloma, but varying degrees of papillary growth may be seen at the surface

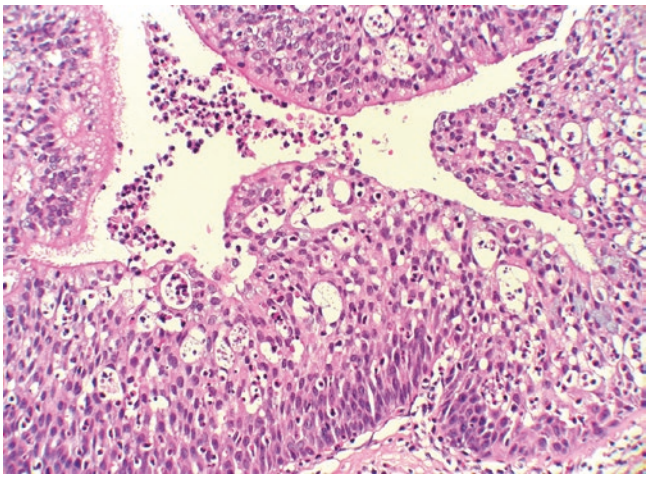

Fig. 2.28 Inverted papilloma: inflammatory infiltrate is present between the epithelial cells, within the dilated lumens of invaginated crypts, and within the numerous microcysts of the respiratory epithelium

Differential diagnosis ISPs must be distinguished mainly from REAH and from squamous cell carcinoma with basaloid features.

Treatment and prognosis If treated only by local surgical excision, recurrence occurs in up to 75% of cases. Therefore, lateral rhinotomy and medial maxillectomy are advisable for tumors of the lateral nasal wall [123]. Carcinoma develops in about 10–15% of inverted papillomas [114, 123, 124]. Carcinoma may coexist with inverted papilloma at the initial presentation or originate subsequently [114, 122, 125–127]. According to the experience of Michaels and Hellquist [128], carcinoma does not usually develop in the course of recurrences of inverted papilloma. The presence of severe atypia or marked keratinization in an inverted papilloma is always suspicious of malignant transformation. In these instances, the entire specimen should be thoroughly examined to exclude an associated carcinoma. Most associated carcinomas are squamous and less often undifferentiated (Figs. 2.29 and 2.30) [129]; other types may also occur such as verrucous carcinoma [130]. Carcinoma associated with ISP has a 60% 10-year survival rate [131].

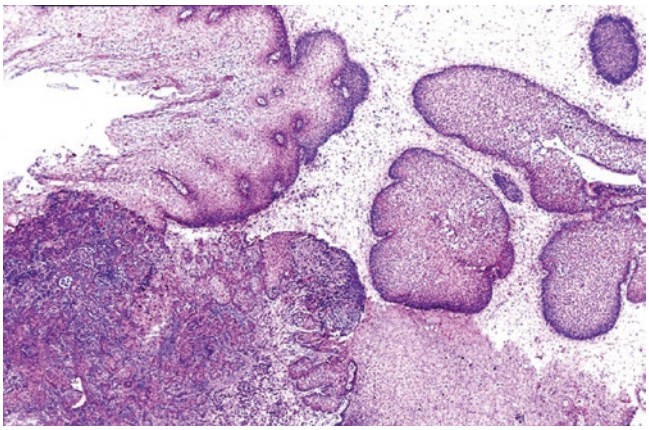

Fig. 2.29 Squamous cell carcinoma ex-inverted papilloma: cords and nests of infiltrating squamous epithelium are seen at the *lower left* (Courtesy of Prof. C. Ereño, Bilbao, Spain)

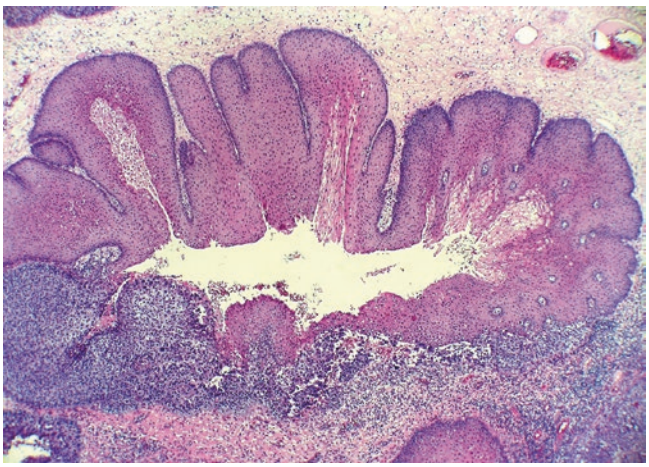

Fig. 2.30 Undifferentiated carcinoma ex-inverted papilloma: confluent nests of undifferentiated carcinoma originate from inverted papilloma at the bottom. The characteristic edematous stroma of inverted papilloma is seen at the *top* (Courtesy of Prof. C. Ereño, Bilbao, Spain)

2.8.1.4 Oncocytic (Schneiderian) Papilloma

Definition Oncocytic (Schneiderian) papilloma (OSP) is papilloma composed of both exophytic fronds and endophytic invaginations lined by multiple layers of columnar cells with oncocytic features.

Synonyms OSP is also known as "columnar" or "cylindrical" cell papilloma [115]

Epidemiology OSP is the least common type of Schneiderian papillomas. It comprises less than 5% of all sinonasal papillomas [109, 114, 132–135]. Both sexes are equally affected. Bilaterality has not been documented.

Macroscopy Tumors are in general small, although occasionally may reach various centimeters in greatest dimension.

Microscopy OSPs are composed of exophytic fronds and endophytic invaginations lined by pseudostratified or multilayered columnar cells with prominent oncocytic features. The cells have uniform hyperchromatic nuclei and abundant eosinophilic, occasionally granular cytoplasm that contains abundant mitochondria and stains for the mitochondrial enzyme cytochrome C oxidase [136]. Goblet cells are not found. Cilia may be occasionally encountered on the superficial epithelial layer. Intraepithelial microcysts containing mucin and neutrophils are usually present. These microcysts are larger than the similar structures also seen in inverted papilloma. The tumor resembles inverted papilloma in its sites of occurrence, the lateral wall of the nasal cavity and the maxillary antrum.

Differential diagnosis OSP must be distinguished from low-grade mucoepidermoid adenocarcinoma and other low-grade adenocarcinomas of the sinonasal tract. Rhinosporidiosis is the main entity to rule out in endemic countries like India and South America, as sporangia of *Rhinosporidium seeberi* may mimic the microcysts of OSP.

Treatment and prognosis The same treatment principles apply for OSP as for ISP [134]. The rate of recurrence of OSP is considered to be 36%, which is slightly lower than in inverted papilloma. The low frequency of these tumors makes it difficult to evaluate its true malignant potential, which seems to be similar to that of inverted papilloma [133]. Atypical hyperplasia and carcinoma in situ changes can be occasionally found (Fig. 2.31). Surgical excision with wide margins is the treatment of choice. Invasive squamous cell carcinoma, high-grade mucoepidermoid carcinoma, and undifferentiated carcinoma have been reported in association with oncocytic papilloma [114, 132, 137–139].

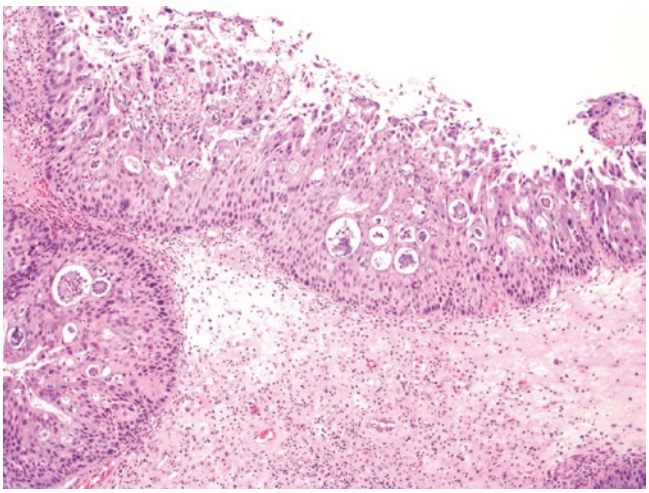

Fig. 2.31 Oncocytic papilloma with atypical cells: papillary fronds formed by columnar cells with frequent atypical nuclei, oncocytic cytoplasm, and presence of microcysts

2.8.2 Salivary Gland-Type Adenomas

2.8.2.1 Pleomorphic Adenoma

Definition Pleomorphic adenoma is a tumor composed of epithelial and modified myoepithelial cells variably mixed with mucoid, myxoid, or chondroid ground substance. Pleomorphism is architectural while cells are monomorphic.

Synonym Mixed tumor

Epidemiology Pleomorphic adenoma is the most frequent benign glandular tumor of the sinonasal region. Most of them arise on the nasal septum and the rest on the lateral nasal wall or turbinates. Origin from the maxillary antrum is rare. Most patients are between 20 and 60 years of age [140, 141].

Macroscopy Tumors are usually polypoid and may measure up to 5 cm.

Microscopy They are unencapsulated. Myoepithelial cells, often of the plasmacytoid hyaline type, tend to predominate over the glands [141].

Differential diagnosis Myoepithelioma is the main type of tumor to differentiate from pleomorphic adenoma.

Treatment and prognosis Wide surgical excision is recommended. The recurrence rate of sinonasal pleomorphic adenoma is much lower than for its counterpart in the major salivary glands [140, 142].

2.8.2.2 Other Salivary Gland-Type Adenomas

Rare examples of sinonasal oncocytoma have been reported, most arise from the nasal septum, although they may also arise from the maxillary sinus [143, 144]. Those examples that have behaved aggressively are more appropriately considered low-grade adenocarcinomas rather than adenomas [141]. Intranasal basal cell adenoma has been also documented [145]. In addition, myoepithelioma [146] and one case of sinonasal myoepithelioma transformed into myoepithelial carcinoma following multiple recurrences were reported [147].

2.8.3 Pituitary Adenomas

Definition Pituitary adenomas are benign tumors expressing the phenotype of cells of the anterior pituitary gland.

Epidemiology The rare pituitary adenomas of the sinonasal region are in most instances extensions from intrasellar tumors [148, 149]. Very unusually, they arise from ectopic pituitary tissue as tumors from the sphenoid sinus or the nasal cavity [150, 151].

Microscopy Extrasellar pituitary adenomas are histologically similar to tumors within the sella [148, 149]. The main growth patterns are diffuse, ribbonlike, papillary, and pleomorphic. Most consist of chromophobe cells (Fig. 2.32). Immunohistochemistry is required for classification according to the hormones produced [152].

Differential diagnosis Main pitfalls to avoid in pituitary adenomas presenting as sinonasal tumors include carcinoma, melanoma, paraganglioma, and olfactory neuroblastoma [152, 153].

Treatment and prognosis Complete surgical removal of pituitary adenomas is mandatory. Radiotherapy is required in incomplete resections as well as an optional dopamine agonist.

2.8.4 Primary Sinonasal Ameloblastoma

Definition Ameloblastoma (AMB) primary of the sinonasal tract is a tumor derived from remnants of odontogenic epithelium, having similar features to its gnathic counterparts (see Chap. 4) and devoided of significant osseous involvement [154].

Epidemiology Primary sinonasal AMBs are rare tumors that present in the nasal cavity and in the maxillary sinus [154–159]. The mean age of patients at presentation is about 60 years; the rate of men versus women is of 4 to 1 [154].

Macroscopy Frequently presents as a polypoid mass of variable size and rubbery consistence.

Microscopy AMB consists of centrally placed islands and nests of epithelial stellate reticulum cells, surrounded by columnar ameloblastic epithelium. The columnar epithelium presents a characteristic nuclear palisading with reverse

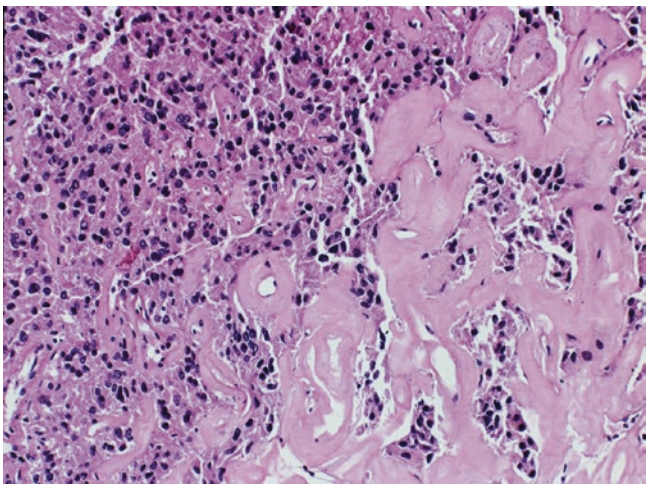

Fig. 2.32 Ectopic pituitary adenoma: cords and nests of chromophobe cells often surrounded by strands of hyaline deposits. Immunohistochemistry was positive for prolactin

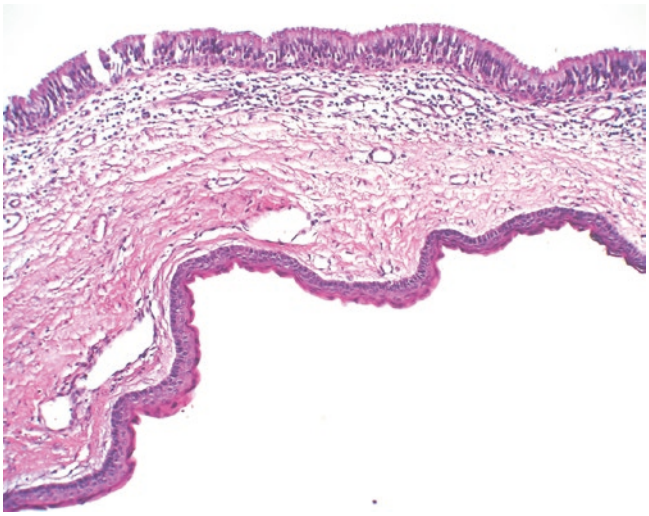

Fig. 2.33 Sinonasal odontogenic keratocyst: corrugated squamous epithelium with palisading of basal cell lines the inner surface of the cyst, while adjacent Schneiderian epithelium covers the lumen of the maxillary sinus

polarity, due to the presence of cytoplasmic subnuclear vacuoles that displace the nuclei away from the basement membrane toward the stellate reticulum. Occasional foci of squamous metaplasia may be found in the stellate reticulum. Immunostaining for calretinin is positive in over 90 % of AMB [160]. Remnant bands of covering respiratory epithelium may be found, which have been considered as a possible source of the tumor [154].

Differential diagnosis AMB must be distinguished mainly from basal cell adenoma, pleomorphic adenoma, basaloid squamous cell carcinoma, adenoid cystic carcinoma, and biphasic synovial sarcoma. Unicystic AMB must be told apart from odontogenic keratocyst (Fig. 2.33).

Treatment and prognosis Excellent results are usually achieved after surgical excision with margins free of tumor. Ameloblastoma is a benign, but locally aggressive tumor that requires long-term follow-up to control the risk of recurrence.

2.9 Benign Sinonasal Soft Tissue and Neural Neoplasms

2.9.1 Hemangiomas

Hemangiomas of the upper respiratory tract may be of the lobular capillary, cavernous or venous types [161].

2.9.1.1 Lobular Capillary Hemangioma

Definition Lobular capillary hemangioma (LCH) is a benign proliferation of capillary blood vessels adopting a lobular configuration [161].

Synonym Pyogenic granuloma.

Epidemiology and pathogenesis The sinonasal mucosa accounts for 29 % of the LCH of the upper aerodigestive tract. Although the cause of LCH is unknown, it has an association with trauma, pregnancy, and oral contraceptives [161].

Clinical features The nasal and the vestibular septum are typical sites for LCH. Nasal obstruction and epistaxis are the most common early symptoms.

Macroscopy LCHs present as red-colored polypoid formations with a collar-like invagination around its basis. They measure up to 1.5–2 cm.

Microscopy LCH consists of lobular arrangements of blood-filled capillaries separated by loose connective tissue. The blood supply is provided by a feeder vessel with branches ramifying to the lobules (Fig. 2.34). Nasal LCHs are covered often by squamous metaplastic epithelium. Superficial stromal edema and ulceration are common accompanying features. At the ulcerated zone, conventional granulation tissue may be found.

Differential diagnosis LCH should be distinguished mainly from conventional polypoid granulation tissue, which has a distinctive radial distribution of capillary blood vessels and lacks lobular arrangements. Other differential diagnoses include papillary endothelial hyperplasia, angiomatoid polyp, bacillary angiomatosis, glomangiopericytoma, Kaposi's sarcoma, and angiosarcoma.

Treatment and prognosis After complete excision, recurrences of LCH are rare.

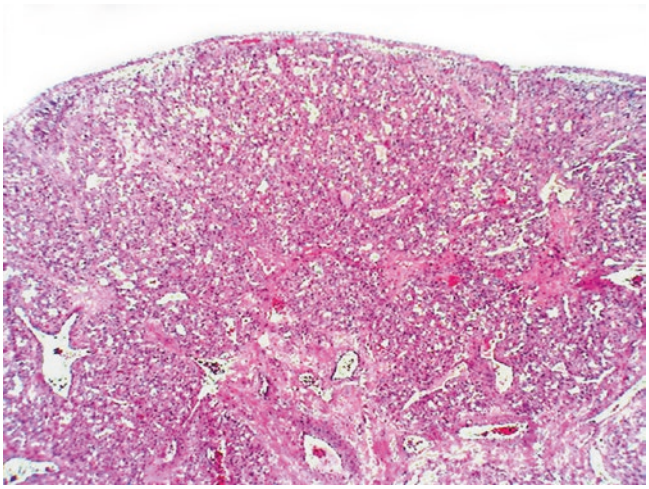

Fig. 2.34 Lobular capillary hemangioma: lobular arrangements of blood-filled capillaries separated by loose connective tissue. The blood supply is provided by a feeder vessel at the base of the lesion

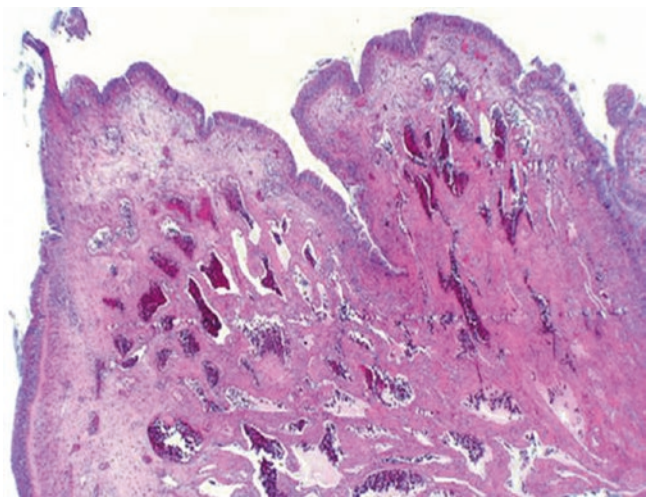

Fig. 2.35 Cavernous hemangioma: multiple, thin-walled, markedly dilated blood vessels separated by fibrous stroma

2.9.1.2 Cavernous Hemangioma

Definition Cavernous hemangiomas are neoplastic proliferations of thin-walled blood vessels with marked luminal dilatation.

Epidemiology Cavernous hemangiomas of the sinonasal tract are commonly intraosseous or involve the turbinates or the lateral nasal wall. They occur mainly in men in the fifth decade of life [163].

Microscopy As elsewhere in the body, they are composed of multiple, large thin-walled, dilated blood vessels separated by fibrous stroma (Fig. 2.35).

Differential diagnosis Cavernous hemangioma of the sinonasal tract has to be distinguished from venous hemangioma, a rare vascular tumor in this location being composed of thick-walled veins with abundant smooth muscle. Other differential diagnoses include sporadic telangiectasia, hereditary telangiectasia (Osler-Weber-Rendu syndrome), vascular malformations, angiomatoid polyps, and papillary endothelial hyperplasia.

Treatment and prognosis Complete removal is the treatment of choice whenever possible. Recurrences occur after incomplete resection.

2.9.2 Fibroma and Fibrous Histiocytoma

2.9.2.1 Fibroma

Definition Sinonasal fibroma is a benign nodular proliferation composed of fibroblasts and collagen.

Epidemiology and pathogenesis Sinonasal fibromas are uncommon lesions, mainly seen in the nasal cavity. Their distinction from reactive fibrosis may be controversial. A few true examples reported in the past [164] continue to be recognized as such nowadays [165].

Macroscopy Sinonasal fibromas are small nodules of polypoid configuration that may measure up to 1 cm.

Microscopy They consist of a proliferation of fibroblastic spindle cells intermingled with bands of collagen. Cytoplasms are inconspicuous and nuclei are bland, although on occasions may depict slight pleomorphism. Mitoses are minimal or absent (Fig. 2.36).

Differential diagnosis Sinonasal fibromas must be distinguished from other benign sinonasal myofibroblastic proliferations. True sinonasal fibromas are only immunoreactive for vimentin.

Treatment and prognosis Complete removal is curative.

2.9.2.2 Fibrous Histiocytoma

Definition Benign fibrous histiocytoma (BFH) is a benign nodular proliferation composed of fibrohistiocytes and collagen.

Epidemiology Since the advent of immunohistochemistry, tumors typed as sinonasal BFHs have become an exceedingly rare entity.

Clinical features BFH presents as a yellow-tan nodule or polyp, most commonly causing nasal obstruction or bleeding [165].

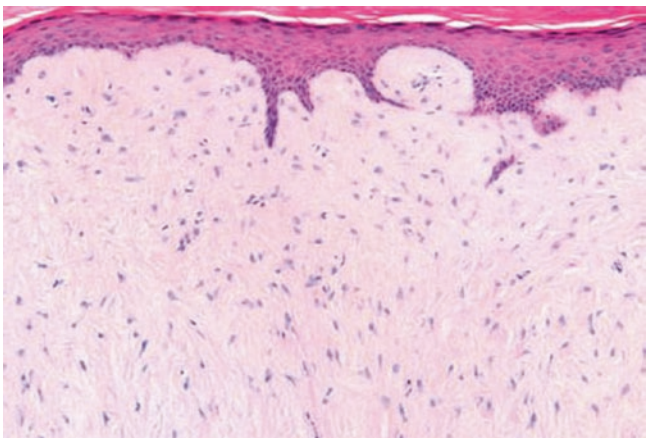

Fig. 2.36 Fibroma of the nasal vestibule: fibroblastic dermal proliferation covered by hyperplastic squamous epithelium

Microscopy BFH is composed of spindle-shaped cells arranged in a storiform pattern admixed with histiocytic cells and multinucleated giant cells.

Differential diagnosis The distinction from other benign sinonasal spindle cell proliferations is largely based on the immunohistochemical findings. BFH is immunoreactive for vimentin and for markers of macrophages such as CD68.

Treatment and prognosis Benign fibrous histiocytoma may recur if incompletely excised.

2.9.3 Leiomyoma and Myofibroma

2.9.3.1 Leiomyoma

Definition Leiomyoma is a benign nodular proliferation composed of smooth muscle cells.

Epidemiology Sinonasal leiomyomas are rare tumors. They occur in adults and preferentially involve the nasal cavities [166].

Clinical features Nonspecific symptoms of nasal obstruction [166]

Microscopy Their morphologic and immunohistochemical profiles are identical to those of leiomyomas of other sites. An origin from blood vessel walls has been postulated. Leiomyomas usually express smooth muscle actin, muscle-specific actin, and desmin.

Differential diagnosis The distinction of leiomyoma from myofibroma is mainly based on immunohistochemical features and on the presence in the latter of a hemangiopericytoma-like vascular network, which is lacking in the former (see also Sect. 2.9.3.2). The distinction of leiomyoma from leiomyosarcoma is based on the absence of atypia and mitoses in the former.

Treatment and prognosis Complete removal of leiomyomas is curative. Huang and Antonescu have proposed to separate a category of smooth muscle tumors of uncertain malignant potential, characterized by the presence of 1–4 mitotic figures/10 high-power fields, which tend to pursue a more aggressive behavior than leiomyoma [167].

2.9.3.2 Myofibroma

Definition Myofibromas are solitary nodular proliferations composed of benign myofibroblasts. Myofibromatosis is the term for the presence of multiple myofibromas.

Epidemiology Most myofibromas are seen in young children. Sinonasal myofibromas are very rare [168].

Microscopy Myofibromas are made up of interlacing fascicles of plump spindle cells, with weakly eosinophilic cytoplasm and bland, round to oval nuclei. The cellular density may vary between the different areas. In the densely cellular areas, the blood vessels may show hemangiopericytoma-like features [169]. Myofibromas express smooth muscle actin and muscle-specific actin and are usually negative for desmin and other markers [170].

Differential diagnoses Sinonasal myofibromas must be differentiated from leiomyomas, as well as from glomangiopericytoma and low-grade myofibroblastic sarcoma.

Treatment and prognosis Complete excision of myofibromas is the recommended treatment. Incompletely removed tumors may recur.

2.9.4 Schwannoma, Neurofibroma, and Neurothekeoma

2.9.4.1 Schwannoma

Definition Schwannoma is a benign tumor, composed of differentiated, neoplastic Schwann cells [162].

Synonym Neurilemmoma

Epidemiology About 4 % of schwannomas of the head and neck region arise in the sinonasal tract [163]. Schwannomas of the sinonasal mucosa are usually not associated with type 2 neurofibromatosis.

Clinical aspects They usually present as polypoid lesions involving the nasal cavity and/or a paranasal sinus, with nonspecific symptoms of obstruction, compression, or extension in the surrounding structures [162].

Microscopy Histologically, the tumor is composed of elongated wavy-shaped monomorphic spindle cells, with eosinophilic cytoplasm and oval nucleus. Antoni type A and type B areas usually coexist within the lesion, and nuclear palisading may be present. Focal degenerative nuclear atypia has been described, while mitotic activity is absent to low. A consistently reported feature of sinonasal schwannomas is the lack of tumor encapsulation which determines an apparently infiltrative growth pattern. Immunohistochemically, sinonasal schwannoma is intensely reactive for S-100 protein and also for vimentin [171].

Differential diagnosis It includes neurofibroma and other spindle cell lesions of the sinonasal mucosa, like angiofibroma, solitary fibrous tumor, and leiomyoma. Particular care should be taken in evaluating cellular schwannomas with a predominance of Antoni type A areas, which should not be confused with malignant spindle cell neoplasms, like malignant peripheral nerve sheath tumor, fibrosarcoma, leiomyosarcoma, and spindle cell melanoma.

Treatment and prognosis Complete removal of sinonasal schwannomas is curative.

2.9.4.2 Neurofibroma

Definition Neurofibroma is a benign tumor of peripheral nerve sheath phenotype with mixed cellular components including Schwann cells, perineural hybrid cells, and intraneural fibroblasts [162].

Epidemiology Neurofibromas of the sinonasal mucosa are rare, usually solitary, and sporadic, not associated with multiple neurofibromatosis, type 1 (von Recklinghausen's disease).

Etiology and pathogenesis Experimental induction of peripheral nerve sheath tumors of the Gasserian ganglion and the orbital and maxillary regions has been achieved after prenatal and postnatal exposure to ethylnitrosourea [172].

Microscopy Neurofibromas appear as unencapsulated lesions composed of a mixture of Schwann cells and fibroblasts embedded in a predominately myxoid stroma. Residual neurites may be found at the center of the lesion [162, 173].

Differential diagnosis Due to the overlap of the histological features, it may be difficult to differentiate neurofibroma from schwannomas of the sinonasal mucosa. Neurofibroma should be distinguished also from myxoma, which is S-100 protein negative.

Treatment and prognosis Complete removal is the treatment of choice for solitary neurofibroma.

2.9.4.3 Neurothekeoma

Definition Neurothekeoma is a rare benign neoplastic proliferation derived from nerve sheaths and arranged in lobules separated by fibrous septa.

Epidemiology The tumor may be seen anywhere in the body. One neurothekeoma of the paranasal sinuses has been reported in a 3-year-old boy [174].

Microscopy A syncytium of spindle and epithelioid-like cells often admixed with osteoclastoid cells and occasional myxomatous areas appears surrounded by fibrous septations that confer the lobular pattern. Tumor cells are usually positive for vimentin and glial fibrillary acidic protein, while reactivity for S-100 protein is variable. Cytokeratin markers are constantly negative.

Treatment and prognosis Complete resection is curative.

2.9.5 Meningioma

Definition Meningioma is a tumor derived from meningothelial cells.

Epidemiology Meningiomas of the sinonasal tract may extend directly from the central nervous system or arise from ectopic extracranial tissue. Although rare, they are more commonly seen in the orbit, ear, and skin of the head and neck than in the sinonasal tract. Sinonasal meningiomas tend to occur in younger patients than intracranial meningiomas [162, 175].

Microscopy Histologically, they are similar to meningiomas elsewhere, being the meningothelial type the most frequent. Aggressive variants of meningioma may be seen mainly within the group of primary intracranial sinonasal meningiomas.

Treatment and prognosis Surgical removal with margins free of tumor is mandatory. Extracraneal sinonasal meningiomas have usually an excellent prognosis. Primary intracranial sinonasal meningiomas require aggressive surgery [176].

2.9.6 Juvenile Angiofibroma

Definition Juvenile angiofibroma (JAF) is a benign and richly vascularized fibrous neoplasm that arises in the posterior nasal cavity and neighboring nasopharynx in young males [177].

Synonym Nasopharyngeal angiofibroma

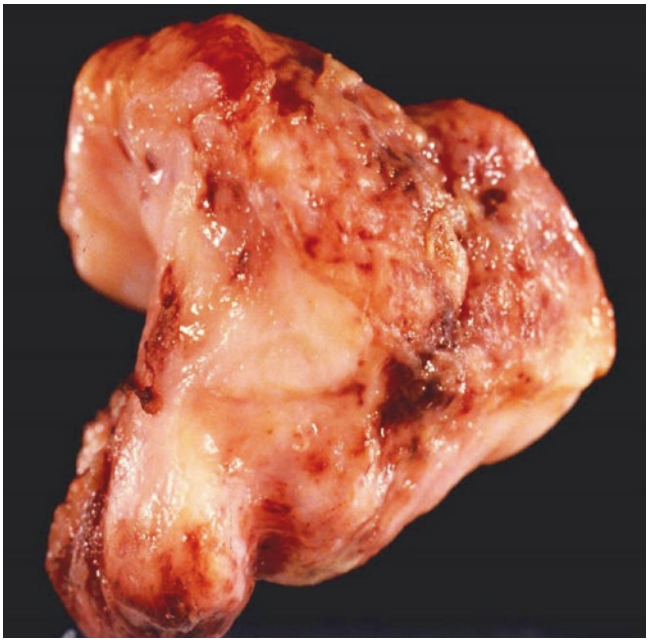

Fig. 2.37 Juvenile angiofibroma: well-demarcated polypoid formation with white-red cut surface and a lobulated contour

Epidemiology JAF arises in the confluence of the posterolateral nasal wall and the lateral nasopharynx and occurs nearly always in young males [177, 178]. Although JAFs almost invariably arise in the nasopharynx and often extend secondarily to the sinonasal region, about 1.5 % of angiofibromas involve the nasal cavity alone [179].

Clinical aspects Although benign JAF has a tendency to recur and is locally destructive, causing pressure necrosis of adjacent soft tissue and bone, it may occasionally extend into paranasal sinuses, into the orbit, and intracranially.

Macroscopy JAFs are sessile or polypoid lobulated formations of rubbery consistency, well demarcated but devoid of a capsule (Fig. 2.37). The cut surface is whitish and a rich vascularization is not always apparent.

Microscopy JAFs are composed of vascular and fibrous elements in varying proportions. The vessels in the superficial portions of the tumor are mainly gaping capillaries which may become compressed with increasing stromal fibrosis. Thick-walled vessels without elastic membranes and with irregular, incomplete, or absent muscle coats and focal intimal thickenings are usually present in the deeper portions of the tumor. These vessels resemble those normally seen in the submucosa of the nasal conchae. The vascular elements are embedded in fibrous tissue which varies in cellularity and collagenization. Stellate fibroblast-like cells are often present close to the blood vessels. The tumor cells express vimentin and the vascular endothelial cells CD31 and CD34. Pericytes

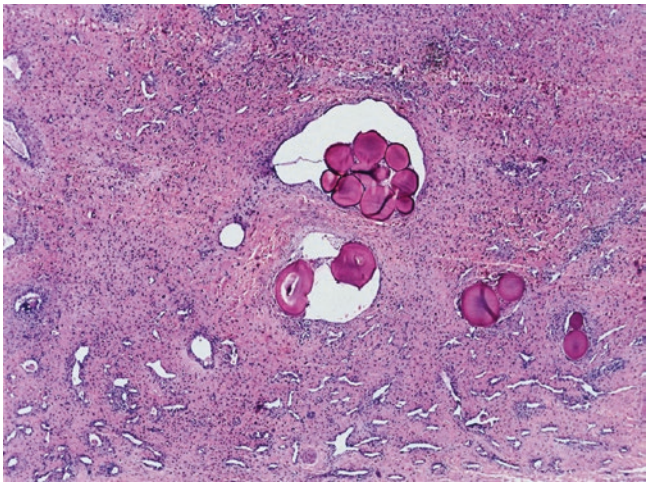

Fig. 2.38 Juvenile angiofibroma: blood vessels embedded in fibrous tissue showing intravascular microembolization, a therapeutic procedure before surgery

that surround parts of small blood vessels stain for smooth muscle actin. The nuclei of fibroblastic cells of juvenile angiofibroma are strongly positive for testosterone receptors [180]. Ultrastructurally the nuclei of angiofibroma contain characteristic dense granules [181]. Occasionally, the fibroblasts may exhibit cytologic atypia, and some of these cells may be multinucleated, but mitosis are rare. Mast cells may be numerous. There may be focal thrombosis, hemorrhage, and chronic inflammatory reaction. With the advent of preoperative selective embolization, iatrogenic emboli may be encountered in resected specimens (Fig. 2.38) [182]. For further reading on this tumor, see Chap. 6.

2.10 Borderline Soft Tissue Neoplasms

2.10.1 Glomangiopericytoma

Definition Glomangiopericytoma (GPC) is a sinonasal tumor with perivascular myoid phenotype, showing features of glomus and pericytes [183, 184]. It is characterized by the proliferation of oval, polyhedral, or spindle-shaped cells arranged about vascular channels provided with a single layer of endothelial cells [185, 186].

Synonyms Hemangiopericytoma-like tumor, sinonasal hemangiopericytoma, and sinonasal glomus tumor

Epidemiology GPCs arise in the nasal cavity as well as in the paranasal sinuses. They show a slight predilection for females. Most of them develop in the seventh decade of life.

Clinical features Nasal obstruction, epistaxis, and difficulty of breathing are usual presenting symptoms. A rare association with osteomalacia has been recently reported [187, 188].

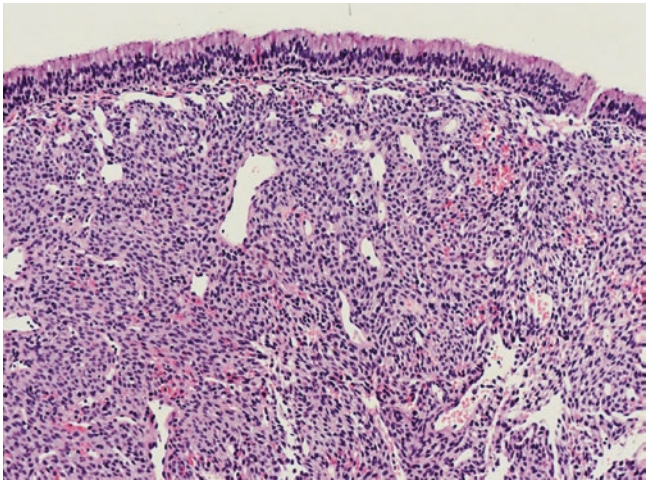

Fig. 2.39 Glomangiopericytoma: rich interconnection of thin-walled blood vessels surrounded by uniform spindle-shaped cells with oval or elongated bland nuclei and pale cytoplasm

Macroscopy GPCs are usually polypoid measuring in average 3 cm and may reach up to 8 cm in size [189].

Microscopy GPC contains numerous thin-walled blood vessels that often may adopt a staghorn configuration and on occasions are surrounded by prominent hyalinization. The tumor cells, typically arranged around the blood vessels, are of uniform size with regular oval or elongated nuclei and pale cytoplasm; mitoses are very rare and without atypia (Fig. 2.39). The cells may also be arranged in short haphazard fascicles or in sheets of closely packed cells containing compressed capillaries. Areas of poor cellularity, myxoid change, and fibrosis are not uncommon. The tumor cells are enmeshed by collagen type IV fibers and entirely situated outside the capillaries which are lined by a single-layer of normal-looking endothelium.

Immunohistochemistry GPCs show diffuse reactivity for actins, factor XIIIA, and vimentin, lacking diffuse staining for other markers. GPC vessels stain for muscle-specific actin [184].

Differential diagnosis It includes lobular capillary hemangioma, solitary fibrous tumor, leiomyoma, myofibroblastic low-grade sarcoma, synovial sarcoma, and leiomyosarcoma [184]. In GPC the tumor cells are enmeshed by collagen type IV fibers; this feature, well shown by reticulin stain or by anti-collagen IV antibodies, helps to distinguish the tumor from angiosarcoma [184]. In due clinical settings, other differential diagnoses to consider are Kaposi's sarcoma and phosphaturic mesenchymal tumor.

Treatment and prognosis Complete surgical removal is the recommended treatment for GPCs achieving an overall 5-year survival of about 90 %; recurrence may occur many years after initial surgery and may rarely metastasize [184, 189]. Aggressive GPCs (malignant glomangiopericytomas) are very uncommon and often show size larger than 5 cm, bone invasion, nuclear atypia, necrosis, increased mitotic number, and proliferation index higher than 10 % [184, 189, 190].

2.10.2 Desmoid-Type Fibromatosis

Definition Desmoid-type fibromatosis (DTF) is a nonmetastasizing unencapsulated myofibroblastic proliferation that has a tendency for local invasion and recurrence.

Synonyms Desmoid tumor, extra-abdominal fibromatosis, and aggressive fibromatosis

Epidemiology DTF rarely arises in the sinonasal tract [164, 191].

Microscopy DTF is composed of interlacing fascicles of bland spindle-shaped myofibroblasts, in a collagenous background of parallel-running fibers. Focal myxoid areas may be found. Immunohistochemistry: Actins and vimentin are positive while desmin only occasionally. Beta-catenin presents intranuclear localization [192].

Differential diagnosis DTF must be distinguished from fibrosarcoma, solitary fibrous tumor, fibroma, and reactive types of fibrosis.

Treatment and prognosis Complete surgical removal is the treatment of choice; positive or close (<1 mm) resection margins are predictive of recurrences [193]. DTFs of the sinonasal tract tend to have lower recurrence rates than those arising in other locations [164].

2.10.3 Solitary Fibrous Tumor

Definition Solitary fibrous tumor (SFT) of the nose and paranasal sinuses is a fibroblastic proliferation with variable cellularity and vascularity having features identical to those of SFT of the pleura [194–196].

Epidemiology The sinonasal region is the second most common location for SFTs of the upper aerodigestive tract, only preceded by the oral cavity. SFTs mainly develop in adults, with slight predominance in women [194–198].

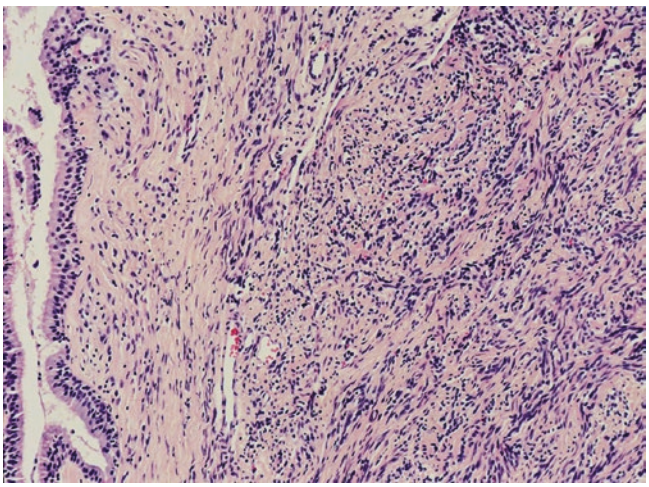

Fig. 2.40 Solitary fibrous tumor: fibroblastic proliferation, collagen production, and variably dilated blood vessels. Identical features to the pleural counterpart

Clinical features Nasal obstruction is the most common presenting symptom.

Macroscopy Sinonasal SFTs are polypoid to nodular formations of firm consistency that usually measure several centimeters [199].

Microscopy SFTs are characterized by a disorderly proliferation of small spindle cells with bland nuclei and inconspicuous cytoplasms that produce abundant amounts of collagen (Fig. 2.40). The proportion of cells and collagen may vary considerably between the different areas. Mitoses are quite uncommon; growth is slow and expansile. Usually vascularization is prominent with blood vessels forming thick collagenized walls; in other areas hemangiopericytoma-like vessels may be found. Mucosal ulceration, necrosis, and invasion are usually absent. Nevertheless, recent examples of malignant SFTs of the upper aerodigestive tract have been reported [200].

Immunohistochemistry SFTs are immunoreactive for CD34, BCL2, CD99, and vimentin [195].

Differential diagnosis The main differential diagnoses of SFT are sinonasal glomangiopericytoma and juvenile angiofibroma. Also, other benign and malignant spindle cell tumors must be distinguished from SFT.

Treatment and prognosis Fully excised SFTs with free margins do not recur. This is the case for sinonasal polypoid SFTs. Broad-based SFTs are difficult to remove and prone to recur. Progression of recurrences is in most cases very slow [199]. The very rare examples of malignant SFT behave aggressively [200].

2.11 Malignant Neoplasms

Malignant sinonasal tumors comprise less than 1 % of all cancers seen in humans and represent about 3 % of all malignancies of the head and neck region [201, 202]. Despite the low rate of malignancy arising in the sinonasal tract, a great variety of histological types of tumors may be found [121, 122]. The advent of electron microscopy and the more recent advances in immunohistochemistry, and in gene technologies, have further refined the criteria for their correct recognition [203], which recent comprehensive publications have made available worldwide [204, 205].

Geographical differences in the relative frequency of certain histological types of malignant sinonasal tumors may be related to variations in the exposure to carcinogens [206]. In Table 2.3, the histological types of malignant sinonasal tumors collected at the Hospital Clinic of the University of Barcelona from 1976 to 2005 are presented in decreasing order of frequency. Keratinizing squamous cell carcinoma, undifferentiated carcinoma, non-keratinizing squamous cell carcinoma, malignant lymphoma, malignant mucosal melanoma, intestinal-type adenocarcinoma, adenoid cystic carcinoma, low-grade adenocarcinomas, and olfactory neuroblastoma are the most frequent histological types. Most common sinonasal carcinogens in humans are cigarette smoking, high-risk HPV, radiation therapy, nickel, chromates, wood dust, boot and shoe dusts, and isopropyl alcohol.

A practical way to start typing malignant sinonasal tumors is to separate them into large and small cell categories. Among the large cell malignant tumors, the most common types are squamous cell carcinoma, non-keratinizing squamous cell carcinoma, malignant mucosal melanoma, intestinal-type adenocarcinoma, and low-grade adenocarcinomas. Sinononasal undifferentiated carcinoma, malignant lymphoma, adenoid cystic carcinoma, and olfactory neuroblastoma are among the most common small cell tumors. Large cell tumors account for approximately 75 % of the malignant sinonasal tumors and the small cell tumors for the remaining 25 %.

For staging of malignant sinonasal tumors, Ref. [207] is recommended, as well as Tables 13 and 14 in Chap. 17.

2.11.1 Keratinizing Squamous Cell Carcinoma

Definition Keratinizing squamous cell carcinoma (KSCC) is a malignant epithelial neoplasm originating from the mucosal epithelium of the nasal cavities or paranasal sinuses with histological evidence of squamous differentiation and keratin production.

Table 2.3 Malignant sinonasal tumors at the Hospital Clínic, University Barcelona Medical School

Histological	Frequency		Men		Women		Mean	Age
Type of tumor	Nr %		Nr %		Nr %		Age	Range
Keratinizing SCC	54	27	38	70	16	30	64	39–87
Undifferentiated carcinoma	26	13	19	73	7	27	60	41–87
Non-keratinizing SCC	19	9.5	15	79	4	21	59	26–84
Malignant lymphoma	19	9.5	15	79	4	21	59	9–89
Malignant melanoma	14	7	7	50	7	50	69	56–89
High-grade adenocarcinoma	13	7	10	77	3	23	59	16–81
Adenoid cystic carcinoma	11	5	7	64	4	36	58	22–69
Low-grade adenocarcinoma	10	5	4	40	6	60	64	28–92
Olfactory neuroblastoma	7	3	3	43	4	57	36	2–67
Mucoepidermoid carcinoma	4	2	3	75	1	25	55	50–61
Malignant fibrous histiocytoma	4	2	3	75	1	25	56	35–65
Plasmacytoma	4	2	3	75	1	25	51	50–65
Rhabdomyosarcoma	4	2	2	50	2	50	30	8–51
Malignant schwannoma	3	1.5	1	33	2	67	57	27–70
Adenosquamous carcinoma	2	1	2	100	–	–	66	61–71
Myoepithelial carcinoma	2	1	2	100	–	–	47	29–66
Kaposi's sarcoma	2	1	2	100	–	–	37	34–40
Teratocarcinosarcoma	1	0.5	1	100	–	–	76	–
Ewing's sarcoma (PNET)	1	0.5	–	–	1	100	23	–
Total	200	100	137	69	63	31	58	2–92

Synonyms Conventional squamous cell carcinoma and squamous cell carcinoma NOS

Epidemiology At the nasal vestibule, KSCC is the most common malignancy [208–210]. Due to early recognition and easy access to treatment, they usually have more favorable prognosis than their counterpart of the sinonasal region.

Sinonasal KSCC comprises up to 45–50 % of the malignant tumors of this region in several series [211, 212]. They predominate in males and the great majority are seen in patients aged over 50 years. The maxillary antrum, the lateral nasal wall, and the ethmoid sinuses are the most common sites (Fig. 2.41) [213]. Other locations such as the nasal septum and the nasal floor are less usual; the frontal and sphenoid sinuses are rarely involved. These tumors grow by local extension, infiltrating the neighboring structures, but lymph node metastases are rare [214].

Etiology and pathogenesis The occupational epidemiology of KSCC has been strongly related to exposure to nickel [215–218] and to a lesser extent to chromium, isopropyl alcohol, and radium [219]. As in other territories of the respiratory tract, a definite association between sinonasal KSCC and cigarette smoking has been documented [220, 221]. Chronic sinonasal inflammation is considered as a predisposing factor. A case of carcinoma of the maxillary antrum after thorotrast exposure has been reported [222].

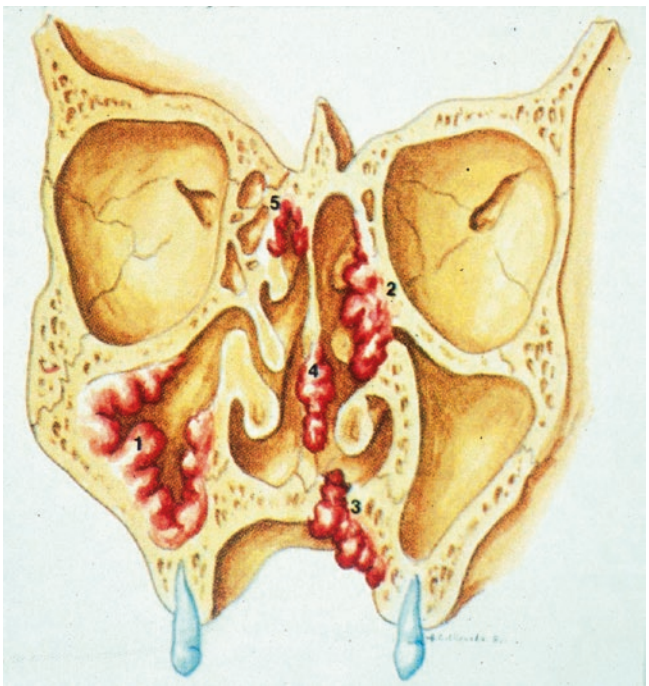

Fig. 2.41 Main locations of sinonasal malignant tumors: *1* maxillary sinus, *2* ethmoid and lateral wall, *3* nasopalatine septum, *4* nasal septum, and *5* roof of the nasal cavity (Courtesy of Prof. J Traserra, Barcelona, Spain. Ref: [213])

Nitrosamines and to a lesser extent formaldehyde are strong nasal carcinogens in laboratory rodents [223, 224].

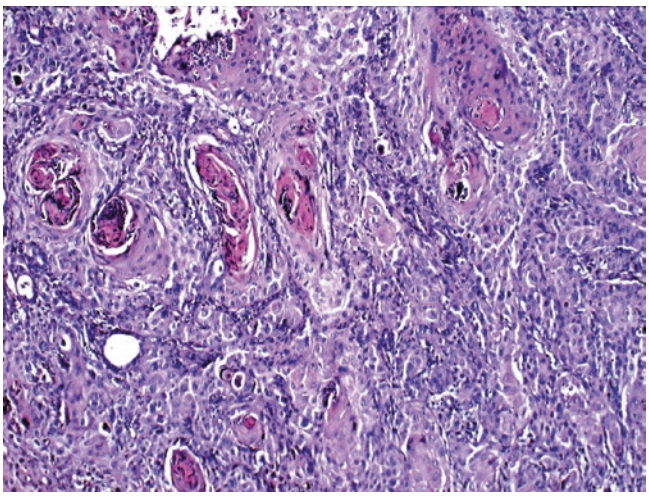

Fig. 2.42 Keratinizing squamous cell carcinoma: proliferation of malignant squamous cells with keratin pearls

Macroscopy Sinonasal KSCCs display gross features similar to those of other upper aerodigestive tract territories.

Microscopy KSCCs originate in the respiratory sinonasal mucosa from areas of preexisting squamous metaplasia and manifest the same range of histological appearances as those arising in other sites. They are characterized by the proliferation of malignant squamous epithelial cells with keratin production and intercellular bridges (Fig. 2.42). Malignancy is graded according to the degree of differentiation, cellular pleomorphism, and mitotic activity. They are divided into well-differentiated, moderately differentiated, and poorly differentiated forms. Most KSCC of the sinonasal tract present as moderately or poorly differentiated tumors. Special types of squamous cell carcinoma (SCC), such as verrucous carcinoma [225], spindle cell carcinoma [226, 227], basaloid squamous cell carcinoma [228, 229], and adenosquamous carcinoma [230, 231], are occasionally found in the sinonasal tract.

Immunohistochemistry and in situ hybridization The prototypical KSCC is p53 positive, p16 negative, and high-risk HPV negative.

Differential diagnosis KSCC has to be mainly differentiated from NKSCC and from other special types of SCC. Well-differentiated carcinomas are uncommon in this territory and when encountered need to be differentiated from pseudoepitheliomatous types of hyperplasia and from verrucous carcinoma.

Treatment and prognosis Complete surgical resection combined with radiotherapy and optional chemotherapy is recommended [232, 233]. Regional lymph node involvement is seen in about 17 % of sinonasal squamous cell carcinomas and distant metastases in about 1.5 % [214]. For neoplasms circumscribed to the nasal cavity, the 5-year survival is slightly above 50 % [234], whereas in neoplasms of the maxillary antrum, the 5-year survival may be as low as 25 % [220].

2.11.2 Non-keratinizing Squamous Cell Carcinoma

Definition Non-keratinizing squamous cell carcinoma (NKSCC) is a malignant epithelial neoplasm originating from the mucosal epithelium of the nasal cavities or paranasal sinuses with squamous differentiation and lack of histological evidence of keratin production.

Synonyms Cylindrical cell carcinoma, transitional cell carcinoma, and Schneiderian carcinoma. The name cylindrical cell carcinoma was first coined by Ringertz in 1938 [235]; it was the preferred term in the 1991 WHO classification [121], until the 2005 WHO classification recommended non-keratinizing squamous cell carcinoma as the most appropriate term [202]

Etiology and pathogenesis NKSCC is etiopathogenetically related with HPV [236–238]. In SCC with biologically active HPV, inactivation of the Rb protein by the HPV E7 protein leads to p16 overexpression because Rb normally represses the transcription of p16. HPV-positive HNSCC also expresses the oncoprotein E6 that binds and degrades wild-type p53 protein [238]. Unlike carcinomas of the uterine cervix, where HPV infection and *TP53* mutations are mutually exclusive events, HPV infection and *TP53* overexpression sometimes occur together in HNSCC, but disruptive *TP53* gene mutations are not encountered in HPV-positive carcinomas [239, 240].

Epidemiology NKSCC affects males more often than women, at younger ages than keratinizing carcinoma, between 40 and 60 years of age, in patients that usually do not drink alcohol nor smoke tobacco [241, 242]. Twenty percent of sinonasal SCC is HPV positive and shows non-keratinizing histological features [236–238]. Eighty-two percent of them were type 16, 12 % types 31/33, and 6 % type 18 [243].

Macroscopy The tumors grow in most cases as exophytic masses showing either corrugated or smooth surface. They may arise from the antrum, the lateral nasal wall, or the ethmoid, being the antrum the most frequent site. They may occur concomitantly with other nonneoplastic polypoid formations.

Microscopy Main histological features of NKSCC are the presence of islands and interlaced cords of squamous epithelial cells with the lack of maturation, absence of keratinization, and moderate to significant degree of atypia (Fig. 2.43).

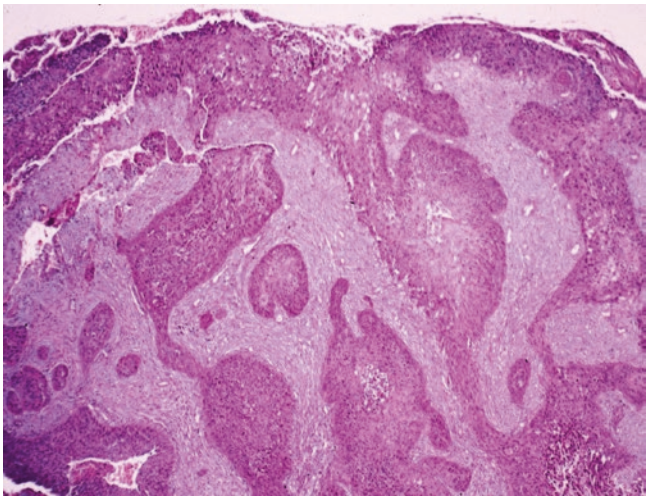

Fig. 2.43 Non-keratinizing squamous cell carcinoma: islands and interlaced cords of squamous epithelial cells with poor maturation, absence of keratinization, and moderate to significant atypia

The tumor invades into the underlying fibrosed tissue with an expanding, smooth and lobulated, generally well-delineated border, although foci of infiltration by irregular small nests or strands may be seen. NKSCC is usually moderately or poorly differentiated, being in the latter case difficult to recognize as SCC. Occasionally, some degree of keratinization may be seen. When keratinization is conspicuous, there may be microscopic overlap with KSCC [202]. Some of these tumors may also overlap with the papillary, basaloid SCC, adenosquamous [244], and lymphoepithelial-like types of SCC.

The papillary variant of NKSCC is composed of papillary fronds, thick ribbons, and polystratified masses of commonly cylindrical cells that give rise quite often to invaginations of the surface epithelium [245]. The tumor cells have a tendency to form palisade arrangements perpendicular to the underlying basement membrane (Fig. 2.44). The nuclei are atypical and show increased mitotic activity, as well as abnormal mitotic figures. The basement membrane remains in most cases conspicuous, despite stromal infiltration, which should not be regarded as carcinoma in situ. Recent studies have shown that not only the papillary type but most of the variants of head and neck SCC may be associated with high-risk HPV [244, 246–248].

Immunohistochemistry HPV-positive NKSCCs are immunohistochemically positive for p16 protein in a diffuse and intense fashion (Fig. 2.45). Positivity must be nuclear, although cytoplasmic positivity is also seen [236–238].

Molecular diagnosis HPV detection may be achieved through various techniques [203], such as DNA-PCR [236] or DNA-ISH (Fig. 2.46) [237], as well as mRNA-PCR [238] or mRNA-ISH assays [249]; the latter have gained increasing relevance as it is not the presence of the virus alone but its transcriptional activity what confers pathogenicity. For a more detailed description of these methods, the reader is referred to Chap. 6.

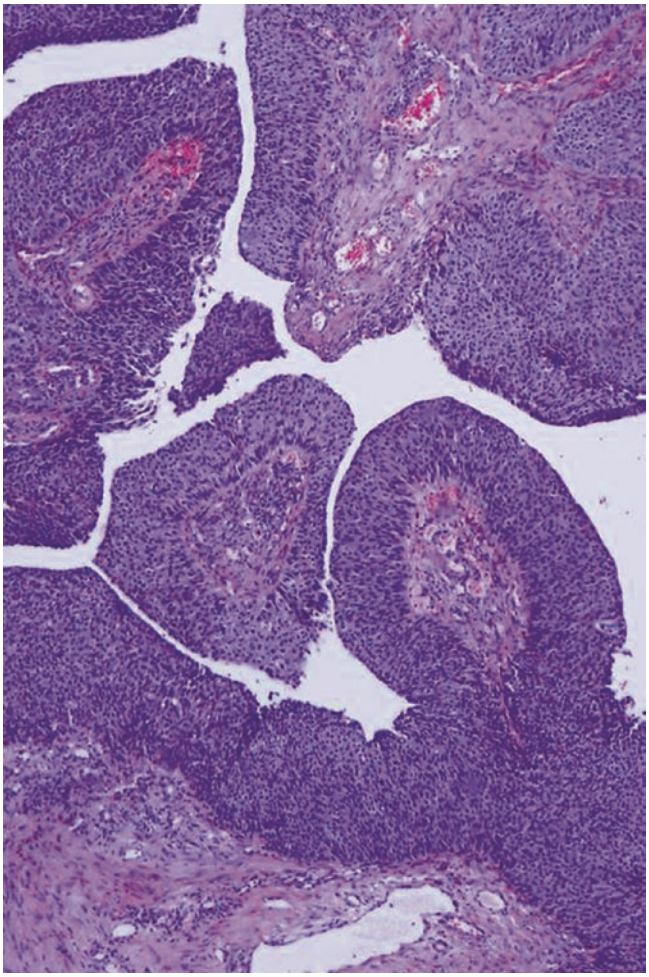

Fig. 2.44 Papillary non-keratinizing squamous cell carcinoma: papillary fronds usually composed of cylindrical cells, with tendency to form palisade arrangements perpendicular to the basement membrane

Differential diagnosis In addition to rule out KSCC and the different sinonasal SCCs that are negative for HPV, it includes the Schneiderian papillomas of the inverted and oncocytic types, especially when they have concomitant carcinomatous changes. Both types of papilloma lack the atypical cellularity constantly seen in NKSCC. When Schneiderian papillomas coexist with NKSCC, or with other types of carcinoma, the two components appear usually demarcated one from the other although in contiguity. When the invaginating crypts of an inverted papilloma are filled with the cords and ribbons of a keratinizing or non-keratinizing SCC, the lesion represents a conventional squamous cell carcinoma arising in an inverted papilloma, which implies a worse prognosis than that of NKSCC.

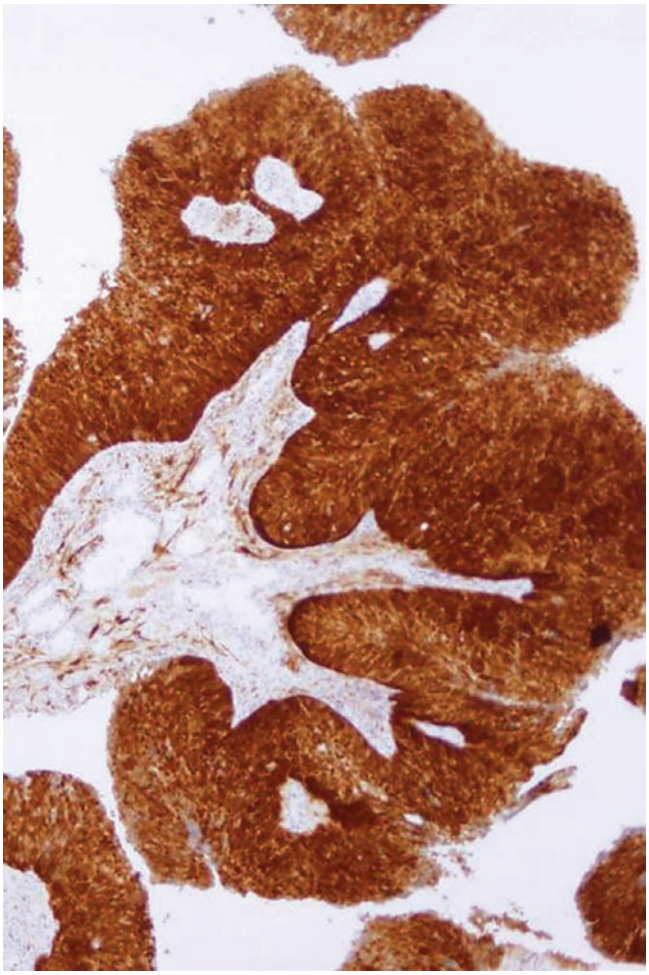

Fig. 2.45 Exophytic non-keratinizing squamous cell carcinoma: diffuse and intense positive immune reaction for p16 protein

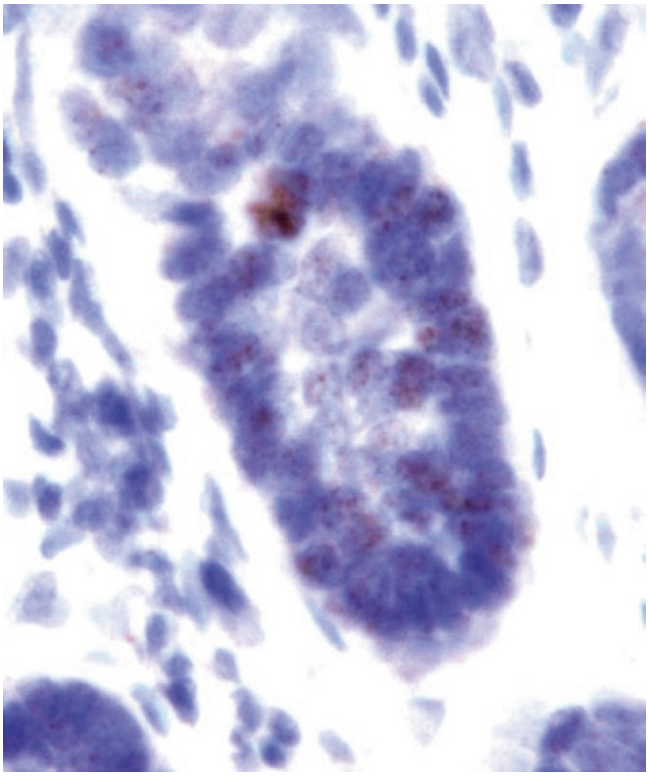

Fig. 2.46 Non-keratinizing squamous cell carcinoma: intranuclear detection of HPV 16 DNA by in situ hybridization

An unusual variant of HPV-related carcinoma with adenoid cystic-like features arising in the sinonasal tract was recently described because of immunohistochemical p16 protein expression. It was characterized by a nested growth with a prominent basaloid component showing myoepithelial differentiation, microcystic spaces, and even ductal structures. Evidence of squamous differentiation, when present, was restricted to the surface epithelium [243]; more experience with this type of tumor may be needed to clearly separate it from the HPV-related basaloid SCC. A unique example of low-grade papillary Schneiderian carcinoma has been very recently reported [250].

Treatment and prognosis NKSCC behaves as locally aggressive tumor, and the recommended treatment is complete surgical excision followed by radiotherapy. NKSCC bears a better prognosis than conventional squamous cell carcinomas [236, 237, 251]. The mechanisms underlying this favorable outcome may involve the combined effects of immune surveillance to viral-specific tumor antigens, an intact apoptotic response to radiation, and absence of widespread genetic alterations associated with smoking [252–255].

2.11.3 Sinonasal Undifferentiated Carcinoma

Definition Sinonasal undifferentiated carcinoma (SNUC) is a high-grade malignant epithelial neoplasm of the nasal cavity and paranasal sinuses, composed of small- to medium-sized cells, lacking evidence of squamous or glandular differentiation, as well as of rosette formation [256–258].

Epidemiology etiology, and pathogenesis SNUC occurs in both sexes over a wide age range, with a median in the sixth decade of life. Cigarette smoking [257] and nickel exposure [218] have been associated with SNUC. Epstein-Barr virus (EBV) and the deletion of the retinoblastoma gene have been ruled out as factors involved in the development of this tumor (Table 2.4). Ionizing radiation is another etiologic factor, for radiotherapy either for retinoblastoma or for nasopharyngeal carcinoma has been associated with SNUC [259]. High-risk HPV has been recently related to SNUC [260].

Clinical aspects The most common symptoms are nonspecific and include nasal obstruction, proptosis, cranial nerve

Table 2.4 Immunohistochemical and molecular features of SNUC

CK	++
EMA	+
NSE	–
Synaptophysin	–
Chromogranin	–
S-100 protein	–
HR-HPV	+/–
EBV	–
del 13q14	–
Other markers	–

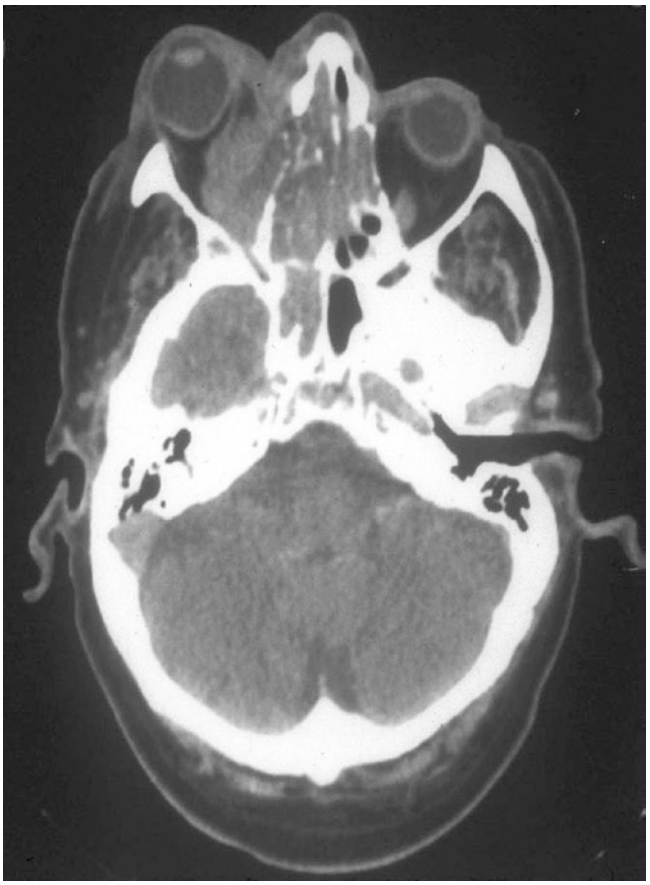

Fig. 2.47 Sinonasal undifferentiated carcinoma: CT scan demonstrating bilateral occupation of the nasal cavity, perforation of the nasal septum, extensive involvement of paranasal sinuses, including left sphenoid and also the left orbit (Courtesy of Prof. J. Traserra, Barcelona, Spain)

palsies, periorbital swelling, diplopia, epistaxis, and periorbital pain [257]. Most tumors present in advanced stage with involvement of multiple paranasal sinuses and invasion of adjacent structures, including the orbit, the cranial cavity and the nasopharynx (Fig. 2.47).

Macroscopy The tumors are often extensive lesions.

Microscopy SNUC is composed of small- to medium-sized, undifferentiated cells, which arise via dysplastic changes from the basal cells of the surface epithelium. The cells are polygonal with distinct borders, showing round to oval, hyperchromatic or vesicular nuclei, with either inconspicuous or slightly prominent nucleoli, surrounded by moderate amount of either amphophilic or eosinophilic cytoplasm. Mitotic figures are common (Fig. 2.48). The tumor forms nests, cords, and sheets of cells that show frequent areas of central necrosis and tendency to vascular and perineural invasion (Fig. 2.49).

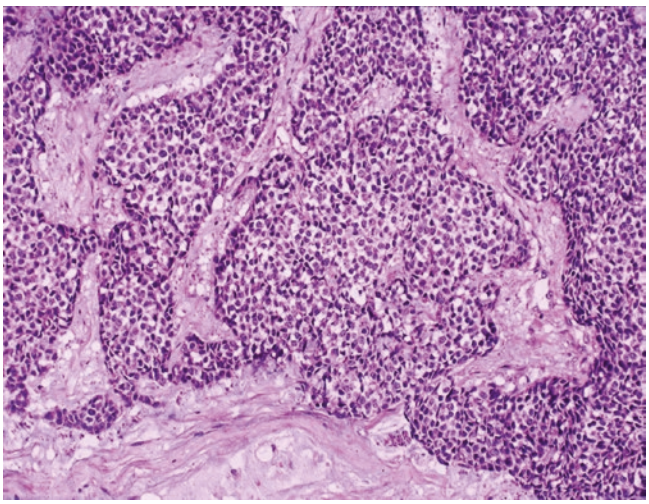

Fig. 2.48 Sinonasal undifferentiated carcinoma: interconnected nests of small- to medium-sized, polygonal undifferentiated epithelial cells with distinct borders. They show round to oval, hyperchromatic, or vesicular nuclei, surrounded by moderate amount of cytoplasm

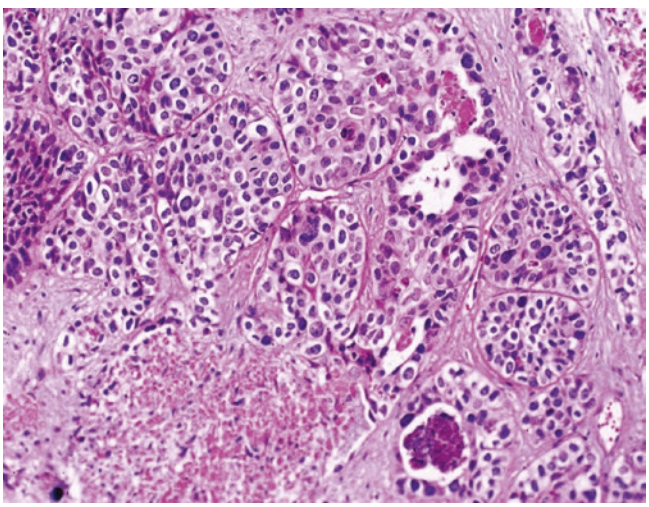

Fig. 2.49 Sinonasal undifferentiated carcinoma: nests of small to intermediate epithelial cells showing pleomorphic atypical nuclei, with either inconspicuous or slightly prominent nucleoli, mitosis, and areas of necrosis

Immunohistochemistry SNUCs are immunoreactive with epithelial markers, such as simple epithelium-type cytokeratins (Fig. 2.50) [261] and epithelial membrane antigen (EMA). Synaptophysin, chromogranin, and other neuroendocrine markers are negative [262]. In a recent report, up to 47 % of SNUCs have been found positive for high-risk HPV [260]. EBV is negative [256, 259] (Table 2.4).

Electron microscopy Ultrastructural studies demonstrate poorly formed desmosomes in quite a number of cells, while the presence of tiny bundles of tonofilaments is very rare. Neurosecretory granules are either absent or very rarely found (Fig. 2.51).

Genetics Few cases of SNUC have been examined cytogenetically, and they showed a complex karyotype [263]. Activating genomic mutations of clinically relevant genes, including *AKT*, *BRAF*, *CDK4*, *CTNB1*, *EGFR*, *FBXW7*, *JAK2*, *c-KIT*, *KRAS*, *PDGFR*, *PI3K*, and *VEGF*, have not been detected [264, 265].

Differential diagnosis The three main differential diagnoses of SNUC are small cell neuroendocrine carcinoma (SCNC), large cell neuroendocrine carcinoma (LCNC), and high-grade olfactory neuroblastoma (ONB). All four entities may share some overlapping clinical and light microscopic features. However, SNUC, SCNC, and LCNC show a marked immunoreactivity for cytokeratins that is not seen in ONB, and on the other hand, SNUC lacks the marked neuroendocrine immunoreactivity seen in SCNC, LCNC, and ONB (Table 2.5). Most lesions categorized in the past as grade IV ONB are now considered to be either SNUC or SCNC. This is important because SNUC, SCNC, and LCNC have worse prognosis than ONB. The recently described NUT carcinoma can be separated from SNUC based on the presence of *NUTM1* gene rearrangement or NUT immunohistochemical positivity. Notably, in the past, NUT carcinomas arising in the sinonasal tract may have been diagnosed as SNUC [266].

In addition, SNUC needs to be distinguished from other primary sinonasal tumors, such as solid adenoid cystic carcinoma, microcytic malignant melanoma, NKSCC, primary sinonasal nasopharyngeal-type undifferentiated carcinoma, lymphoma, and others.

Treatment and prognosis SNUCs are very aggressive tumors, with frequent local recurrence and spread to lymph node and distant sites. In most instances, the tumor is so large and the infiltration is so extensive that complete surgical resection cannot be achieved. Neck involvement in advanced local disease is considered a poor prognostic sign [267]. Combined treatments, including surgery and adjuvant radiotherapy, or surgery, radiotherapy, and chemotherapy,

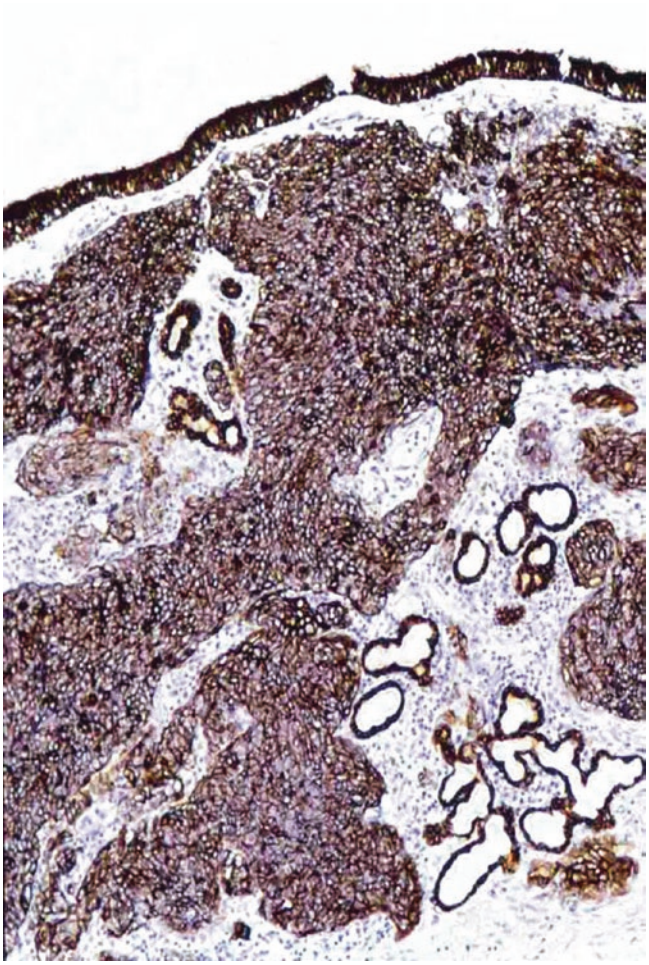

Fig. 2.50 Sinonasal undifferentiated carcinoma: strong immune reaction of the tumor cells with the marker of low molecular weight cytokeratins CAM 5.2

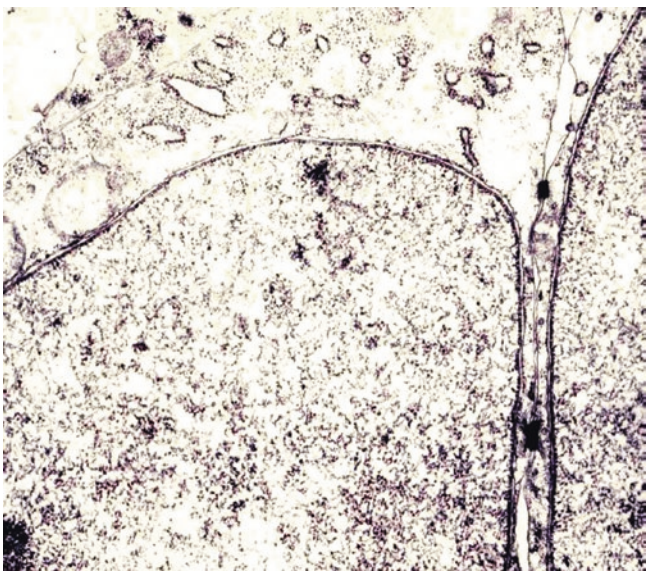

Fig. 2.51 Sinonasal undifferentiated carcinoma: ultrastructurally, poorly formed desmosomes are seen joining the cells (Courtesy of Prof. J.A. Bombi, Barcelona, Spain)

Table 2.5 Summary of the immunohistochemical and molecular features of selected sinonasal round cell tumors

Entity	CK	SYN	CHR	CD56	S100	HMB45	CD45	CD99	Desmin	P63	Calretinin	EBV	Molecular diagnostics
Sinonasal undifferentiated carcinoma	7+, 8 + 5/6–, 13–	– (focal +)	– (focal +)	–	–	–	–	–	–	Rarely +	–	–	–
Nasopharyngeal-type undifferentiated carcinoma	Pan +, 5/6 +, 13 +	–	–	–	–	–	–	–	–	+	–	+	–
Neuroendocrine carcinoma	Pan +, 5/6 –	+	+	+	–	–	–	–	–	–	–	–	–
Basaloid squamous cell carcinoma	Pan+, 5/6+	–	–	–	–	–	–	–	–	+	–	–	–
NUT carcinoma	Pan+, 7+	–	–	–	–	–	–	–	–	+	ND	–	t(15;19)
Ectopic pituitary adenoma	Pan+	+	+	+	–	–	–	+	–	–	+	–	–
Olfactory neuroblastoma	–	+	+	+	Sustentacular cells	–	–	–	–	–	+	–	–
Melanoma	– (rarely +)	– (rarely +)	– (rarely +)	– (rarely +)	+	+	–	–	– (rarely +)	–	– (rarely +)	–	–
Lymphoma	–	–	–	+ in NK/T cell	–	–	+	–[a]	–	–	–	+ in NK/T cell	–
Rhadomyosarcoma	– (rarely +)	– (rarely +)	–	– (rarely +)	–	–	–	– (rarely +)	+	–	ND	–	t(2;13) alveolar
Ewing's sarcoma	– (rarely +)	– (focal +)	–	– (focal +)	– (focal +)	–	–		–	–	–	–	t(11;22)
Metastatic neuroblastoma	–	+	+	+	–	–	–	–	–	–	–	–	*MYCN*

CK cytokeratins, *SYN* synaptophysin, *CHR* chromogranin, *ND* not determined

[a]Lymphoblastic lymphoma and anaplastic large cell lymphomas are positive for CD99

seem to offer the best chance of cure compared with either modality alone [268, 269]. High-dose chemotherapy and autologous bone marrow transplantation have been considered as a form of treatment [270]. Prognosis of SNUC is dismal, with a median survival of 4 months to 1 year [257, 258]. In a recent meta-analysis of outcome, 26.3 % of patients were alive with no evidence of disease, 21.0 % were alive with disease, and 52.7 % were dead of disease [267].

2.11.4 Primary Sinonasal Nasopharyngeal-Type Undifferentiated Carcinoma

Definition A poorly differentiated squamous cell carcinoma or histologically undifferentiated carcinoma accompanied by a prominent reactive lymphoplasmacytic infiltrate, morphologically similar to nasopharyngeal carcinoma, that originates in the sinonasal tract.

Synonym Sinonasal lymphoepithelioma.

Although nasopharyngeal carcinoma (NPC) almost invariably arises in the nasopharynx [271], "bona fide" primary sinonasal nasopharyngeal-type undifferentiated carcinomas (PSNPC) have been recently reported [259]. Due to the undifferentiated appearance of cells in NPC and PSNPC, these tumors may be lumped together with SNUC if unaware of their differences [256, 259, 261]. SNUC does not arise in the nasopharynx, but bulky lesions may extend into this region. Also NPC may extend from the nasopharynx into the sinonasal region. The distinction between these tumors can generally be made on purely histological grounds, since SNUC lacks the lymphoplasmacytic cell infiltrate seen in most cases of NPC and PSNPC. Immunohistochemistry and in situ hybridization are of great help in difficult cases. All three, NPC, PSNPC, and SNUC, react positively for low molecular weight cytokeratins and EMA. In contrast, NPC and PSNPC are positive for EBV, whereas SNUC is negative. Until very recently, confusion of NPC and PSNPC with SNUC has led to the belief that some SNUCs were related to EBV. The sharp distinction of these entities is crucial because NPC and PSNPC have a better prognosis and are more responsive to radiation therapy than SNUC. HPV-related lymphoepithelial-like NKSCC is another entity to be distinguished from PSNPC.

2.11.5 Neuroendocrine Neoplasms

Sinonasal neuroendocrine carcinomas are rare neoplasms accounting for about 5 % of all tumors in this region. Like in the lungs, they encompass four entities: carcinoid, atypical carcinoid, small cell neuroendocrine carcinoma, and large cell neuroendocrine carcinoma [272]. For a detailed discussion of neuroendocrine neoplasms, the reader is referred to Chap. 11.

2.11.5.1 Carcinoid

Definition Sinonasal carcinoids are very rare well-differentiated neuroendocrine neoplasms that present bland cytology, lack of necrosis, and have <2 mitoses per 10 high-power fields [272].

Differential diagnosis, treatment, and prognosis are similar to carcinoids in other territories of the respiratory tract.

2.11.5.2 Atypical Carcinoid

Definition Sinonasal atypical carcinoids are moderately differentiated neuroendocrine carcinomas that present generally mild cytologic atypia, can have patchy necrosis, and have mitotic activity between 2 and 10 per high-power field. Although very rarely seen, a tumor with low mitotic activity, <2 per 10 high-power fields, that has bona fide necrosis is also considered atypical carcinoid [272].

Differential diagnosis, treatment, and prognosis are similar to those of atypical carcinoids in other territories of the respiratory tract.

2.11.5.3 Small Cell Neuroendocrine Carcinoma

Definition Small cell neuroendocrine carcinoma (SCNC) of the sinonasal tract is a high-grade, poorly differentiated, malignant epithelial tumor with histological features similar to small cell carcinoma of the lung [121].

Synonyms Small cell carcinoma, oat cell carcinoma, and poorly differentiated neuroendocrine carcinoma

Epidemiology This type of tumor has been well documented in various head and neck territories, mainly in the parotid gland and in the larynx. In the sinonasal tract, where they are distinctly uncommon, SCNC mainly arises from the ethmoid and maxillary sinuses and from the nasal cavity [273–277]. They may occur in pediatric age after treatment of retinoblastoma [278].

Etiology and pathogenesis Sinonasal SCNC is considered to derive from cells with neuroendocrine differentiation occasionally found in the seromucous glands.

Microscopy SCNC gives rise to nests, cords, and sheets of small undifferentiated cells, with molded nuclei and scanty cytoplasm (Fig. 2.52). More often than not, there is ulceration of the mucosa, but sometimes SCNC exclusively infiltrates beneath the surface epithelium. Foci of necrosis may be found and mitotic figures are frequently seen. Occasionally

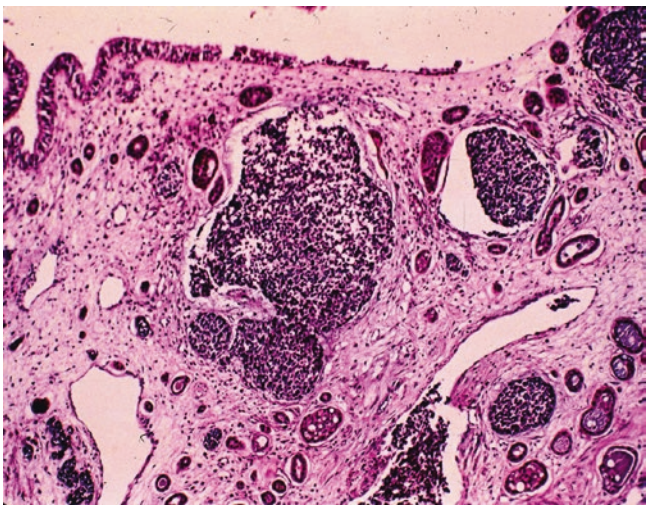

Fig. 2.52 Small cell neuroendocrine carcinoma: nests, cords, and islands of small undifferentiated cells, with compact nuclei and scanty cytoplasm, are seen to infiltrate beneath the surface epithelium. They appear interspersed in the lamina propria with the seromucous glands of a low-grade adenocarcinoma

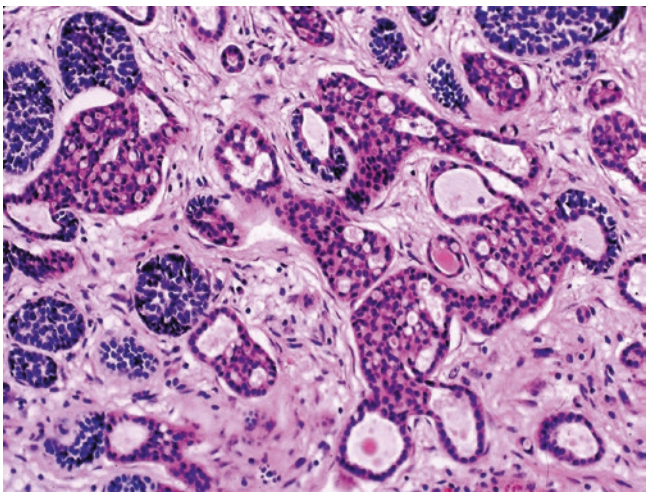

Fig. 2.53 Small cell neuroendocrine carcinoma: cords and nests of a small undifferentiated carcinoma, with molded nuclei and scanty cytoplasm, are seen in continuity with the seromucous glands of a low-grade adenocarcinoma, as if the former tumor was originating from the latter

the tumor grows surrounding the seromucous glands of the lamina propria, as if it was originating from them (Fig. 2.53). We have observed one case of SCNC originating at the base of a papillary NKSCC (Fig. 2.54). Variable degrees of neuroendocrine differentiation may be demonstrable by electron microscopy or immunohistochemistry (Fig. 2.55) [279]. Before placing a tumor within this category, a primary tumor from the lung must be ruled out.

Immunohistochemistry SCNC exhibits positive reaction for low molecular weight cytokeratins and EMA, as well as

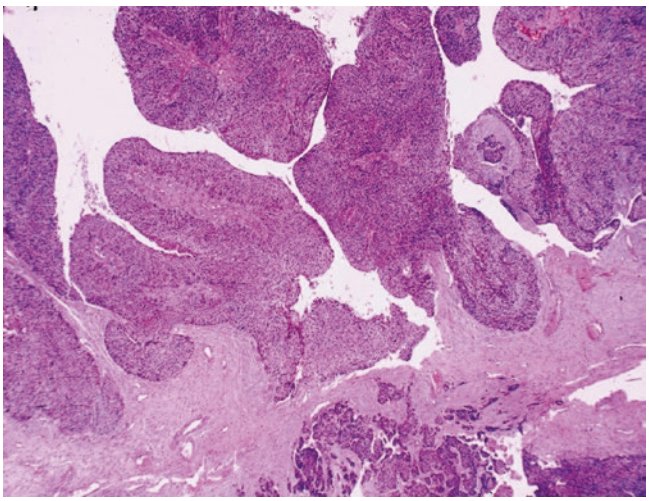

Fig. 2.54 Small cell neuroendocrine carcinoma: the tumor is at the base of a papillary non-keratinizing squamous cell carcinoma

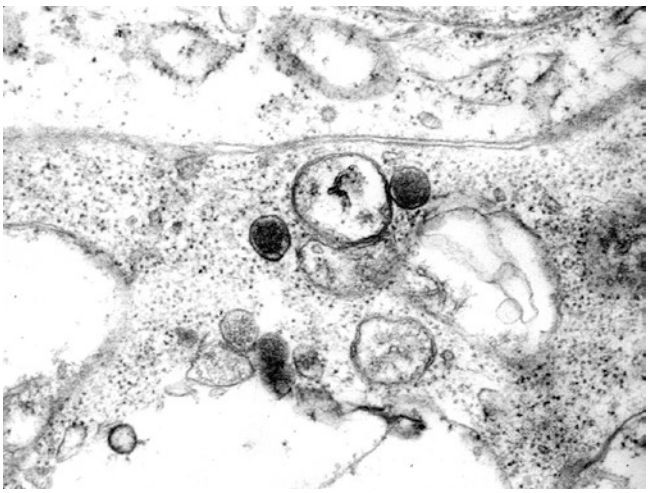

Fig. 2.55 Small cell neuroendocrine carcinoma: ultrastructurally, neurosecretory granules are found after diligent search (Courtesy of Prof. J.A. Bombi, Barcelona, Spain)

variable positivity for CD 56, synaptophysin, and chromogranin. At least two neuroendocrine markers should be positive [280].

Differential diagnosis Sinonasal SCNCs have been less precisely characterized than in other head and neck territories, and so far no unanimous consensus has been reached in regard to the way they have to be separated from other small cell tumors, either round or undifferentiated, occurring in this region [274–277, 281–284]. Table 2.5 provides the current criteria most widely accepted for their recognition. In addition, large cell neuroendocrine carcinoma has to be distinguished from SCNC [285, 286].

Treatment and prognosis Combination of surgery and radiotherapy, plus chemotherapy, is the treatment of

SCNC. Its prognosis seems to be somewhat better than for SNUC or for similar tumors of the lung.

2.11.5.4 Large Cell Neuroendocrine Carcinoma

Definition Large cell neuroendocrine carcinoma (LCNC) of the sinonasal tract is a high-grade, poorly differentiated, malignant epithelial tumor with histological features similar to its counterpart of the lung [272].

Epidemiology Only a few cases of LCNC have been reported in the sinonasal region [285, 286].

Microscopy LCNC is a tumor with high mitotic activity, but instead of having cells with high nuclear to cytoplasmic ratios, molding, and crush artifact, it has moderate to abundant cytoplasm.

Differential diagnosis Mitotic activity is the major histological feature that distinguishes LCNC from carcinoid and atypical carcinoid, >10 mitoses/10 HPFs in the former versus <10 mitoses/10 HPFs in the latter two.

Treatment and prognosis Surgery supplemented with postoperative radiotherapy and chemotherapy is the primary treatment for LCNC, which in general has the same poor prognosis as SCNC [272].

2.11.6 NUT Carcinoma

Definition NUT carcinoma is a rare, highly aggressive variant of poorly differentiated squamous cell carcinoma, which is defined by a rearrangement of the nuclear protein in testis (NUT) gene *NUTM1* on chromosome 15q14 [287].

Epidemiology It is a rare tumor, but the exact incidence is unknown, because most cases have gone unrecognized due to the lack of specific diagnostic features. In two recently published studies, it represented 18 % of poorly differentiated carcinomas of the upper aerodigestive tract [288] and 2 % of sinonasal carcinomas [266].

Clinical aspects NUT carcinoma can occur at all ages, but patients with sinonasal involvement are mainly young adults. Presenting symptoms are nonspecific and include nasal mass and pain, proptosis, and toothache. The tumors involved in most cases both the nasal cavities and the paranasal sinuses [266, 288].

Microscopy Histologically, NUT carcinoma is composed of undifferentiated basaloid cells, with monotonous appearance, round to ovoid nuclei, and often clear cytoplasm. Areas of abrupt squamous differentiation can be present, in which mature keratinizing cells are juxtaposed to undifferentiated neoplastic cells. In some instances, squamous differentiation may be more pronounced [289]. Areas of necrosis and the presence of neutrophilic infiltrate are commonly observed. The surface epithelium may show areas of squamous metaplasia, but no evidence of dysplastic changes has so far been reported [266, 288].

Immunohistochemistry A monoclonal antibody to NUT for use in immunohistochemistry is currently available [290], which may help to separate NUT carcinoma from other poorly differentiated sinonasal carcinomas. This antibody is considered to be enough sensitive and specific, so that the demonstration of the *NUTM1* rearrangement is no longer considered necessary [291]. Other immunohistochemical markers, which are consistently positive in NUT carcinoma, are cytokeratins, EMA, and p63, while no or limited immunoreactivity has been observed with muscle, neuroendocrine, and melanocytic markers. The presence of HPV and EBV infection has never been identified, either using immunohistochemistry, in situ hybridization, or polymerase chain reaction [292].

Genetics NUT carcinoma is characterized by a translocation involving the *NUTM1* gene on chromosome 15q14 and, in most cases, the *BRD4* gene on chromosome 19p13.1 [293]. The remaining cases either present a *BRD3-NUTM1* fusion [t(9;15)(q34.2;q14)] or a yet uncharacterized fusion (so-called NUT-variant). The fusion gene encodes for a protein which is thought to be involved in a block of epithelial differentiation and squamous maturation [294].

Differential diagnosis It is difficult if not impossible to separate NUT carcinoma from other poorly differentiated carcinomas on pure morphological grounds. It has been suggested that some features that may support the inclusion of NUT carcinoma in the differential diagnosis are neoplastic cells with monotonous appearance, which vary in size from small to medium but are not large, and the presence of areas of focal "abrupt" keratinization [291]. The differential diagnosis in the sinonasal tract includes SNUC, poorly differentiated squamous cell carcinoma, basaloid squamous cell carcinoma, and neuroendocrine carcinoma. Among non-epithelial neoplasms, olfactory neuroblastoma, Ewing's sarcoma, rhabdomyosarcoma (RMS), and hematolymphoid tumors can be considered in the differential diagnosis. The diagnosis of NUT carcinoma requires either the immunohistochemical demonstration of nuclear reactivity for NUT, which has a characteristic speckled pattern [290], or the demonstration of *NUTM1* rearrangement by FISH or PCR. Other markers that may be useful in the differential diagnosis with the abovementioned entities are p63, which is diffusely positive in NUT carcinoma, but not in SNUC and neuroendocrine carci-

noma, and neuroendocrine markers, which are not expressed or only focally expressed by NUT carcinoma.

Treatment and prognosis NUT carcinoma has an extremely aggressive clinical course with short survival periods. According to a recent report, intensive local therapy, with complete surgical resection and radiation, seems to be associated with improved progression-free and overall survival. The type of translocation does not seem to affect prognosis [295].

2.11.7 SMARCB1–Deficient Sinonasal Basaloid Carcinoma

Recently, some tumors initially diagnosed as SNUCs as well as NKSCCs and myoepithelial carcinomas of the sinonasal tract have been shown to share a common alteration resulting in inactivation of the *SMARCB1* (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1, also *INI-1*) tumor suppressor gene along with rhabdoid features, whether in isolated cells or in confluent sheets of polygonal cells with a plasmacytoid appearance. SMARCB1 deficiency can be easily identified through immunohistochemistry. Although the number of cases reported of these aggressive basaloid carcinomas is limited, it is likely that they represent a distinctive type of sinonasal carcinoma [296, 297].

2.11.8 Sinonasal Adenocarcinomas

Sinonasal adenocarcinomas comprise a wide spectrum of glandular tumors accounting for approximately 20 % of all sinonasal malignancies [298]. They show a remarkable wide range of histological appearances, and they arise both from mucosal seromucous glands and surface epithelium. The 2005 WHO classification recognizes three major adenocarcinoma subtypes: intestinal type, non-intestinal type, and salivary gland type.

2.11.8.1 Intestinal-Type Adenocarcinoma

Definition Intestinal-type adenocarcinoma (ITAC) is a tumor with histological features resembling colorectal dysplastic adenoma or adenocarcinoma [299, 300]. It is considered to originate through intestinal metaplasia of the ciliated respiratory cells lining the Schneiderian membrane. Metastasis from gastrointestinal adenocarcinoma should be ruled out before a tumor is labeled as a primary of this region.

Epidemiology and etiology ITAC is the most common type of sinonasal adenocarcinoma representing about 6–13 % of malignancies developing in the sinonasal tract [301–303]. It is strongly associated with exposure to different types of dust, mainly hardwood but also softwood dusts, as well as leather dust [304–310]. About 20 % of sinonasal ITACs seem to be sporadic, without evidence of exposure to industrial dusts [298, 305]. The incidence has remained relatively stable in the last decades, although recently a decrease in the incidence of sinonasal adenocarcinomas has been observed in males [298, 311]. Males are more frequently affected than females, and the peak age is in the fifth and sixth decades, for both sexes. The most common location is the ethmoidal region [312], followed by the nasal cavities and other sinuses.

Macroscopy ITACs have a fungating appearance with either polypoid or papillary features. Occasionally, they may have a gelatinous consistency resembling a mucocele.

Microscopy Histologically, ITAC is mainly composed of columnar mucin-secreting cells and of goblet cells [312]. Some well-differentiated tumors may also contain resorptive cells, argentaffin cells, and Paneth cells (Fig. 2.56). Endocrine-amphicrine enteric differentiation may occasionally be found [313]. Metaplastic and atypical changes have been observed in adjacent preneoplastic epithelium [314]. These tumors depict different histological patterns that may be predominantly (a) papillary, (b) glandular, (c) compact, (d) mucinous, and (e) mixed [312, 315]. Papillary tumors mainly consist of elongated outgrowths lined by intestinal-type cells with markedly atypical pseudostratified nuclei (Fig. 2.57). Although most of them are high-grade tumors, low-grade forms mimicking colonic villous adenoma may occasionally occur (Fig. 2.58) [316]. The glandular pattern resembles common-type intestinal adenocarcinoma (Fig. 2.59). Compact or solid forms show poorly differentiated nests of cells in which glandular formation is rarely seen. In the mucinous pattern, more than 50 % of the tumor is composed of dilated mucin-filled glands lined by columnar mucin-secreting epithelium and lakes of mucin containing fragmented epithelial elements (Fig. 2.60). Other mucinous tumors show mucin-filled cells with the pattern of "signet ring" cell carcinoma. Various attempts have been made to correlate histopathological grading and typing with clinical behavior [317–319].

In rare instances, ITAC may be combined with small cell neuroendocrine carcinoma. In these cases, the two components are distinct and differ morphologically and immunohistochemically [320].

Immunohistochemistry and electron microscopy Both technologies have confirmed the enteric differentiation of the tumor cells [321]. Wide-spectrum cytokeratin markers are positive, whereas CEA is only occasionally positive

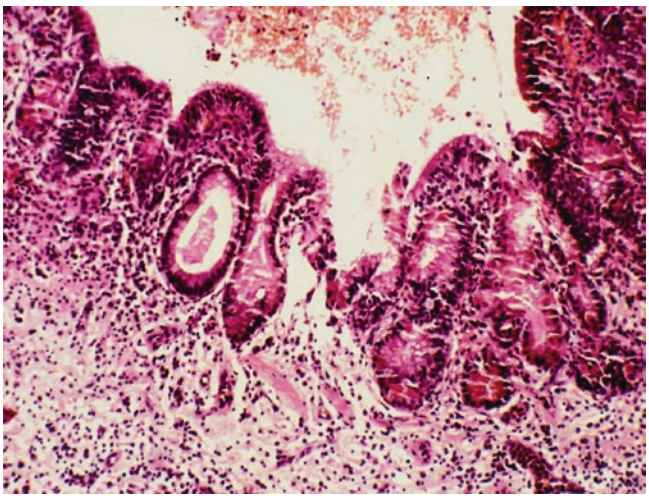

Fig. 2.56 Intestinal-type adenocarcinoma: in situ adenocarcinoma with abundant presence of Paneth cells

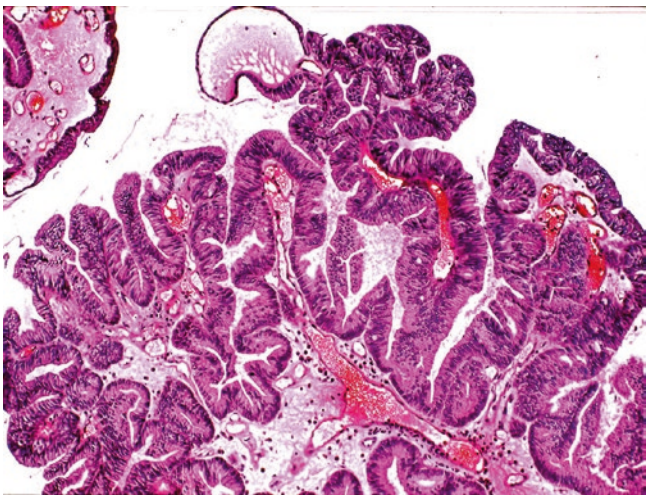

Fig. 2.58 Intestinal-type adenocarcinoma: low-grade variant mimicking villous adenoma. Notice the presence of small intestine-type resorptive cells at the *left*

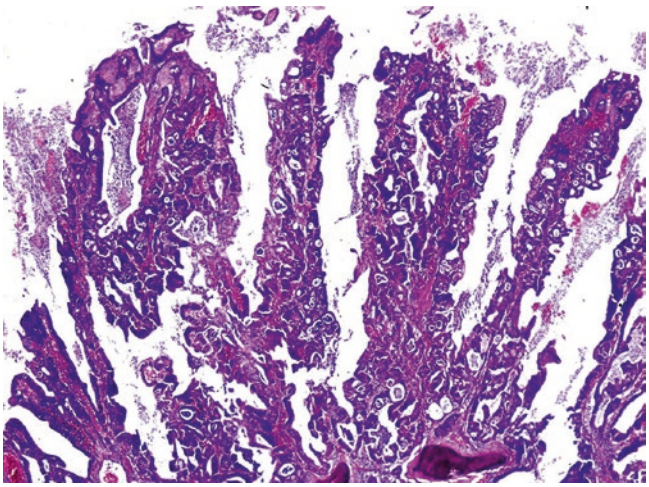

Fig. 2.57 Intestinal-type adenocarcinoma: high-grade variant of papillary outgrowth of intestinal-like malignant epithelium. Destruction of sinonasal bone at the *bottom*

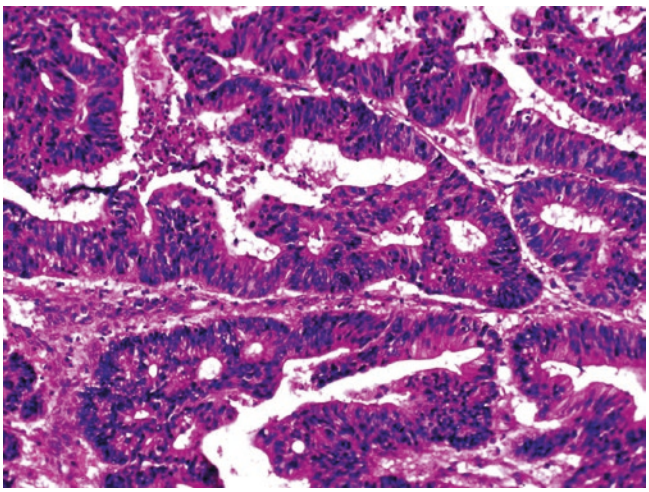

Fig. 2.59 Intestinal-type adenocarcinoma: the glandular pattern resembles common-type intestinal adenocarcinoma

[322]. Cytokeratin 7 is frequently but not constantly positive, while most ITACs express cytokeratin 20 and CDX2, two markers related to intestinal differentiation [323]. Villin is another marker of enteric differentiation which is positive in these tumors [280]. Neuroendocrine markers, including chromogranin and synaptophysin, are frequently detected in individual cells or small clusters of cells, representing interspersed neuroendocrine or amphicrine cells [322, 324].

Genetics The genotypic features of ITAC show a significant overlap with those present in colorectal adenocarcinoma, particularly with colorectal adenocarcinomas developing through the MSI-negative pathway [325]. Commonly altered genes include *TP53*, *CDKN2A*, and deleted in colon cancer (*DCC*), while, at variance with colorectal adenocarcinoma, the APC-beta-catenin pathway is likely to have a marginal involvement in the development of ITAC. In addition, activating *KRAS* mutations occur at a lower frequency (10–13%) than in colorectal cancer [326, 327]. The epidermal growth factor receptor (EGFR) is overexpressed in approximately 15% of ITACs, and most of these cases show either chromosome 7 polysomy or *EGFR* gene amplification by FISH analysis [328]. Conversely, activating mutations of *EGFR* and *BRAF* genes are rare or absent.

Differential diagnosis ITAC can be differentiated from other adenocarcinomas on the basis of histological morphology and with the help of immunohistochemical markers of intestinal differentiation. These markers are characteristically expressed by ITACs but not by sinonasal non-intestinal-type adenocarcinomas.

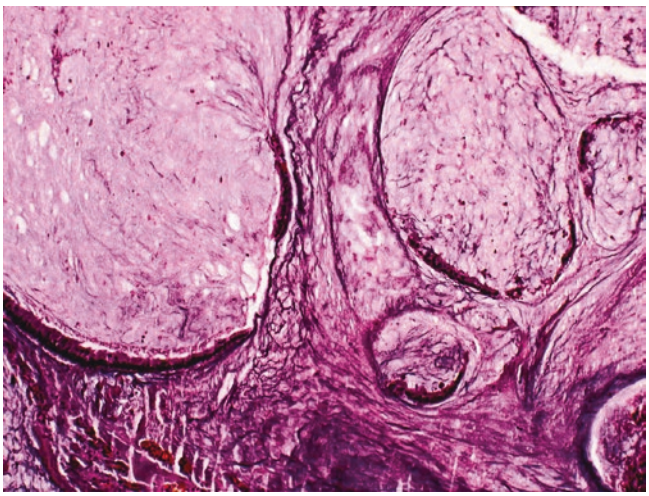

Fig. 2.60 Intestinal-type adenocarcinoma: dilated glands lined by columnar mucin-secreting epithelium, containing lakes of mucin and fragmented epithelial elements. A mucocele was clinically suspected

Features such as cytologic atypia, high mitotic rate, and areas of necrosis, which are common findings in most ITACs, help to distinguish the high-grade variants from rare low-grade ITACs and from mucoceles. The lack of epidermoid and squamous differentiation separates these tumors from mucoepidermoid and adenosquamous carcinomas.

In rare occasions, adenocarcinomas originating in the gastrointestinal tract may metastasize to the sinonasal region, and this is usually a late event in the clinical course of the tumor. In these cases, the differential diagnosis with primary ITAC is mainly based on the clinical history, because no histological or immunohistochemical feature is distinctive enough to allow separation.

Treatment and prognosis Treatment of choice is complete surgical resection followed by radiotherapy. A good response to chemotherapy has been observed in some cases. Interestingly, chemotherapy was highly effective in tumors bearing wild-type or a still-efficient p53 protein, but ineffective in those carrying a disabled p53 protein, indicating that p53 status represents a promising predictive biomarker to chemotherapy in ITAC [329]. Prognosis of ITAC is generally poor and largely depends on T stage. Recurrences and subsequent deeply invasive local growth are frequent; however, lymph node and distant metastases are rare [310, 312, 318].

2.11.8.2 Non-intestinal-Type Adenocarcinomas

Sinonasal non-intestinal-type adenocarcinomas (non-ITAC) are an uncommon and heterogeneous group of tumors defined, by exclusion, as glandular malignancies that do not show signs of intestinal differentiation and do not resemble any salivary gland tumor type. Possibility of a metastatic adenocarcinoma should also be ruled out. They can be further distinguished in high- and low-grade subtypes.

High-Grade Non-intestinal-Type Adenocarcinomas

Definition High-grade non-ITACs are characterized by neoplastic non-intestinal and non-salivary type of glands showing moderate to marked cell pleomorphism, high number of mitotic figures, and foci of necrosis.

Epidemiology High-grade non-ITACs are rare tumors that develop more commonly in men, and, although they occur over a wide age range, they are much more common in older individuals [330].

Macroscopy They arise more often in the nasal cavity and maxillary sinus and appear as large destructive tumor masses, with areas of necrosis and hemorrhage.

Microscopy High-grade non-ITACs appear as poorly differentiated tumors, with predominantly solid growth pattern, and poorly formed gland structures. They show a great deal of heterogeneity, and different patterns have been recognized. The blastomatous pattern resembles primitive gland differentiation seen in teratocarcinosarcoma, with ribbons and trabeculae of neoplastic cells with numerous rosette-like gland structures sometimes containing mucus. In the apocrine subtype, the infiltrating glands resemble those of ductal carcinoma of the breast or high-grade salivary duct carcinoma. The oncocytic/mucinous can be associated with oncocytic Schneiderian papilloma and is formed by oncocytic and mucinous cells, growing as solid sheets and sometimes showing extracellular mucus accumulation [330]. The poorly differentiated/undifferentiated adenocarcinomas are predominantly solid with occasional cribriform nests and papillary structures.

Immunohistochemistry These tumors are consistently positive for cytokeratin cocktails and cytokeratin 7, while cytokeratin 20 and CDX2 are negative. Occasional cases have shown focal positivity for synaptophysin, S-100 protein, and p63.

Differential diagnosis High-grade non-ITAC can be distinguished from low-grade non-ITAC for the presence of prominent cytologic atypia, brisk mitotic activity, and/or necrosis. ITAC can be ruled out based on the lack of morphological resemblance to colorectal adenocarcinoma and for the absence of positivity to intestinal markers, such as cytokeratin 20, CDX2, and villin. Other poorly differentiated high-grade neoplasms to be considered in the differential diagnosis include salivary duct carcinoma and teratocarcinosarcoma.

Treatment and prognosis Although definitive specific treatment recommendations are lacking due to the rarity of this type of tumors, complete surgical excision is the treatment of choice, which may be followed by radiotherapy [331]. The prognosis, however, remains poor, with most patients experiencing local recurrence and death from disease.

Low-Grade Non-intestinal-Type Adenocarcinomas

Definition Low-grade non-intestinal-type adenocarcinomas (low-grade non-ITACs) are characterized by neoplastic non-intestinal and non-salivary type of glands showing absence or minimal cell pleomorphism, absence or minimal number of mitotic figures, and absence of necrosis.

Epidemiology Low-grade non-ITACs occur over a wide age range, with a mean of 60 years. There is no significant gender predilection. No relation with occupational activities has been documented in these tumors. The nasal cavity is the most commonly affected site, followed by the ethmoid sinus.

Macroscopy Given their rarity, precise data are not available.

Microscopy Different histological patterns may be recognized: papillary, tubular, tubulopapillary, glandular, mucinous, trabecular, cribriform, psammomatous, and clear cell. The papillary pattern is characterized by complex papillary fronds lined by bland columnar cells (Fig. 2.61). They may occasionally mimic oncocytic (columnar) cell papilloma. Recognition of invasion may be difficult in these cases. Quite similar tumors also develop in the nasopharynx [332]. The tubulopapillary carcinoma consists of a proliferation of cuboidal to columnar of epithelial cells, forming tubules at the center and papillae at the surface [333]; it has to be differentiated from the terminal tubulus adenocarcinoma of the nasal seromucous glands (Fig. 2.62) [334]. Tumors with glandular pattern may simulate adenoma; nevertheless, the presence of closely packed glands, forming back-to-back arrangements, indicates the true malignant nature of the lesion [335]. Papillary structures may be occasionally noted within dilated glandular structures. In a recent report, one-third of the cases were associated with respiratory epithelial adenomatoid hamartoma, but the significance of this finding is yet to be determined [336]. The clear cell pattern is best exemplified by the nasal renal cell-like adenocarcinoma, which consists of cuboidal to polyhedral cells with abundant clear cytoplasms, forming either solid or glandular patterns that mimic clear cell renal carcinoma [337].

Immunohistochemistry Neoplastic cells are positive for broad-spectrum cytokeratins and cytokeratin 7, but not for cytokeratin 20, CDX2, MUC2, or villin. S-100 protein can also be detected [336]; positivity for myoepithelial markers has been reported in a few cases [338].

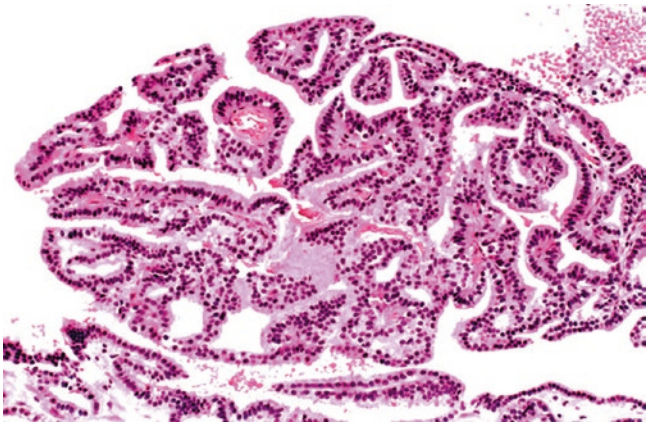

Fig. 2.61 Low-grade non-intestinal-type adenocarcinoma: the papillary variant is made up of cuboidal to columnar epithelial cells, forming papillae supported by delicate fibrovascular stalks

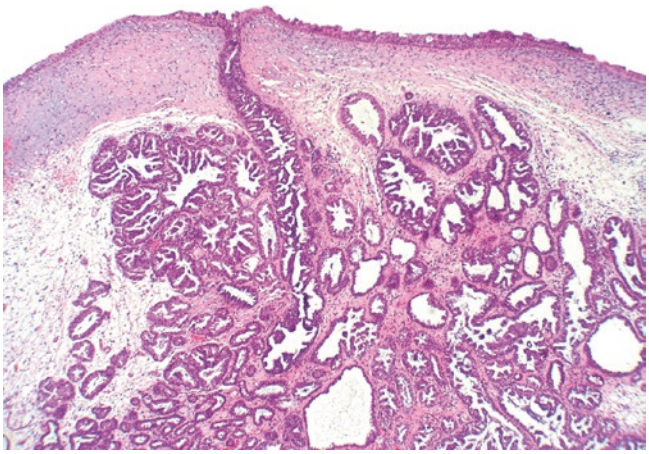

Fig. 2.62 Low-grade non-intestinal-type adenocarcinoma: the terminal tubulus variant shows tubulopapillary glands in continuity with a duct that drains into the luminal surface

Genetics There are only sporadic reports of genetic analysis in these tumors. *TP53* gene was not mutated in two cases examined by Franchi et al. [338].

Differential diagnosis *L*ow-grade non-ITACs have to be distinguished both from benign and malignant lesions (Table 2.6). The main differential diagnosis is with sinonasal seromucinous hamartoma. The lack of lobular architecture; presence of epithelial tufting and papillae, "back-to-back" glands, areas with cribriform or trabecular pattern; and invasion of normal structures support the diagnosis of low-grade non-ITAC [42]. Respiratory epithelial adenomatoid hamartoma is characterized by gland-like structures lined by ciliated respiratory-type epithelium that originate from the

Table 2.6 Differential features of selected glandular lesions of the sinonasal tract

Lesion	Clinical features	Salient histopathologic features	Immunohistochemistry
Respiratory epithelial adenomatoid hamartoma (REAH)	Polypoid lesion; posterior nasal septum, ethmoid sinus, nasopharynx; wide age range, male predominance	Back-to-back glands lined by ciliated columnar cells, periglandular hyalinization; occasional mucinous change of proliferating epithelium	CK7+, CK20–, CDX2–, p63+ in the basal compartment, S100-
Seromucous hamartoma	Polypoid lesion; posterior nasal septum, nasopharynx; wide age range, slight male predominance	Lobular growth of small serous glands, uncommon mucinous glands; areas resembling REAH occasionally seen	S100+, CK7+, CK20–, CDX2–, myoepithelial markers -
Intestinal-type adenocarcinoma (ITAC)	Exophytic lesions, often with necrotic-hemorrhagic or mucoid appearance; ethmoid sinus, nasal cavity; adult subjects, male predominance; association with woodworking and leatherworking	Columnar cells, goblet cells, signet ring cells; papillary, glandular, solid, and alveolar-mucinous architecture	CK20+, CK7 variably positive, CDX2+
Low-grade non-ITAC	Papillary/exophytic lesions; nasal cavities and ethmoid sinus; wide age range, predominantly adult patients, no gender predilection	Different growth patterns, more frequently tubulopapillary, cribriform, clear cell; back-to-back glands, mild atypia, low mitotic activity, infiltration of the mucosa and bone	CK7+, CK20–, CDX2–, myoepithelial markers occasionally +
High-grade non-ITAC	Large destructive lesions, with exophytic appearance; hemorrhage and necrosis often present; nasal cavities and maxillary sinus; adult patients	Predominantly solid growth pattern, poorly formed glands, marked atypia, pleomorphism, necrosis, brisk mitotic activity	CK7+, CK20–, CDX2–

surface epithelium of the sinonasal tract. In addition, mucinous tumors have to be distinguished from mucoceles [339, 340]. Low-grade non-ITAC can be separated from high-grade non-ITAC based on the lack of marked cellular pleomorphism, necrosis, and brisk mitotic activity. Tubulopapillary ITAC can be ruled out with the help of immunohistochemistry for markers of intestinal differentiation, including cytokeratin 20, CDX2, MUC2, and villin. Salivary-type adenocarcinomas to be ruled out include acinic cell carcinoma [341] and low-grade salivary duct carcinoma of salivary glands [342]. The renal cell-like adenocarcinoma has to be separated from the salivary-type tumors with clear cells and from metastatic renal carcinoma [337, 343] and from the very rare clear cell variant of olfactory neuroblastoma, another mimicker of renal cell carcinoma [344]. Immunohistochemistry may help in this latter distinction, because vimentin and RCC are usually positive in clear cell renal carcinoma, while both markers are negative in sinonasal renal cell-like adenocarcinoma [337, 343].

Treatment and prognosis The main treatment is surgery, but radiotherapy has been employed in some cases, especially in case of positive margins [331]. Low-grade non-ITACs tend to recur locally, but distant metastases are rare. Only few deaths from disease have been recorded [336].

2.11.8.3 Salivary-Type Adenocarcinomas

A wide range of salivary gland-type tumors may occur in the sinonasal region (see Chap. 5). However, with the exception of adenoid cystic carcinoma, they are quite rare, and most of them have been reported as single cases. They derive from the seromucous glands of the Schneiderian mucosa. Most sinonasal salivary-type tumors are malignant and account for 8–10 % of all malignancies in this territory [345].

Adenoid Cystic Carcinoma

Definition Adenoid cystic carcinoma (AdCC) is a malignant small cell tumor composed of ductal epithelial cells surrounded by modified myoepithelial cells, giving rise to tubular, cribriform, and solid patterns.

Epidemiology AdCC is the most common malignant salivary type of tumor of the upper respiratory tract and comprises 5–10 % of all sinonasal malignancies [302, 346, 347]. AdCC is most common in the maxillary antrum, followed by the nasal cavity [348], although ethmoid, sphenoid, and frontal sinuses may also be involved [142, 349, 350]. Invasion of the skull base by an AdCC has been recently documented [351].

Macroscopy AdCCs present as unencapsulated masses of white to gray color and variable size.

Microscopy Sinonasal AdCC is identical to that arising at other head and neck sites (Fig. 2.63). Over 50 % present a cribriform growth pattern and less often solid or tubular growths [352]. Perineural growth and bone invasion are frequently observed (Fig. 2.64). Rarely, sinonasal AdCC may arise in a preexisting pleomorphic adenoma [353]. Examples of so-called dedifferentiated AdCC have also been reported in the sinonasal region [354]. These tumors consist of a con-

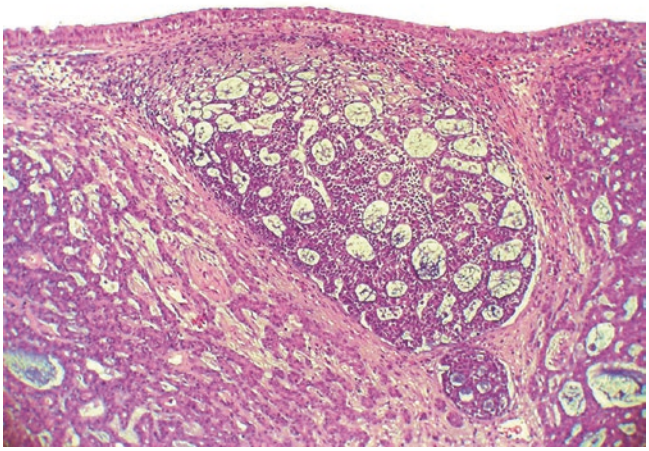

Fig. 2.63 Adenoid cystic carcinoma: typical cribriform growth pattern is seen infiltrating beneath the respiratory mucosa

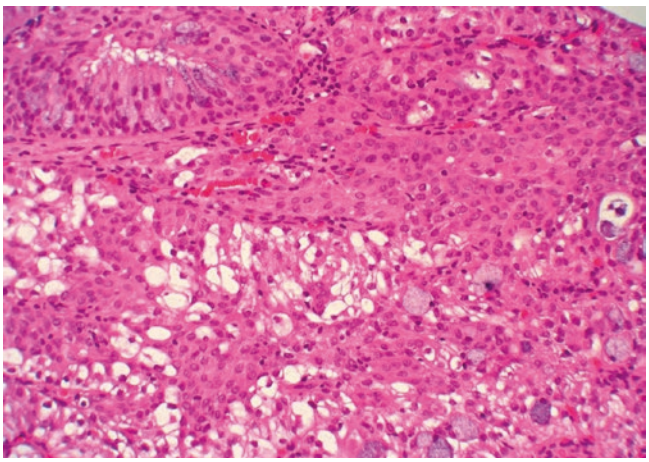

Fig. 2.65 Mucoepidermoid carcinoma: mucous-secreting cells, clear cells, and intermediate cells are the main components. At the *upper left*, entrapped respiratory epithelium is seen

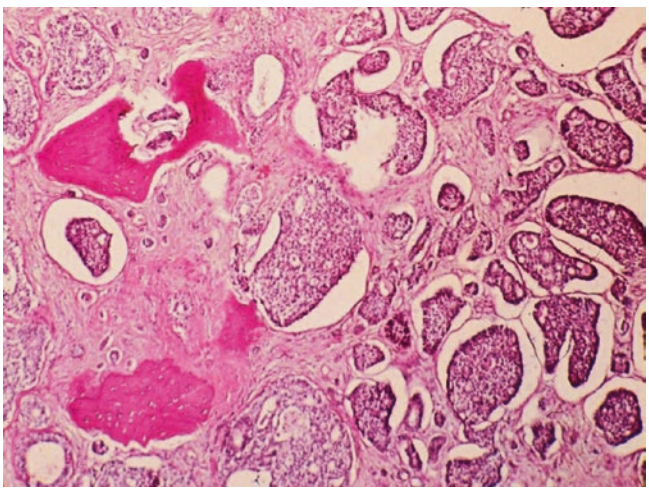

Fig. 2.64 Adenoid cystic carcinoma: the tumor invades and destroys adjacent bone

ventional low-grade AdCC component, with tubular or cribriform architecture, which is clearly separated from a high-grade undifferentiated or poorly differentiated carcinoma component.

Differential diagnosis Sinonasal AdCC must be mainly distinguished from other salivary gland-type tumors which occur in this territory, particularly pleomorphic adenoma, polymorphous low-grade adenocarcinoma, epithelial-myoepithelial carcinoma, and basaloid squamous cell carcinoma with cribriform pattern.

Treatment and prognosis Wide surgical resection is the usual treatment, which may be followed by radiotherapy. AdCC follows a protracted but relentless course, which at the outset may be silent. The majority of patients present with locally advanced disease; lymph node and distant metastases are a rare late event. The overall 5-year survival is around 60 %, but the 10–20-year survival is poorer [355]. Patients with cribriform pattern may have a longer survival than patients with solid-type tumors [352]. Spontaneous regression of an AdCC of the nasal cavity has been recently reported [356].

Mucoepidermoid Carcinoma

Definition Mucoepidermoid carcinoma (MEC) is a malignant glandular tumor characterized by mucous, intermediate, and epidermoid cells. They are subdivided in low- and high-grade categories.

Epidemiology Sinonasal MEC is rare, representing less than 1 % of all sinonasal carcinomas [357, 358]. Patients are usually adult, and there is no gender predilection [359]. The majority of tumors arise in the nasal cavity, followed by the maxillary sinus.

Microscopy The diagnosis of sinonasal MEC requires the identification of mucous, squamous, and intermediate cells (Fig. 2.65). Infiltration of the mucosa and bone is usually identifiable. The presence of cystic spaces is a frequent feature, while necrosis and atypical mitotic figures are rarely seen. The majority of tumors are in the low-grade category [359] although high-grade MEC may be encountered in the sinonasal tract [360].

Differential diagnosis In the sinonasal tract, the differential diagnosis of MEC includes mainly squamous cell carcinoma and adenosquamous carcinoma. Non-intestinal-type adenocarcinomas with clear cells and/or mucous production should also be ruled out.

Treatment and prognosis Most tumors present in low stage and recurrences develop in about one-third of patients after surgical resection [359].

Acinic Cell Carcinoma

Definition Acinic cell carcinoma (ACC) is a low-grade malignant neoplasm composed of cells with serous acinar differentiation.

Epidemiology ACC is uncommon in the sinonasal tract, and only a small number of cases have been documented in the nasal cavity [341, 361–365] and in the maxillary sinuses [366–368]. Most of them are single case reports.

Microscopy ACC is composed of four cell types, acinar, vacuolated, clear, and nonspecific glandular. They may give rise to the following main patterns: solid, microcystic, papillary cystic, and follicular [369].

Differential diagnosis The main tumors to distinguish from ACC are oncocytoma (Figs. 2.66 and 2.67), all the clear cell salivary-type tumors that may arise in the sinonasal tract and metastatic renal cell carcinoma [345]. Although mammary analogue secretory carcinoma (MASC) should be considered another differential diagnosis in cases of non-parotid ACCs, the single potential ethmoidal case so far studied was negative for the *ETV6-NTRK3* translocation, which excluded MASC, and showed PAS-diastase-resistant zymogen granules typical of ACC [370].

Treatment and prognosis Surgical resection alone gives in most cases excellent results.

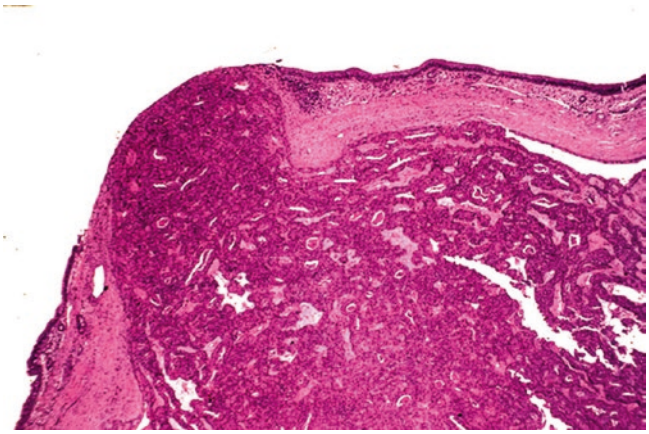

Fig. 2.66 Low-grade oncocytic carcinoma: the tumor infiltrates the sinonasal mucosa just beneath the epithelium which is focally eroded (Courtesy of Prof. H. Ostertag, Hannover, Germany)

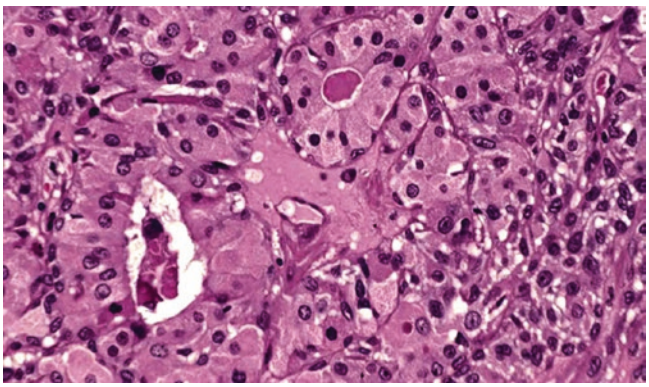

Fig. 2.67 Low-grade oncocytic carcinoma: the oncocytes form glands and cords and contain nuclei with conspicuous nucleoli and discrete atypia (Courtesy of Prof. H. Ostertag, Hannover, Germany)

Epithelial-Myoepithelial Carcinoma

Definition Epithelial-myoepithelial carcinoma (EMC) is a low-grade malignant tumor composed of variable proportions of two cell types which typically form duct-like structures. There is an inner layer of duct lining cells and an outer layer of clear cells [339].

Epidemiology EMC is quite rare in the sinonasal tract. Cases have been reported to involve the nasal cavity and maxillary sinus [371–377].

Microscopy The inner layer of the duct-like structures consists of small dark-staining cuboidal cells. The outer clear cells stain strongly for glycogen and are also positive for p63, vimentin, and smooth muscle actin; the inner luminal ductal cells are positive for cytokeratin cocktails and also for CK 19. There is considerable variation in the proportion of duct lining cells and clear cells, and not uncommonly, the latter are the predominant feature, forming sheets or nests of clear cells rather than ductal structures. The tumor is cytologically bland and mitoses are rare. Perineural and vascular invasion may be present and recurrence and metastases may develop.

Differential diagnosis Main differential diagnoses of EMC are myoepithelioma, pleomorphic adenoma, myoepithelial carcinoma, and adenoid cystic carcinoma.

Treatment and prognosis Wide surgical resection and adjuvant radiotherapy currently achieve excellent results in patients with EMC.

Other Salivary-Type Adenocarcinomas

Carcinoma ex pleomorphic adenoma [142, 378], myoepithelial carcinoma [147, 379], polymorphous low-grade adenocarcinoma (Figs. 2.68, 2.69, and 2.70) [380], and basal cell adenocarcinoma [381] have been reported in the sinonasal

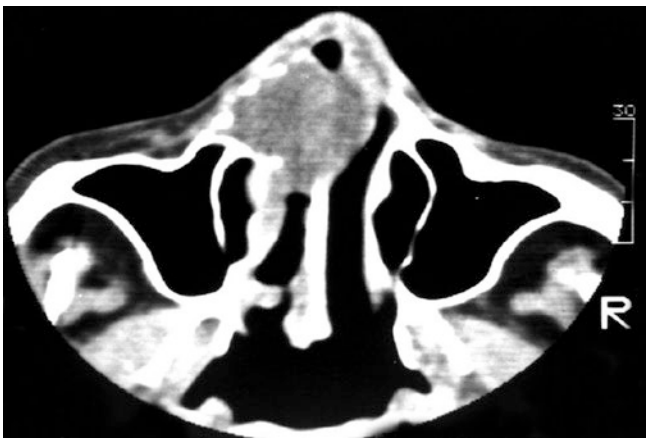

Fig. 2.68 Polymorphous low-grade adenocarcinoma: CT scan showing an irregularly nodular lesion destroying the anterior nasal septum

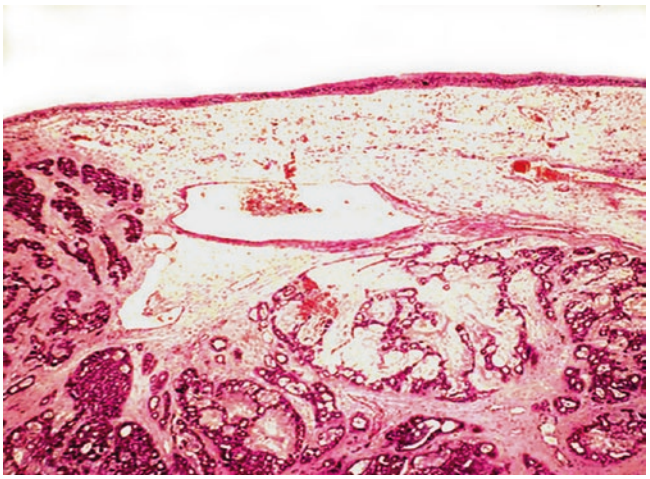

Fig. 2.69 Polymorphous low-grade adenocarcinoma: variegated glandular arrangements composed of tubules with bland cellularity are seen beneath the respiratory epithelium

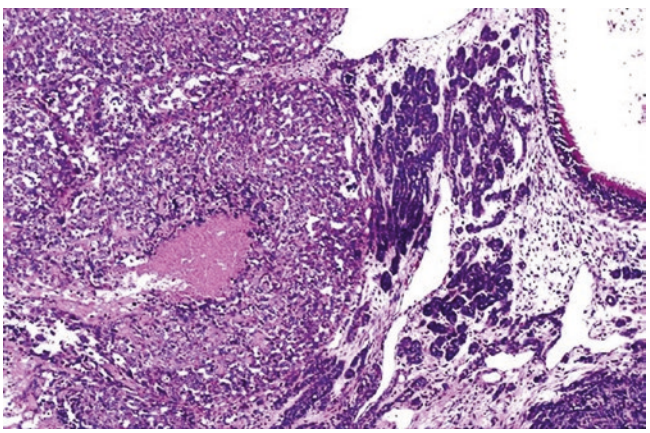

Fig. 2.70 Polymorphous low-grade adenocarcinoma with high-grade component: an undifferentiated carcinoma with a focus of central necrosis is seen in the immediate neighborhood of the usual low-grade component (Courtesy of Prof. J. Lloreta, Barcelona, Spain)

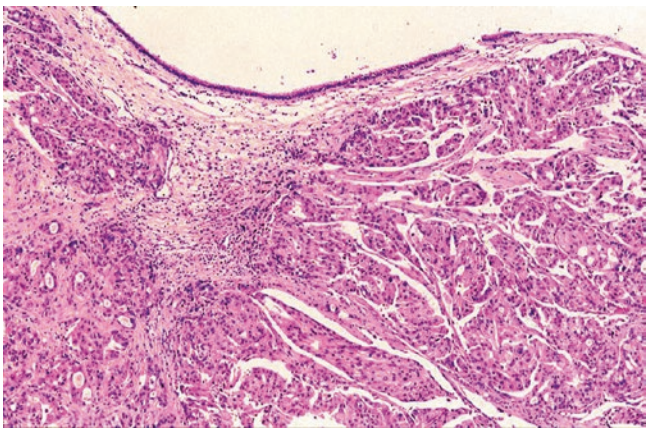

Fig. 2.71 Salivary duct carcinoma: markedly atypical glandular growth beneath the respiratory epithelium

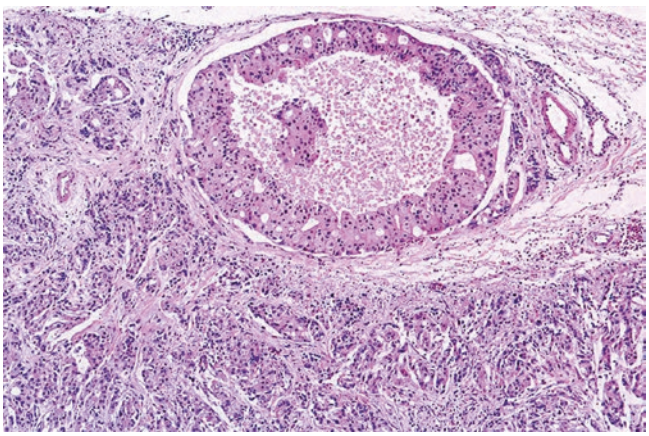

Fig. 2.72 Salivary duct carcinoma: the ductal pattern, with comedo type of necrosis, was convincingly evident in the metastasis to a submandibular lymph node

tract. Two to 10% of salivary duct carcinomas (SDCs) arise from the seromucous glands of the upper respiratory tract [382]. We have seen one example of SDC originating in the maxillary sinus, in which the characteristic ductal pattern, with comedo type of necrosis, was only evident in the metastases to the submandibular lymph nodes. The primary tumor was initially classified as adenocarcinoma NOS (Figs. 2.71 and 2.72).

2.11.9 Primary Malignant Mucosal Melanoma

Definition Primary malignant mucosal melanoma (PMMM) of the sinonasal tract is a neoplasm derived from the melanocytes in the Schneiderian mucosa [383–385].

Epidemiology The head and neck region is the most commonly involved site in which PMMMs develop, being the sinonasal tract its most frequent location [386, 387]. Sinonasal PMMMs account for less than 1 % of all melanomas and for less than 5 % of all sinonasal malignancies [386–389]. Although most series report a similar gender distribution, others indicate a slightly increased incidence in males [390, 391]. The tumors develop primarily between the fifth and eighth decades of life with a median age of presentation at approximately 60 years [391, 392]. They originate from melanocytes present in the mucosa of the respiratory tract (Fig. 2.73) [384, 385, 388]. In our experience, it is not uncommon to see melanoma arising in an area of squamous metaplasia (Fig. 2.74). In contrast to Caucasian, black Africans often show visible pigmentation at sites corresponding with the common locations of intranasal melanomas, for which they have a higher incidence [393].

Clinical aspects The signs and symptoms of presentation of sinonasal PMMMs are not specific. Epistaxis and nasal obstruction are frequent when located in the nasal cavity. PMMMs of the head and neck occur most frequently in the nasal cavity, where the lateral nasal wall and nasal septum are the most common sites of origin of the sinonasal tract. Melanomas arising from the lateral nasal wall account for almost half of the total. Middle and inferior turbinates and nasal vestibule are other possible sites. The maxillary sinus is the most commonly affected paranasal cavity, followed by the ethmoid, frontal, and sphenoid sinuses. Concurrent nasal and paranasal lesions are infrequent. The sinonasal PMMMs are usually advanced at presentation and the precise site of origin may be difficult to localize. Sinonasal PMMMs metastasize less frequently to lymph nodes but more frequently to the lungs and brain [390, 391, 394].

Etiology Unlike cutaneous melanomas, sinonasal PMMMs are not clearly related to ultraviolet radiation. Inhaled and ingested carcinogens, particularly products of smoking and formaldehyde, have been implicated in the pathogenesis, similar to other malignancies of the nasal cavity [395, 396].

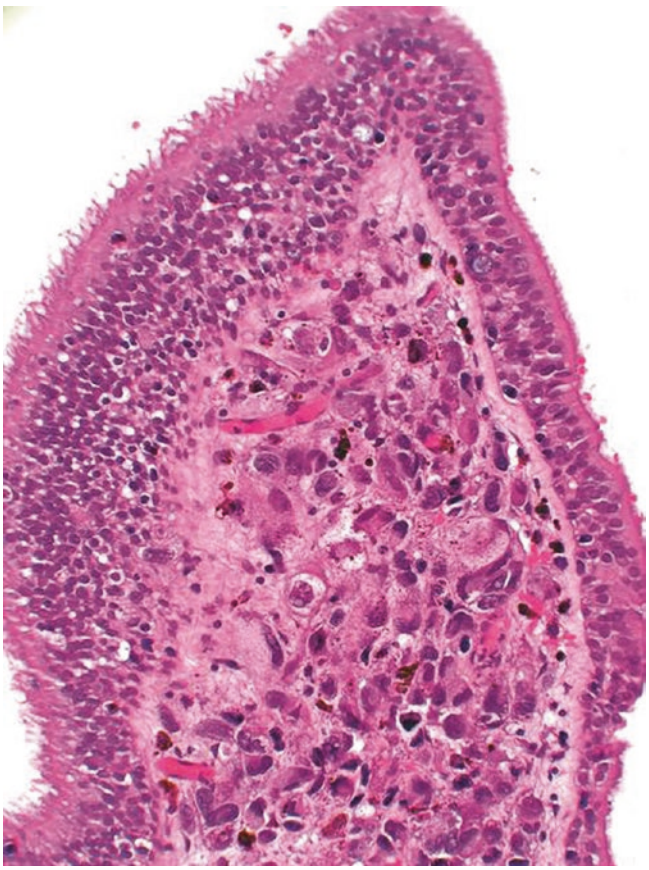

Fig. 2.73 Malignant mucosal melanoma: sheet of pigmented malignant melanocytes distributed in the lamina propria underneath ciliated respiratory epithelium

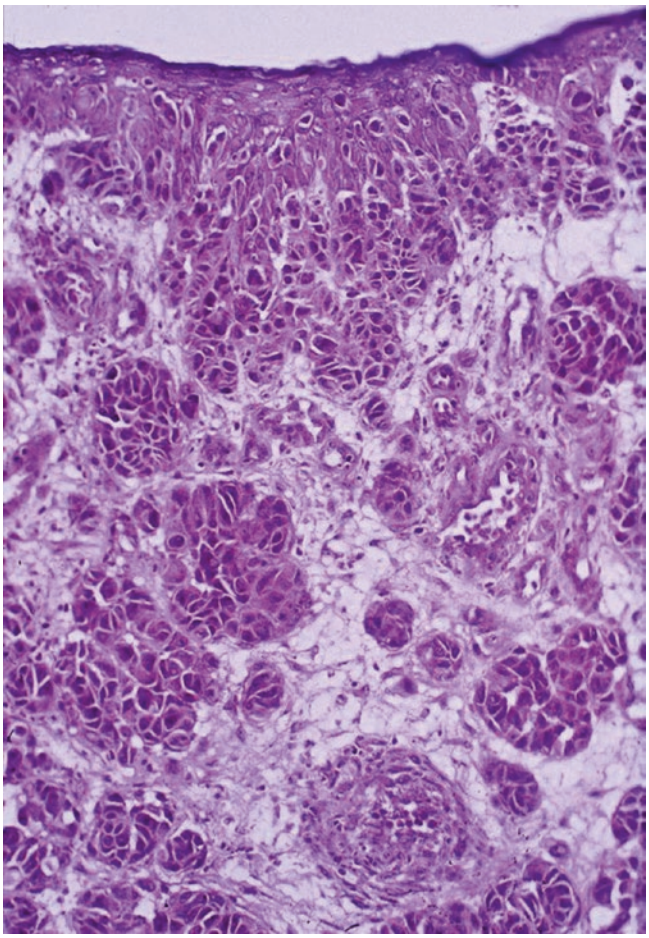

Fig. 2.74 Malignant mucosal melanoma: nests of nonpigmented, downward invasive malignant melanocytes arising from metaplastic squamous epithelium showing junctional activity

Genetics An increased frequency of c-KIT (CD117) aberrations has been observed in PMMMs, while this is not the case in cutaneous melanomas [397]. Conversely, *BRAF* mutations that are increased in cutaneous melanomas are uncommon in PMMMs [398, 399]. Recently, it has been reported that in sinonasal PMMMs *NRAS* mutations and *CCDN1* amplification are more frequent than *KIT* or *BRAF* mutations [398]. Loss of p16 expression, *CDKN2A* mutations, and loss of heterozygosity are observed in up to 50 % of PMMMs [400, 401].

Macroscopy Sinonasal malignant melanomas are either pigmented (black-brown) or nonpigmented (pink-tan) lesions. In the nasal cavity, they commonly arise in the anterior portion of the septum and present as tan-brown polypoid formations, with occasional ulcerated and hemorrhagic areas (Fig. 2.75). When arising within sinuses, they present as extensive and widely infiltrative tumors. The development of intranasal malignant melanoma in inverted papilloma has been reported [402].

Microscopy The histological features of sinonasal melanomas may be as polymorphic as in their cutaneous counterpart. Metastatic disease needs to be ruled out, before they are labeled as primary tumors. Primary melanomas may be recognized by the presence of junctional activity or by the finding of an intraepithelial component in the adjacent mucosa; nevertheless, these features are usually lost in sinonasal mucosa because of the thinness of the surface epithelium and frequent ulceration in advanced stages of the disease. Melanomas are composed of medium- to large-sized cells that may be polyhedral, round, fusiform, pleomorphic, microcytic, or a mixture of them. Usually, they have finely granular cytoplasm and nuclei with one or more eosinophilic nucleoli. Mitotic activity is prominent. A rare balloon cell variant with clear cytoplasm may mimic the various sinonasal salivary gland-type clear cell tumors. Osteocartilaginous differentiation has also been observed [403]. The cells of sinonasal melanoma grow in either solid, loosely cohesive, storiform, pseudo-alveolar, or organoid patterns [388]. Two-thirds of sinonasal melanomas contain some intracytoplasmic brown pigment [388], which has to be confirmed as melanin (Fig. 2.76). In the sinonasal tract, nonpigmented melanomas are not uncommon; in our series in Barcelona, up to 40 % of the sinonasal melanomas are amelanotic (Fig. 2.77). When melanin is scarce or is not found, diagnosis may be difficult, and special techniques are mandatory. Electron microscopy reveals the presence of premelanosomes and/or melanosomes (Fig. 2.78).

Immunohistochemistry The cells of melanotic and amelanotic malignant melanomas are negative for cytokeratin and positive for vimentin, S-100 protein, Melan-A, HMB-45, tyrosinase, microphthalmia-associated transcription factor

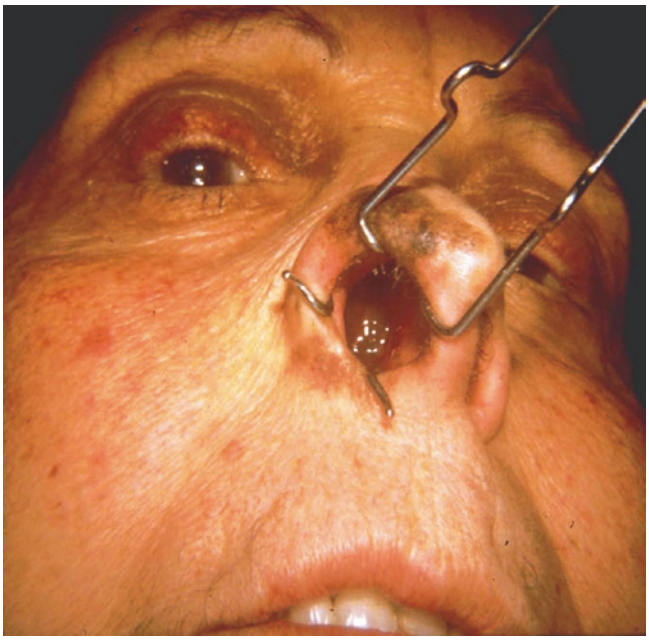

Fig. 2.75 Malignant mucosal melanoma: darkly pigmented polypoid lesion of the anterior nasal cavity in contiguity with a similarly pigmented lesion of the nasal skin (Courtesy of Prof. J. Trasserra. Barcelona, Spain)

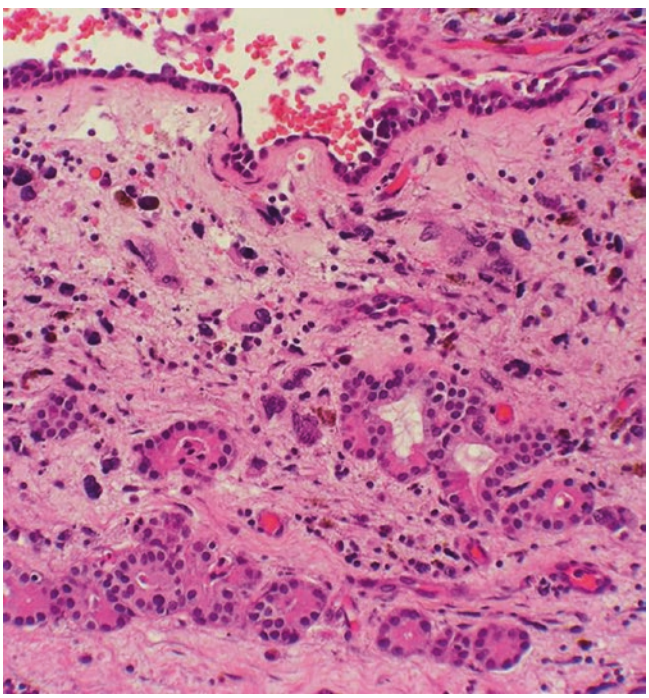

Fig. 2.76 Malignant mucosal melanoma: the tumor grows in the lamina propria beneath the respiratory epithelium and between seromucous glands. The presence of intracytoplasmic brown pigment is recognized

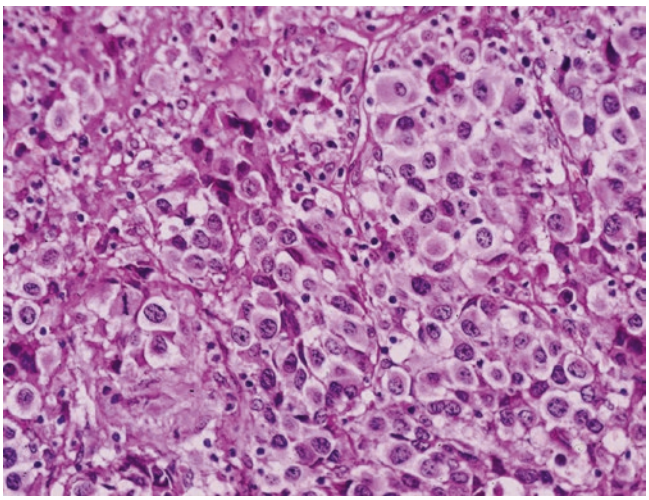

Fig. 2.77 Malignant mucosal melanoma: diffuse growth of nonpigmented malignant melanocytes with delicate fibrous septa forming theca arrangements. This pattern should not be mistaken for non-keratinizing squamous cell carcinoma

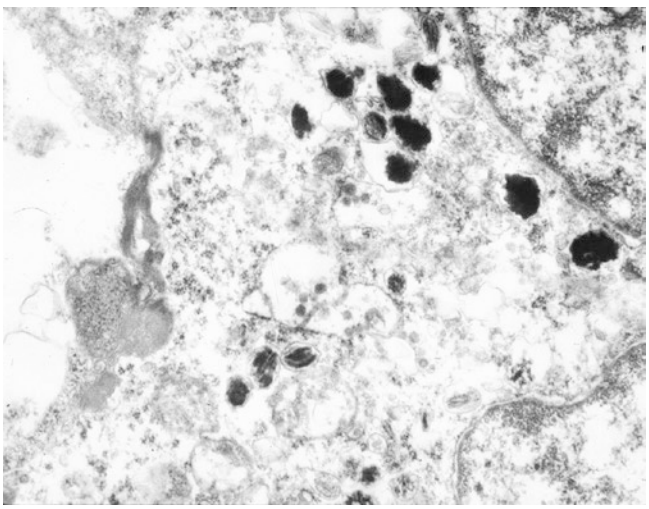

Fig. 2.78 Malignant mucosal melanoma: the ultrastructural hallmark is the presence of melanosomes and premelanosomes in the cytoplasm. The former appear near the nuclear membrane and the latter more peripherally. Desmosomes and tonofilaments are absent (Courtesy of Prof. Bombi, Barcelona, Spain)

(MITF), and SOX10 [404–408]. Loss of p16 expression is seen in 74 % of sinonasal melanomas [409].

Differential diagnosis The recognition of amelanotic malignant melanoma of the sinonasal tract requires ruling out a large list of entities. Epithelioid melanomas have to be mainly distinguished from non-keratinizing squamous cell carcinoma, but also from clear cell carcinomas as well as from epithelioid malignant schwannoma [410] and from metastatic renal cell carcinoma. Microcytic melanoma may mimic SNUC and other small round cell tumors (Table 2.5). Spindle cell melanoma may be mistaken for a variety of spindle cell sarcomas.

Treatment and prognosis The mainstay of treatment is radical surgical resection. Adjuvant radiotherapy seems to improve locoregional control but does not improve overall survival. Systemic therapy should be considered only for patients with metastatic or unresectable locoregional disease [394]. Patients with primary nasal melanomas had significantly better 5-year survival than do patients with melanomas from other head and neck sites [411]. The prognostic significance of the level of local invasion, as established for cutaneous melanomas, does not apply to mucosal melanomas because of the absence of histological landmarks analogous to the papillary and reticular dermis; nevertheless, invasion deeper than 0.5 mm is associated with decreased survival [388].

Although many of the patients do not show initial lymph node involvement or disseminated metastases [388, 412, 413] and have stage I disease at the time of initial diagnosis, the prognosis is bad due to high recurrence rate [389]. This recurrence appears to be related to multicentricity of the tumors and to the anatomic characteristics of the region that preclude adequate resection, which is the treatment of choice [414, 415]. Patients with lower Ki-67 scores showed better survival than those with higher Ki-67 scores [416]. The utility of radiotherapy is controversial but it can be of use in unresectable cases or to control recurrences [415, 417]. Immunotherapy and chemotherapy are also used for metastatic disease [414]. Five-year survival of sinonasal PMMM ranges reportedly between 17 and 47 % [389, 394, 414, 415, 418]. In our series in Barcelona, the 5-year survival is of 35 %, which is similar to that of sinonasal SCC.

2.11.10 Olfactory Neuroblastoma

Definition Olfactory neuroblastoma (ONB) is a malignant tumor unique to the nasal cavity composed of neuroblasts derived from the olfactory mucosa that share neuroepithelial and neuroendocrine features [419–422].

Synonyms Esthesioneuroepithelioma, esthesioneurocytoma, and esthesioneuroblastoma

Epidemiology ONB is an uncommon malignant tumor representing about 2–3 % of all sinonasal neoplasms [383]. ONB can affect patients of all ages and both sexes are equally involved [423]. Although a bimodal age presenta-

tion has been previously suggested, recent reports show an even incidence across all ages with peaks in the fifth and sixth decades [272]. This is clearly different from adrenal neuroblastoma, with most cases arising in children under 4 years of age.

Clinical aspects Nasal obstruction, rhinorrhea, and epistaxis are the most common presenting symptoms. The site of origin of ONB is confined to the olfactory mucosa that lines the upper part of the nasal cavity [424]. Occasionally ONB involves predominantly the superior aspect of the cribriform plate and grows as an intracranial tumor [425, 426]. Ectopic foci of the olfactory mucosa, the Jacobson's or vomeronasal organ, sphenopalatine ganglion, ganglion of loci, and autonomic ganglia of the nasal mucosa are very rare potential sites of origin of ONB [427]. Before establishing a diagnosis of "ectopic" ONB, an extremely rare entity that implies absence of involvement of the olfactory membrane, other sinonasal small round cell tumors have to be carefully ruled out (Table 2.5).

Genetics ONB is characterized by a marked genomic instability with frequent chromosomal losses and gains [428]. ONB lacks the t(11;22) translocation characteristic of PNET [429]. It also lacks the molecular genetic changes of adrenal neuroblastoma, which, in children, may metastasize to the sinonasal region.

Macroscopy ONBs are often unilateral, presenting as smooth polypoid or fungating masses of fleshy consistency and yellow to pink color (Fig. 2.79).

Microscopy ONBs exhibit one of two main patterns of growth that bear diagnostic and prognostic implications [344]. This pattern approach is a valuable complement of the initial scheme proposed by Hyams et al. [114] to grade ONB in four groups (Table 2.7). The low-grade pattern comprises grades I–II of Hyams and the high-grade pattern grades III–IV. The low-grade pattern is seen most often, and it presents lobular arrangements with well-defined groups of tumor cells separated by abundant edematous and variably vascularized stroma (Fig. 2.80). Prominent vascularization may cause bleeding at the time of biopsy. The neoplastic neuroblasts are typically small, showing round to oval nuclei with stippled chromatin, absent or small nucleoli, and minimal cytoplasm; occasionally clear cell type cytoplasms may be found. Neuroblasts are commonly separated by a neurofibrillary matrix formed by neuronal cell processes, in which axons may be demonstrable (Fig. 2.81). This background, seen in about 85 % of ONB, is the most helpful diagnostic feature. Homer Wright pseudorosettes are quite characteristic of ONB; however, they are less commonly seen. They form when the tumor cells surround the neurofibrillary matrix in collar-like arrangements. Perivascular pseudorosettes, formed by tumor cells arranged around capillaries, are of no diagnostic value, for they may be found in several types of neoplasms.

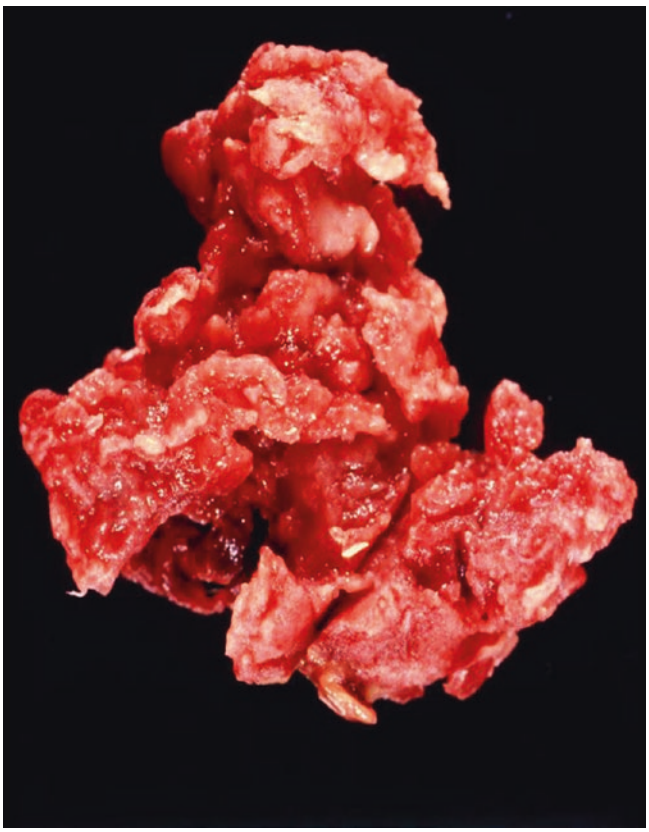

Fig. 2.79 Olfactory neuroblastoma: polypoid mass of fleshy consistency and pink color

Table 2.7 Olfactory neuroblastoma

Histological grades	I	II	III	IV
Lobular architecture	Present	Present	±	±
Mitotic activity	Absent	Present	Prominent	Marked
Nuclear pleomorphism	Absent	Moderate	Prominent	Marked
Fibrillary matrix	Prominent	Moderate	Slight	Absent
Rosettes	H-W ±	H-W ±	Flexner ±	Absent
Necrosis	Absent	Absent	Occasional	Common
Calcification	±	±	Absent	Absent

Hyams grading scheme

H-W Homer Wright rosettes, ± present or absent

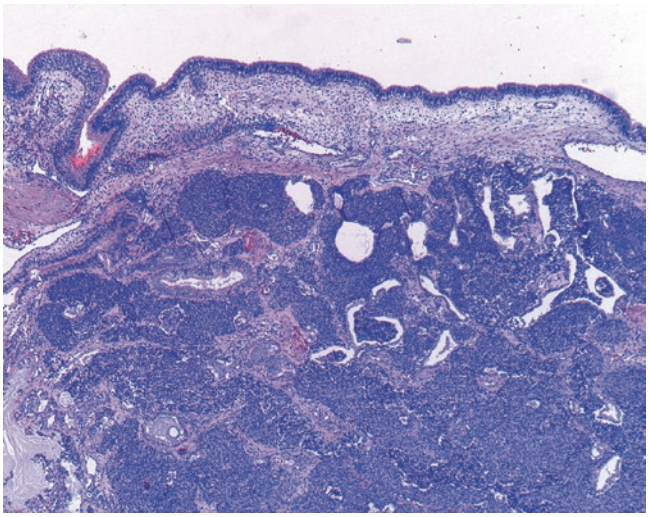

Fig. 2.80 Olfactory neuroblastoma: beneath an intact olfactory mucosa, the low-grade pattern displays well-defined lobular arrangements of tumor cells separated by edematous and variably vascularized stroma

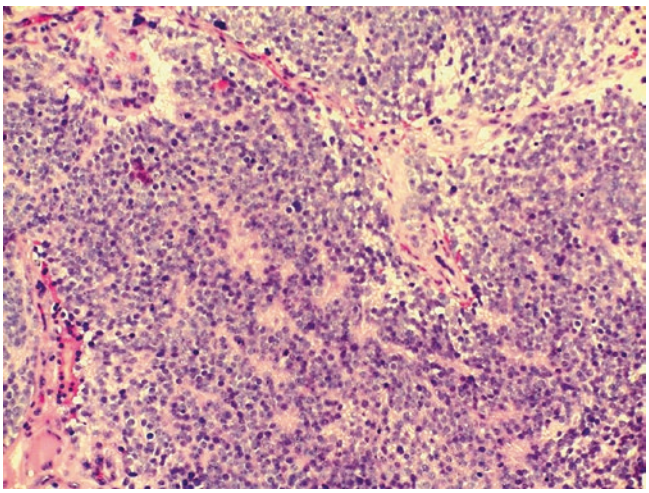

Fig. 2.82 Olfactory neuroblastoma: the high-grade pattern presents diffuse sheets of cells, often with irregular nuclei and compact chromatin. The stroma is scant but well vascularized

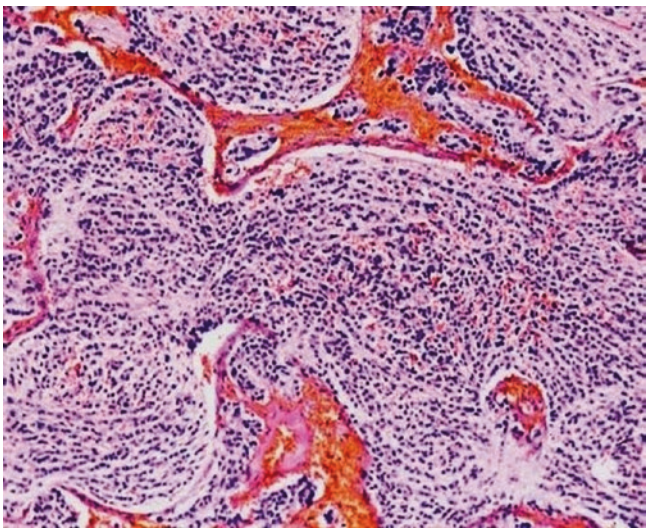

Fig. 2.81 Olfactory neuroblastoma: the neuroblasts appear separated by a neurofibrillary matrix formed by neuronal cell processes

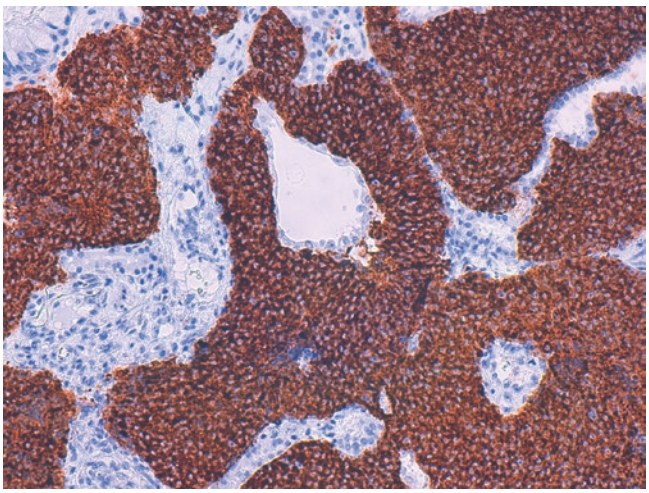

Fig. 2.83 Olfactory neuroblastoma: the neuroblasts that surround entrapped glands show strong and diffuse positivity for synaptophysin

Less frequently seen is the high-grade pattern of ONB, in which the tumor grows as diffuse sheets of cells with presence of foci of necrosis and scanty but highly vascular stroma (Fig. 2.82). True olfactory Flexner-Wintersteiner rosettes are only seen in grade III ONBs; this uncommon type of rosettes depicts well-defined lumina lined by columnar cells resembling olfactory epithelium. These cells generally have basally located nuclei and merge with the adjacent neuroblasts without any intervening basal lamina. Grade IV ONBs are anaplastic tumors and usually show pleomorphic nuclei, prominent eosinophilic nucleoli, increased mitotic rate, and conspicuous necrosis [427]. Very rarely, ONB may exhibit melanocytic or rhabdomioblastic differentiation [430–432]. Exceptional examples of mixed ONB and carcinoma have also been reported [433].

Immunohistochemistry and electron microscopy ONB shows diffuse positivity for synaptophysin, CD56, and NSE (Fig. 2.83). Chromogranin is less often positive. In tumors with a nesting pattern, S-100 protein is positive in the peripheral sustentacular cells. Cytokeratin is generally negative, although in ONB with nesting pattern, a few tumors may exhibit focal staining for low molecular weight CKs. EMA is negative. Neurofilament protein and other markers of neural

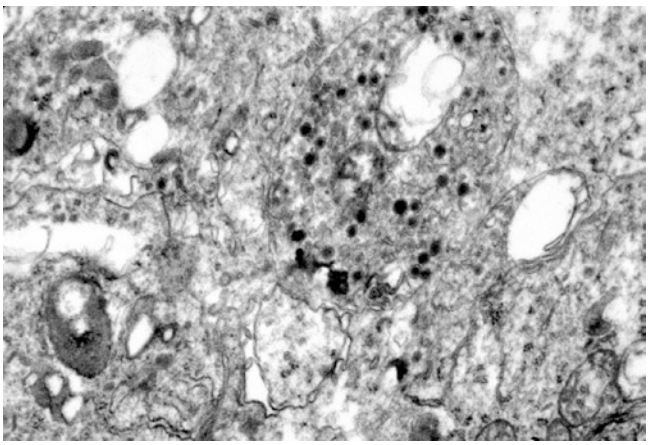

Fig. 2.84 Olfactory neuroblastoma: ultrastructural presence of neuroblastic differentiation, with neuritic processes, neurotubules, and membrane-bound dense-core granules, is seen (Courtesy of Prof. J. A. Bombí, Barcelona, Spain)

Table 2.8 Olfactory neuroblastoma

Kadish* – Morita** Staging		
Stage	Distribution (%)	5-year survival (%)
A* Confined to the nasal cavity	18	90
B* Involves the nasal cavity and paranasal sinuses	32	70
C* Beyond sinonasal cavities	50	40
D** Cervical lymphadenopathy and distant metastasis		<40

differentiation are more often expressed in tumors with diffuse, sheetlike pattern [421, 434–436]. Electron microscopy shows evidence of neuroblastic differentiation, demonstrating neuritic processes, neurotubules, and membrane-bound dense-core granules (Fig. 2.84) [437–439]. The human analogue of achaete-scute gene *HASH1*, expressed in immature olfactory neurons, is also expressed in olfactory neuroblastoma [440]. Conversely the olfactory marker protein [441], expressed exclusively in mature olfactory neurons, is not. ONB lacks CD 99 (MIC-2) expression [429, 442]. In a recent study, intense expression of olfactory-specific sensory transduction proteins was found in ONB, indicating that ONB and olfactory sensory neurons share the same lineage and that the detected transduction proteins could serve as specific tumor markers [443].

Differential diagnosis ONB must be distinguished from a wide variety of small round cell tumors arising in the sinonasal region (Table 2.5). While the diagnosis of low-grade ONBs is usually straightforward, particular care has to be taken before diagnosing high-grade ONBs, as glands of sinonasal non-intestinal-type adenocarcinomas should not be mistaken for the Flexner-Wintersteiner rosettes of ONB grade III; likewise the diffuse sheets of cells seen in either sinonasal undifferentiated carcinoma or in small cell neuroendocrine carcinoma may mimic grade IV ONB. Furthermore, ONBs with rhabdomyoblastic differentiation or mixed with carcinoma have to be differentiated from teratocarcinosarcoma and those with melanocytic differentiation from malignant mucosal melanoma [444].

Treatment and prognosis Complete surgical excision with cribriform plate resection, often followed by radiation therapy and/or chemotherapy, seems to be the treatment of choice [419, 445, 446]. In advanced ONB, high-dose chemotherapy and autologous bone marrow transplantation have been used [447, 448]. Staging of ONB is based on the Kadish system [449], in which stage A disease is confined to the nasal cavity, stage B is confined to the nasal cavity and paranasal sinuses, and stage C shows local or distant spread beyond the nasal cavity or sinuses; most tumors present in stage C. This correlates with survival, which is about 90 % for stage A, 70 % for stage B, and 40 % for stage C [449]. Recognizing the poor prognostic implications of regional and distant metastatic disease, adding cervical lymphadenopathy and distant metastasis as a fourth, stage D category was suggested [445], which showed a worse disease-free survival specifically for the D category [450] (Table 2.8). Necrosis is the single histological feature that seems to correlate with poor survival [420]. About two-thirds of recurrences are in the form of local disease, whereas locoregional recurrences, with intracranial extension or involvement of cervical lymph nodes, represent about 20 %, and distant metastases account for the rest [423, 451]. Distant metastases mainly involve the bone and lung [448].

2.11.11 Ewing's Sarcoma/Primitive Neuroectodermal Tumor (EWS/PNET)

Definition An exceedingly rare sinonasal tumor composed of poorly differentiated small round cells that shows varying degrees of neuroectodermal differentiation and originates from a pluripotential neuroectodermal cell progenitor [452, 453].

Epidemiology Approximately 9 % of extraosseous EWS/PNETs arise in the head and neck region [279], and about 20 % of them develop in the sinonasal tract, being the most common site the maxillary sinus, followed by the nasal cavity (Fig. 2.85) [453–455].

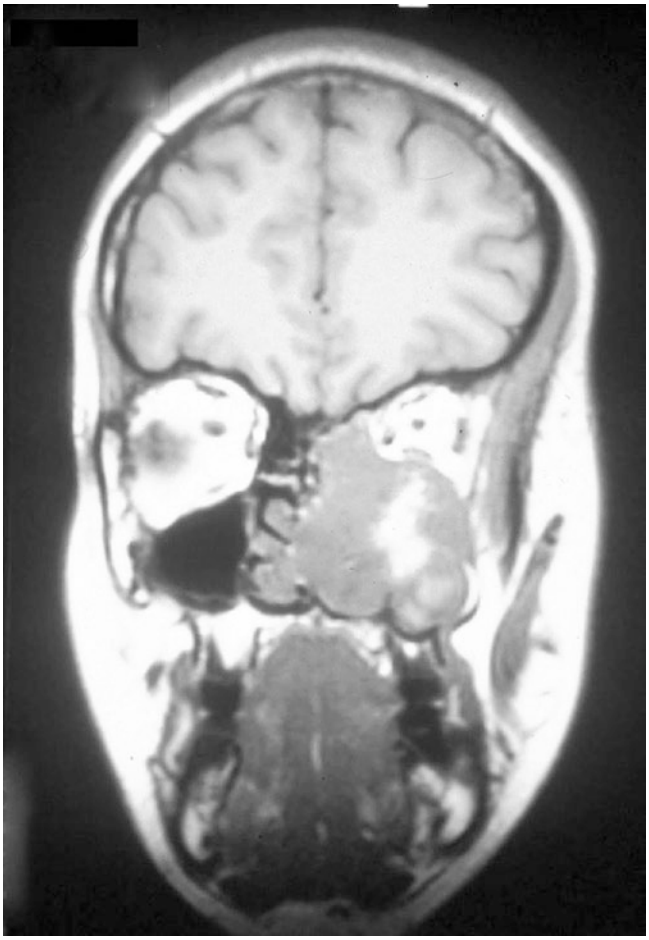

Fig. 2.85 Primitive neuroectodermal tumor: MRI displaying a destructive mass involving the right maxillary sinus, expanding to the orbit and to both sides of the nasal cavity

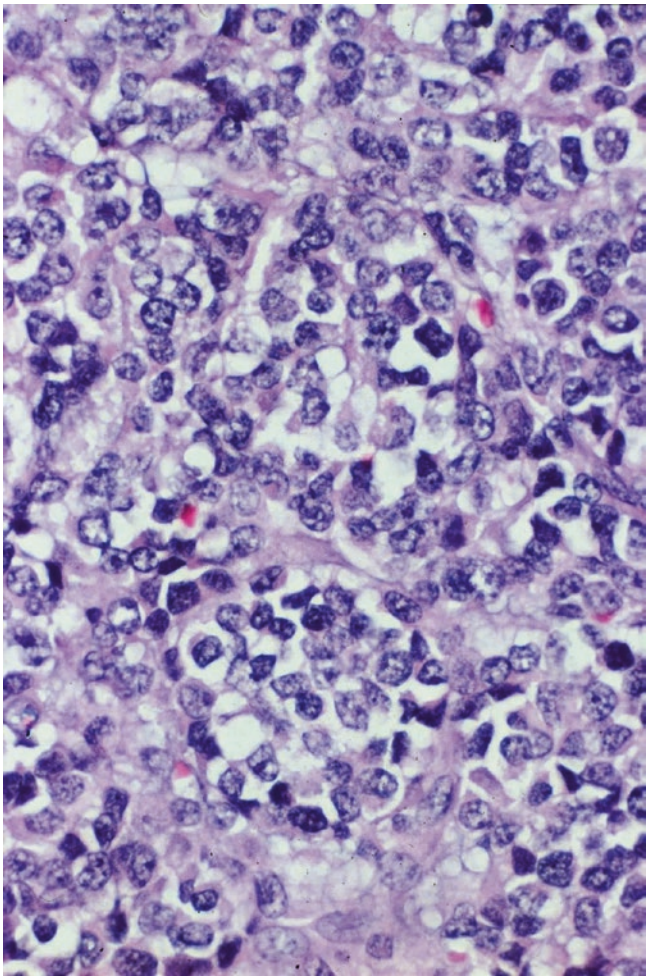

Fig. 2.86 Primitive neuroectodermal tumor: monotonous proliferation of small, round, undifferentiated cells requiring immunohistochemistry for correct typing

Etiology EWS/PNET has been reported following radiotherapy for retinoblastoma [456–458].

Macroscopy Sinonasal EWS/PNET may present as a soft polypoid mass.

Microscopy EWS/PNET is composed of uniform, small, undifferentiated, primitive neuroectodermal cells (Fig. 2.86) [459]. Unusually, pseudorosettes and true rosettes may be found in these tumors.

Electron microscopy EWS/PNET displays rudimentary neuritic differentiation, as well as scanty microtubule formation; dense-core granules are much less abundant than in olfactory neuroblastoma (Fig. 2.87).

Immunohistochemistry The great majority of EWS/PNET will react strongly with antibodies against CD99 (Fig. 2.88). This marker is of considerable value but it is by no means specific. A growing number of other neoplasms expressing

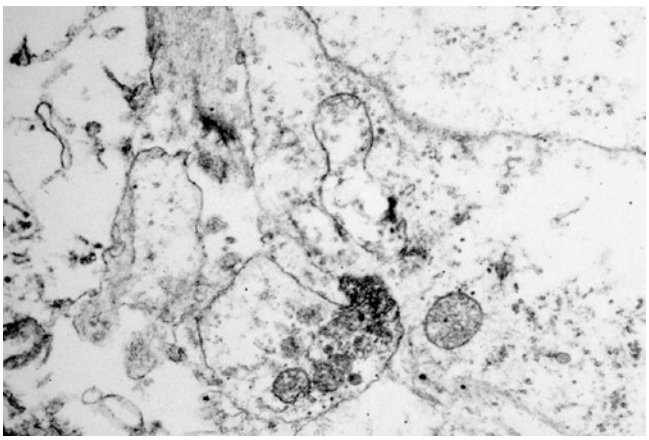

Fig. 2.87 Primitive neuroectodermal tumor: ultrastructurally, a rudimentary neuritic differentiation is seen with scanty microtubule formation. Dense core granules are much less abundant than in olfactory neuroblastoma (Courtesy of Prof. J. A. Bombí, Barcelona, Spain)

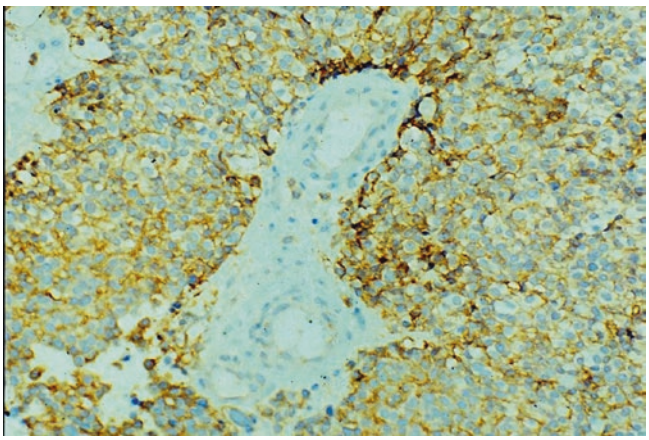

Fig. 2.88 Primitive neuroectodermal tumor: CD99-positive immune reaction seen at the cellular membrane. Molecular study confirmed diagnosis

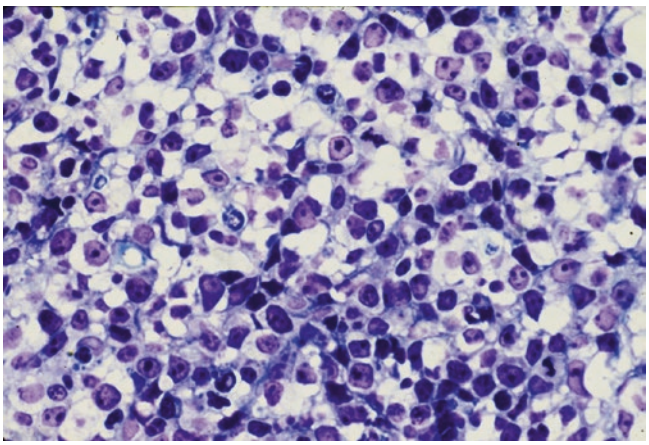

Fig. 2.89 Malignant lymphoma: diffuse large B-cell lymphoma proliferation. Giemsa stain

this protein have been documented. Among these are T-cell lymphomas [442].

Genetics The standard translocation t(11; 22) (q24; q12) of PNET [460] results in the fusion of the *EWS-FLI1* genes. The detection of the chimeric transcript by techniques of molecular biology confirms the diagnosis [461–463].

Differential diagnosis Olfactory neuroblastoma, sinonasal undifferentiated carcinoma, lymphoma, rhabdomyosarcoma, and primary malignant mucosal melanoma are the main entities to rule out [262]. We have seen one example of EWS/PNET arising from the maxillary antrum, which ultrastructurally showed rudimentary neuritic differentiation, as well as scanty microtubule formation. This raised the differential diagnostic dilemma of "ectopic olfactory neuroblastoma"; nevertheless, the tumor cells were CD99 positive and showed the t(11;22)(q24;q12) translocation, findings that are characteristically negative in ONB [429].

Treatment and prognosis Multimodal therapy, which includes chemotherapy, radiotherapy, and surgery, offers the best results. Head and neck EWS/PNET has better prognosis than tumors of other sites. The overall 5-year survival rates reach between 60 and 70 % [453].

2.11.12 Malignant Lymphomas

Definition Malignant lymphomas are small round cell tumors with phenotypic features of B/T cells and variable differentiation.

Epidemiology Sinonasal malignant lymphomas (SNML) account for approximately 13 % of all upper aerodigestive tract lymphomas [205] and for 6 % of all sinonasal malignancies [464]. In our own series, they account for 9.5 % (Table 2.3). In western countries, about 50 % of SNML are of B-cell-type and the other 50 % mostly shows NK-/T-cell lineage [465], whereas other reports point to more variable rates [466–469]. Differently, in oriental populations, most primary lymphomas of the nasal cavity and nasopharynx are of NK-/T-cell lineage [470–473].

Microscopy Sinonasal B-cell lymphomas are in general composed of a diffuse proliferation of large lymphoid cells or of a diffuse mixed pattern of small and large cells (Fig. 2.89). They infiltrate and expand the subepithelial soft tissue and may extend into the underlying bone. Sinonasal B-cell lymphomas lack epitheliotropism, polymorphous cell infiltrate, angiocentricity, prominent necrosis, and fibrosis. They are usually positive for B-cell markers (CD20 and CD79a) and negative for NK-/T-cell markers. κ-light chain restriction is seen more often than λ restriction. EBV markers are often negative.

Sinonasal NK-/T-cell lymphomas were labeled in the past decades with terms such as "lethal midline granuloma," "polymorphic reticulosis," and angiocentric T-cell lymphoma, among others. Patients may present either with an obstructive mass or with midfacial destructive lesions. Histologically, an angiocentric and angiodestructive infiltrate with extensive necrosis and epitheliotropism is frequently seen. In extranodal NK-/T-cell lymphoma, cells may be small, medium sized, large, or anaplastic and may show a conspicuous admixture of inflammatory cells (Fig. 2.90). Pseudoepitheliomatous hyperplasia of the covering epithelium may occur, and when exaggerated, it should not be confused with squamous cell carcinoma [471]. Extranodal NK-/T-cell lymphoma is almost always associated with EBV positivity. The most typical immunophenotype is CD2+, CD56+, surface CD3−, and cytoplasmic CD3ε+. Most cases

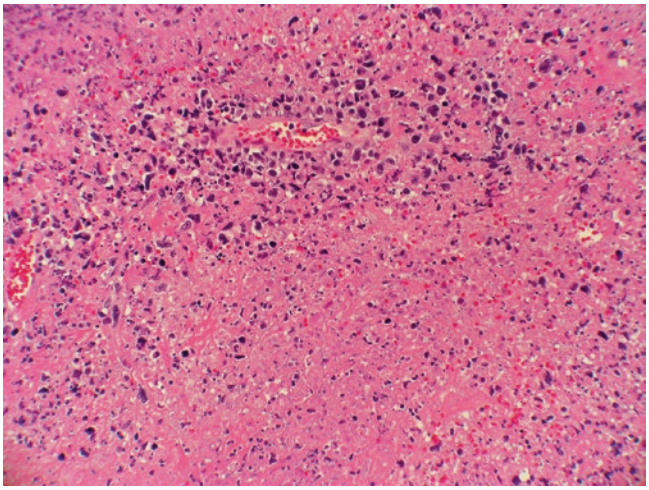

Fig. 2.90 Malignant lymphoma: NK-/T-cell lymphoma nasal type with angiocentric infiltrate of atypical lymphocytes and extensive necrotic areas

are also positive for cytotoxic granule associated proteins (granzyme B, TIA-1, and perforin). Other T- and NK-cell-associated markers are usually negative. Sinonasal lymphomas demonstrating CD3ε+, CD56−, cytotoxic molecule+, and EBV+ are also included within the NK/T category. No specific cytogenetic abnormalities have been identified [474].

Differential diagnosis SNML of either B-cell or T-cell derivation needs a careful distinction of other small round cell tumors (Table 2.5) and with extramedullary plasmacytoma [475, 476], as well as with extramedullary tumors composed of myeloid or lymphoid blasts [477].

Treatment and prognosis Radiotherapy and chemotherapy CHOP regime has been the standard treatment for advanced sinonasal diffuse large B-cell lymphomas [478]. The addition of the anti-CD20 monoclonal antibody Rituximab® has led to a marked improvement in survival [479].

The treatment and prognosis of nasal NK-/T-cell lymphoma are variable. Initial treatment with radiotherapy alone or combined with multiagent chemotherapy is used. Some patients respond well to therapy and others die of disease despite aggressive therapy [472]. In recent years, the survival has improved with more intensive therapy [480]. For a detailed discussion of lymphoid lesions, the reader is referred to Chap. 13.

2.11.13 Extraosseous Plasmacytoma

Definition A mass-forming lesion of monoclonal plasma cells that occurs outside the bone and bone marrow, without evidence of underlying multiple myeloma [481].

Epidemiology More than 80 % of extraosseous plasmacytomas develop in the head and neck region, and 44 % of them involve the sinonasal region [482].

Clinical aspects Full examination of the patient is required to exclude disseminated disease.

Microscopy Plasmacytoma of the sinonasal tract usually appears as a diffuse infiltration of mature plasma cells of the mucosa; occasionally, tumor cells are less differentiated, and diagnosis may be difficult exclusively on histologic basis [475, 476, 483, 484].

Immunohistochemistry Staining for CD138 and κ and λ chains may be helpful. CD19 is nearly always negative and CD56 and CD117 are often aberrantly expressed.

Differential diagnosis Mucosal lymphomas with plasmacytic differentiation, particularly extranodal marginal zone (MALT) lymphoma, may be misinterpreted as extramedullary plasmacytoma.

Treatment and prognosis Most extraosseous plasmacytomas are cured with local radiation therapy. Regional recurrences occur in one-fourth of patients; distant extraosseous metastasis may occasionally occur [482].

2.11.14 Malignant Soft Tissue Tumors

Malignant soft tissues tumors of the sinonasal tract are very rare neoplasms and account for about 5 % of all the malignancies in this territory (Table 2.3). Only the most salient of these entities are covered here. For a detailed discussion of soft tissue tumors, the reader is referred to Chap. 12.

2.11.14.1 Fibrosarcoma

Definition A malignant mesenchymal tumor composed of fibroblast with variable collagen production and in prototypical cases a herringbone pattern [485].

Synonym Adult fibrosarcoma.

Epidemiology Most of head and neck fibrosarcomas occur in the sinonasal tract and are seen across a wide age range [486–489]. They are considered the second most common soft tissue sarcoma after rhabdomyosarcoma in the head and neck [490].

Clinical aspects Fibrosarcomas most commonly cause obstruction and epistaxis [163]. An ethmoid sinus fibrosarcoma arising as a frontal sinus mucocele has been reported [491].

Microscopy The histological appearance is that of a spindle cell lesion, with fascicles or bundles of neoplastic cells intersecting at various angles, sometimes with a herringbone pattern. Most sinonasal fibrosarcomas have a low-grade appearance, with moderate cellularity and low mitotic rate [492]. In accordance, the behavior is more often characterized by repeated local recurrences, while distant metastases are rare.

Differential diagnosis It includes desmoid-type fibromatosis, leiomyosarcoma, nerve sheath tumors, spindle cell carcinoma, and desmoplastic melanoma.

Treatment and prognosis Surgery is the recommended treatment, often followed by radiotherapy [490].

2.11.14.2 Undifferentiated Pleomorphic Sarcoma

Definition The name undifferentiated pleomorphic sarcoma (UPS) has nowadays replaced the until recently used term malignant fibrous histiocytoma (MFH), which was commonly employed as a diagnosis of exclusion for sarcomas mainly composed of myofibroblasts or undifferentiated mesenchymal cells [490].

Epidemiology : A considerably decrease in the frequency of the diagnosis of MFH has occurred following the advent of immunohistochemistry. About 3 % of MFH develop in the head and neck, and 30 % of these arise in the sinonasal region. They most often are seen in adulthood [490].

Etiology MFH represents the most common post-radiation sarcoma, although they are predominantly sporadic.

Microscopy MFH is a high-grade sarcoma, histologically consisting of a proliferation of spindle cells arranged in storiform pattern, intermixed with atypical pleomorphic, often multinucleated giant cells. In the sinonasal tract, it presents as a highly aggressive and destructive lesion, with bone invasion and extension in adjacent structures [166].

Differential diagnosis Before a diagnosis of malignant fibrous histiocytoma is rendered, other pleomorphic malignant tumors, like leiomyosarcoma, osteosarcoma, and sarcomatoid carcinoma, should be excluded by means of immunohistochemical or ultrastructural analysis.

Treatment and prognosis Complete surgical resection is the recommended treatment, often followed by radiotherapy. Prognosis is related to the extension of the tumor.

2.11.14.3 Leiomyosarcoma

Definition Leiomyosarcoma (LMS) of the sinonasal tract is an extremely rare malignant neoplasm, with identical histological and immunophenotypic appearance to its soft tissue counterpart [166].

Epidemiology Sinonasal LMS accounts for less than 1 % of all soft tissue tumors in this region [490].

Microscopy As in other territories, LMS is composed of right-angle intersecting bundles of spindle cells with eosinophilic cytoplasm and "cigar-shaped" nuclei. Foci of necrosis, increased mitotic activity, and cellular atypia are present.

Immunohistochemistry Cells of LMS are reactive for smooth muscle and/or muscle-specific actin, desmin, h-caldesmon, and vimentin.

Differential diagnosis Sinonasal LMS must be distinguished from leiomyoma, glomangiopericytoma, and other spindle cell malignant tumors.

Treatment and prognosis Sinonasal leiomyosarcoma can be regarded as a locally aggressive neoplasm with limited metastatic potential that should be treated by surgery alone if the tumor is limited to the nasal cavity [493]. Adjuvant radiochemotherapy may be used in advanced tumors.

2.11.14.4 Rhabdomyosarcoma

Definition Rhabdomyosarcoma (RMS) is a malignant mesenchymal tumor of skeletal muscle phenotype [490].

Epidemiology RMS is the most common sinonasal malignancy of the pediatric age, [494, 495]. The most common histologic subtypes are the embryonal and the alveolar [494]. RMS is predominantly seen in children and young adults, but they may also occur in older adults, specially the alveolar subtype [490].

Macroscopy The botryoid variant of embryonal RMS has a characteristic grapelike or polypoid appearance, while the other subtypes/variants show an indistinct fish-flesh appearance.

Microscopy Embryonal RMS is characterized by the presence of small, eosinophilic polygonal, or spindled cells with hyperchromatic nuclei and occasional cytoplasmic cross striations; the cell population is usually dense or intermingled with myxoid stroma. Alveolar RMS has fibrous septa, separating clusters of loosely cohesive small round cells with hyperchromatic nuclei and scant eosinophilic cytoplasm; the presence of multinucleated giant cells is a typical feature (Fig. 2.91). Other variants of RMS include sclerosing, spindled, botryoid, and pleomorphic forms [490, 496].

Immunohistochemistry The diagnosis of RMS can be confirmed by immunostaining for myogenin and MyoD1,

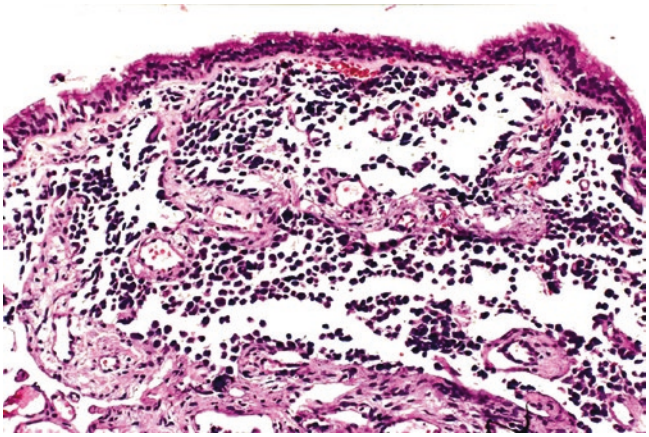

Fig. 2.91 Rhabdomyosarcoma: the alveolar variant depicts fibrous septa separating clusters of loosely cohesive small round cells with hyperchromatic nuclei and scant eosinophilic cytoplasm; notice the presence of multinucleated giant cells, another typical feature

which are nuclear markers with high specificity for skeletal muscle differentiation. Less specific are desmin, muscle-specific actin, and myoglobin.

Genetics Alveolar RMS typically harbors t(2;13) or t(1;13) translocations resulting in *PAX3-FOXO1A* or *PAX7-FOXO1A* gene fusions. Embryonal RMS harbors more complex genetic alterations, such as loss of the tumor suppressor *CDKN2A*, mutation/amplification of *FGFR4*, gain of *GLI1*, and mutations in the myogenic transcription factor *MYOD1* [497–499].

Differential diagnosis It includes all sinonasal undifferentiated small round cell tumors. Furthermore, it must be kept in mind that rhabdomyoblastic differentiation may be encountered in tumors other than RMS [444]. This fact is important because RMS is treated by specific chemotherapy protocols that may be different than those of other tumors in the differential diagnosis.

Treatment and prognosis Treatment includes a combination of radiotherapy and chemotherapy, with surgical resection reserved for residual disease. The risk for neck involvement is high. With the advent of more aggressive therapy, the overall 5-year survival has increased from 40 to 70 % [500]. Adult age and alveolar subtype are adverse prognostic factors in RMS.

2.11.15 Malignant Peripheral Nerve Sheath Tumors

Definition A malignant tumor of nerve sheath phenotype [490].

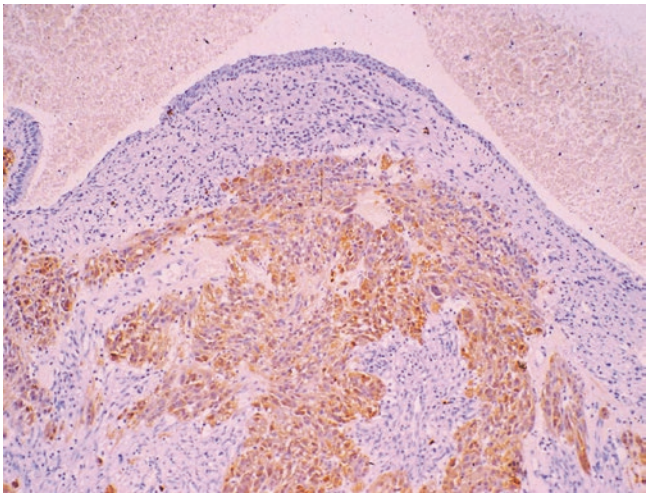

Fig. 2.92 Epithelioid MPNST marked S-100 protein positivity in a large cell malignant neoplasm, mimicking amelanotic melanoma. Additional immunohistochemistry and electron microscopy resulted confirmatory

Synonyms Neurofibrosarcoma, malignant schwannoma, and neurogenic sarcoma

Epidemiology The head and neck is one of the more common anatomic areas to be affected by malignant peripheral nerve sheath tumors (MPNSTs) [501]. MPNST of the sinonasal tract is a very rare neoplasm [502]. Most arise de novo or less often in the context of neurofibromatosis type 1 (NF1) [503–505]. There is female predominance for the novo sinonasal MPNST [492] and male predominance in NF1-associated MPNST [490, 506].

Etiology Radiation and immunosuppression may be causative agents of MPNSTs [507].

Microscopy MPNSTs typically grow in a herringbone-type fascicular pattern. MPNST is a highly cellular spindle cell proliferation and exhibits nuclear hyperchromasia, pleomorphism, elevated mitotic rates, and necrosis. Typically, dark hypercellular areas alternate with light, less cellular ones, conferring a so-called "marbleized" appearance [501]. Sinonasal MPNSTs are often low grade, in contrast to those arising at other sites [492]. In poorly differentiated MPNSTs, the diagnosis can be based on the identification of a preexisting neurofibroma. The epithelioid variant of MPNST has been described in the sinonasal tract and may mimic amelanotic malignant melanoma (Fig. 2.92) [410]. Some tumors may show morphological and immunohistochemical features of skeletal muscle differentiation and are designated as "malignant Triton tumor" [492, 508]. The majority of malignant Triton tumors occur in the setting of NF1. About a third of malignant Triton tumors involve the head and neck [444].

Immunohistochemistry In MPNSTs the nerve sheath markers S100 and SOX10 are positive, but usually have focal distribution; in contrast, epithelioid MPNSTs stain diffusely for S-100.

Genetics Both NF1 alleles are inactivated in MPNSTs ex neurofibroma associated with NF1.

Differential diagnosis It includes fibrosarcoma, leiomyosarcoma, synovial sarcoma, spindle cell carcinoma, and malignant melanoma. Furthermore, positivity for S-100 in a spindle cell sarcoma as present in MPNST may also be shown by the recently described sinonasal biphenotypic sarcoma, a low grade sarcoma that shows immunohistochemically demonstrable neurogenic and myogenic differentiation [509].

Treatment and prognosis Surgical removal is the mainstay treatment that may be followed by radio- and chemotherapy.

2.11.16 Biphenotypic Sinonasal Sarcoma

A new entity known as low-grade, biphenotypic, sinonasal sarcoma has been recently recognized [509]. In addition to distinguishing histological and immunohistochemical features, this tumor is often hallmarked by a recurrent *PAX3-MALM3* gene fusion [510]. It occurs in adults, predominantly women, being characterized by a submucosal proliferation of spindle cells with scant mitotic activity and concomitant neural and myogenic differentiation. Benign glandular proliferation is often present; the majority of glands are lined by respiratory type of epithelium, but areas of oncocytic and squamous metaplasia may be encountered. Hemangiopericytoma - like blood vessels are conspicuous. Focal rhabdomyoblastic differentiation may be seen. Bone invasion emphasizes the infiltrative nature of the lesion. Local recurrence rate approaches 45%, but no patient has developed metastases or died of disease.

2.12 Germ Cell Tumors

Teratoma is the principal benign germ cell tumor of the sinonasal region and shows histological features similar to its counterparts in the gonads and in other extragonadal locations. Malignant tumors with histological features similar to germ cell tumors of the gonads arise on rare occasions in the sinonasal tract. Immature teratomas and teratomas with malignant transformation are tumors of infancy and early childhood, whereas sinonasal endodermal sinus tumors and sinonasal teratocarcinosarcoma have only been documented in adults [511].

2.12.1 Dermoid Cyst

Definition A dermoid cyst is a developmental lesion histogenetically and histologically composed of ectoderm and mesoderm, but no endoderm [511].

Synonyms Nasal dermoid sinus cyst and cystic dermoid

Epidemiology Dermoid cysts of the nose comprise 3 % of all dermoids and 5.5–12 % of those of the head and neck region [512, 513]. A male predominance has been described for cystic dermoids. More than half are detected in children 6 years old or less, and approximately a third are present at birth [514]. Dermoid cysts of the head and neck are located more often in the subcutaneous tissue of the lateral supraorbital ridge and nose. In the nose, they occur most commonly in the bridge and always in the midline. The glabella, nasal tip, and columella are less common sites [512–515]. A few cases have been described as originating in the paranasal sinuses [516].

Etiopathogenesis The most likely explanation for the ontogeny of dermoid cysts is the retention of ectodermal tissue along the lines of closure at junctions of bones, soft tissues, and embryonic membranes [515].

Clinical features Nasal dermoid cysts manifest as a midline nasal pit, fistula, or subcutaneous infected mass. They may cause broadening of the nasal bridge and occasionally cellulitis or purulent discharge. On palpation, the cysts are soft to fluctuant with a pale yellowish-pink color noted beneath the thinned but intact epithelium; when keratin debris and sebum fill the lumen, they may have a doughy consistency [512–515]. Most patients do not have other malformations, but 6–41 % have associated congenital malformations [517, 518]. Computed tomography and magnetic resonance imaging scans are valuable in determining the intracranial and nasal components of a lesion and excluding encephalocele [512–516].

Macroscopy The cysts may range in size from a few millimeters to 12 cm in diameter. The lumen contains cheesy, yellow-white material.

Microscopy Dermoid cysts are lined with mature keratinizing squamous epithelium and frequently contain appendages of the skin in the cyst wall but no endoderm.

Differential diagnosis This lesion is differentiated from a teratoma by the limited variety of tissue types and the absence of endodermal components. Epidermal inclusion cysts may resemble cystic dermoids but do not contain adnexa. Epidermal inclusion cysts occur more frequently in

adults, in contrast to dermoids, which are more commonly found in children and young adolescents [514–516]. Dermoid cysts should be clinically differentiated from encephalocele, which occurs in the same anatomic area.

Treatment and prognosis Dermoid cysts are treated by complete surgical excision, regardless of the extent of the lesion. The recurrence rate has been reported to be less than 7 % [512–515].

2.12.2 Mature Teratoma

Definition Mature teratomas are tumors composed of a variety of mature tissues that are foreign to their sites of occurrence. There are typically tissues derived from two or three germ layers [511].

Synonyms Teratoid tumor, benign teratoma and mature cystic teratoma

Epidemiology Teratomas of the head and neck region account for only 6 % of all teratomas [519, 520]. Mature teratomas in the sinonasal tract are even more unusual [521]. The majority of sinonasal teratomas occur in neonates and infants, and an equal sex distribution has been reported [521, 522]. Stillbirth, prematurity, fetal malpresentation, dystocia, and maternal polyhydramnios are frequent accompaniments. The orbit, oropharynx, and neck are classic locations for mature teratomas of the head and neck, but these tumors have been found rarely in the sinonasal tract [521]. In the sinonasal tract, the maxillary antrum and nasal cavity are affected more often than is the sphenoid sinus [519, 523–525].

Etiopathogenesis The exact origin of teratomas is not yet known, although numerous theories have been presented. The most popular theories of their origin are that they derive from primordial germ cells or from primitive somatic cells that escaped the influence of organizers and inducers [520].

Clinical features Manifestations of teratomas depend on the specific location of the tumors. Signs and symptoms usually result from compression of adjacent organs and tissues. Facial deformity, nasal obstruction, and a nasal mass are common manifestations of sinonasal teratomas. The occasional calcifications seen in computed tomography and magnetic resonance imaging scan provide the most valuable aids in resolving the differential diagnosis [519, 521, 524]. Teratomas may be associated with other skull deformities, anencephaly, hemicrania, and palatal fissures [519].

Macroscopy The tumors are usually cystic, but they can be solid or multilocular. They are commonly encapsulated masses that measure up to 7 cm at their largest dimension.

Microscopy Teratomas are composed of varied admixtures of mature skin, appendages of the skin, fat, glial tissue, smooth muscle, cartilage, bone, minor salivary glands, and respiratory and gastrointestinal epithelium. Neural tissues may be seen more often in sinonasal teratomas than in other teratomas.

Differential diagnosis Although the variegated histological appearance of mature teratomas is usually diagnostic, nasal glial heterotopia and meningocele should be considered in the differential diagnosis. The presence of immature elements or any other germ cell tumor excludes mature teratoma.

Treatment and prognosis Complete surgical excision has been curative in the few cases of sinonasal mature teratomas reported in the literature.

2.12.3 Immature Teratoma

Definition Immature teratomas are composed of variable quantities of immature tissue elements, mostly neuroepithelial, that appear interspersed with mature tissues derived from the three embryonic germ layers [511].

Synonym Teratoma with immature elements

Epidemiology Immature teratomas are tumors of infancy and childhood [121].

Etiopathogenesis The histogenesis of this type of tumor remains unsettled, as it is the case for mature teratomas. Either the displaced, persistent germ cell theory or the possibility of an alternative progenitor cell has been discussed [526].

Clinical features Symptoms are not specific. Nasal discharge and airway obstruction are common. Imaging procedures show expansive growth without invasive destruction.

Macroscopy In contrast to mature teratomas that are usually cystic, immature teratomas tend to be either solid-nodular or a combination of solid and cystic tumor masses; however, this is not a consistent observation.

Microscopy The distinction between mature and immature teratomas is based on their microscopic appearances.

The tumor may contain cystic spaces lined by mature ciliated pseudostratified epithelium and immature areas with primitive neuroepithelial rosettes lined with multilayered neuroblasts. Mitotic figures are frequently present in the immature arrangements; however, cellular atypia is not found.

Differential diagnosis In infants and children, a teratoma with malignant transformation has to be excluded. In adult patients, thorough sampling of the specimen is mandatory to rule out teratocarcinosarcoma.

Treatment and prognosis Complete surgical excision is usually an effective treatment. Despite the immaturity of its tissue elements and of the presence of mitotic figures, immature teratomas rarely behave in a malignant fashion [526].

2.12.4 Teratoma with Malignant Transformation

Definition Teratoma with malignant transformation is a neoplasm containing benign tissue elements of all three germinal layers and, in addition, a specific type of malignant tumor [511].

Synonym Malignant teratoma

Epidemiology In the head and neck, malignant transformation of a teratoma is a distinctly uncommon observation. Involvement of the sinonasal tract by such a lesion is extremely rare. Kuhn et al. reported of a case of squamous cell carcinoma arising in a benign teratoma of the maxilla of a 13-month-old boy [527]. Petrovich et al. reported a nasal malignant teratoma in a 63-year-old man [528].

Clinical features A fluctuating left facial swelling occurred during a period of 9 months prior to the diagnosis. On computed tomography scans, thickened left maxillary sinus mucoperiostium and a soft tissue defect were observed over the alveolar ridge. Metastatic disease was not found.

Macroscopy A soft tissue mass of 2.0-cm diameter in the left maxillary alveolar ridge with displacement of unerupted teeth has been noted [527].

Microscopy The tumor was composed of variable mature tissue elements of ectodermal, mesodermal, and endodermal derivation consistent with extragonadal teratoma. An additional finding was the presence of an atypical squamous proliferation with the features of squamous cell carcinoma [527].

Differential diagnosis It includes immature teratoma with pseudocarcinomatous proliferation of the squamous epithelium and odontogenic cyst.

Treatment and prognosis The tumor reported by Kuhn et al. was locally aggressive and recurred after surgery. There was no evidence of further recurrence 2 years after chemotherapy [527].

2.12.5 Yolk Sac Tumor

Definition Yolk sac tumor (YST) of the sinonasal tract is a primary malignant neoplasm found to arise in this location that has histological features indistinguishable from yolk sac tumor of the gonads [511].

Synonyms Endodermal sinus tumor, yolk sac carcinoma, and orchioblastoma

Epidemiology Only 20 % of YSTs are extragonadal [529]. Head and neck YSTs are very rare, and similarly to the gonadal counterpart, they have two distinct peaks of incidence; the most common one is seen in the early years of life and the less frequent in adult age [526, 529–533].

Pathogenesis The development of a germ cell malignancy does not need always to be explained by the neoplastic transformation of a primordial germ cell. Alternatively, YSTs of the adult may evolve from precursor somatic neoplastic cells by a process of divergent differentiation toward structures resembling the fetal yolk sac [534].

Clinical aspects While the YSTs that develop in infancy and childhood may be associated or not to a teratoma, those occurring in adult patients may associate or not to a somatic carcinoma [534–538]. The two sinonasal YSTs reported in adults developed in men aged 43 and 59 years [534, 537]. Both tumors occupied the paranasal sinuses with focal orbital and cranial destruction. YSTs are known to secrete alpha-fetoprotein (AFP). A case of sinonasal YST admixed with choriocarcinoma has been documented [535].

Macroscopy YSTs tend to be gray white to yellow, focally hemorrhagic.

Microscopy The most characteristic pattern of growth of YSTs is composed of pseudopapillary structures with numerous glomeruloid or perivascular Schiller-Duval bodies and labyrinthine cavities and channels lined by flattened to cuboidal epithelium with various degrees of atypia (Fig. 2.93). Another common pattern is the reticular or microcystic, in which eosinophilic hyaline globules, PAS

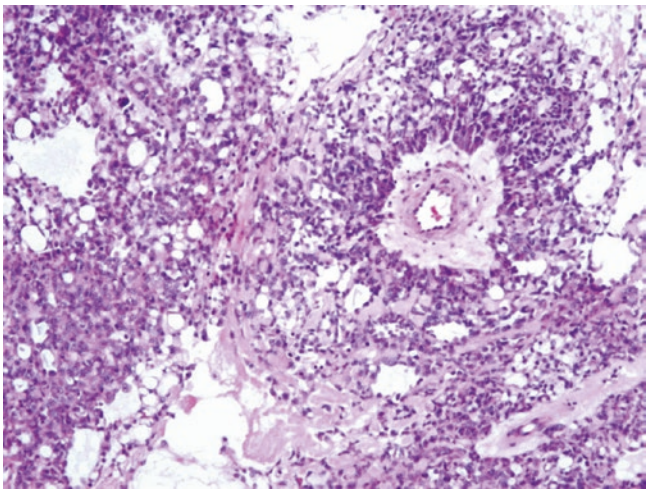

Fig. 2.93 Yolk sac tumor: perivascular Schiller-Duval body and pseudopapillary structures forming labyrinthine cavities and channels lined by flattened to cuboidal epithelium

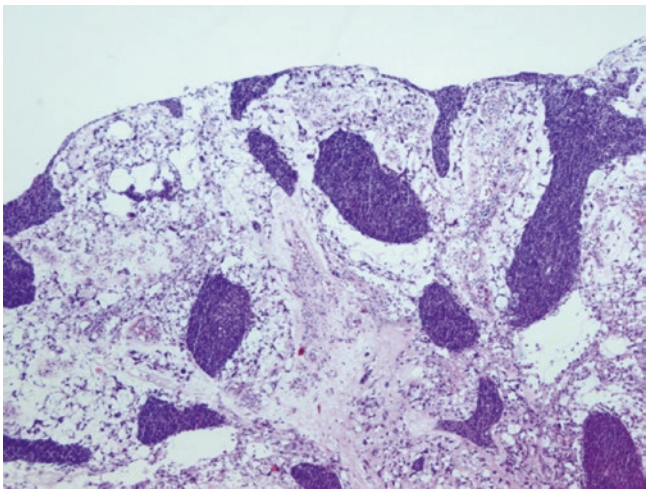

Fig. 2.94 Yolk sac tumor: nests and cords of immature cells displaying the solid pattern of growth are surrounded with reticular and microcystic structures (Courtesy of Dr. Prantl, Munich, Germany)

positive and diastase resistant, are found intracellularly and extracellularly. The solid pattern is composed of densely cellular nests and cords of immature elements that may differentiate into somatic endodermal derivatives (Fig. 2.94). Another pattern is the polyvesicular vitelline. Surrounding these tissue patterns, there are variable amounts of reactive stromal component.

Immunohistochemistry AFP is the characteristic marker of the pseudopapillary and reticular structures of YSTs. The solid pattern mainly immunoreacts with wide-spectrum cytokeratin cocktails and CK20. Beta-HCG is negative in pure YSTs, unless combined with choriocarcinoma [535].

Differential diagnosis Sinonasal YSTs must be distinguished from other germ cell tumors occurring in this region, mainly from teratocarcinosarcoma. The solid pattern of YSTs must be distinguished from somatic malignancies with undifferentiated carcinoma component and from basaloid non-keratinizing squamous cell carcinoma.

Treatment and prognosis Complete excision, whenever possible, followed with radiochemotherapy is the recommended treatment of YSTs. Owing to the aggressive behavior of these gonadal or extragonadal tumors in adult patients, platinum-based therapy should be added [533].

2.12.6 Teratocarcinosarcoma

Definition Sinonasal teratocarcinosarcoma (SNTCS) is a complex malignant sinonasal neoplasm combining features of teratoma and carcinosarcoma. Benign and malignant epithelial, mesenchymal, and neural elements are typically present, including immature tissue with blastomatous features [511, 539].

Synonyms Teratocarcinoma, teratoid carcinosarcoma. blastoma

Epidemiology SNTCS is very rare [540]. Patients are exclusively adults, with ages ranging from 18 to 79 years (mean 60 years) [539–544]. There is a marked male predominance. SNTCS almost exclusively arises in the ethmoid sinus and maxillary antrum, although it may arise in other head and neck territories [539, 545, 546].

Etiology and pathogenesis SNTCS is unlikely to be a germ cell tumor, but probably arises from pluripotent stem cells of the neuroepithelium that not only reproduce the neuroectodermal features of olfactory neuroblastoma but also have the capacity to differentiate into divergent types of somatic cells [543]. In contrast with malignant gonadal teratomas, which are frequently found in patients at younger age, SNTCS does not contain areas of embryonal carcinoma, choriocarcinoma, or seminoma as seen in many germ cell tumors [539].

Clinical aspects Patients present with a short history of nasal obstruction and epistaxis. Imaging studies reveal a nasal mass occasionally accompanied by opacification of the paranasal sinuses. Bone destruction may be seen [539].

Macroscopy Tumors are usually bulky, soft to rubbery, and red tan to purple. A mass filling the nose and projecting for about 3 cm from the naris has been documented [547].

Microscopy SNTCS is made up of multiple tissue types derived from two or three germ layers, often forming cystic spaces and exhibiting variable degrees of maturity and undifferentiated/primitive component (Fig. 2.95). In addition there are carcinomatous and sarcomatous components [540, 544]. The epithelial component includes keratinizing and non-keratinizing squamous epithelium, pseudostratified columnar ciliated epithelium, and glandular structures lined by either cuboidal or columnar cells that may show mucous differentiation (Fig. 2.96). Nests of immature squamous cells containing clear cells which are "fetal appearing" are a common finding and an important diagnostic clue [539]. The carcinomatous component is usually glandular, but sometimes squamous. Neuroepithelial elements with rosettes and neuroblastoma-like areas are in most instances present (Fig. 2.97). These epithelial and neuroepithelial elements occur in close relationship with each other and with mesenchymal elements. The most prominent mesenchymal elements are immature cells with oval or elongated nuclei. The mesenchymal cells may exhibit skeletal muscle differentiation with cross striations and bizarre formations (Fig. 2.98). Foci of cartilage, smooth muscle, adipose tissue, and fibrovascular tissues may also be present. There may be proliferation of small round cells that are difficult to classify. Mitotic activity and cytological features of malignancy are demonstrable in the undifferentiated areas of both the epithelial and mesenchymal elements [540].

Immunohistochemistry The undifferentiated/primitive component often shows positive immunoreaction for CD99 and occasionally for synaptophysin and S-100 protein [543]. The spindle cell component is consistently positive for vimentin and sometimes for desmin, myoglobin, and glial fibrillary acidic protein. The neuroepithelial component is positive for neuron-specific enolase and occasionally for chromogranin, alpha-fetoprotein, cytokeratin, and neurofilaments [540]. The epithelial component is positive for cytokeratins, epithelial membrane antigen, and occasionally S-100 protein and glial fibrillary acidic protein.

Differential diagnosis Small biopsies and/or inadequate sampling of SNTCS specimens may lead to erroneous diagnoses of olfactory neuroblastoma, squamous cell carcinoma, undifferentiated carcinoma, adenocarcinoma, malignant salivary gland-type tumors, and adenosquamous carcinoma [539].

Treatment and prognosis Aggressive initial therapy with a combination of surgical resection, radiotherapy, and chemotherapy is usually recommended [539]. SNTCSs are locally aggressive tumors, with rapid invasion of soft tissues and bone, and metastasize to regional lymph nodes and

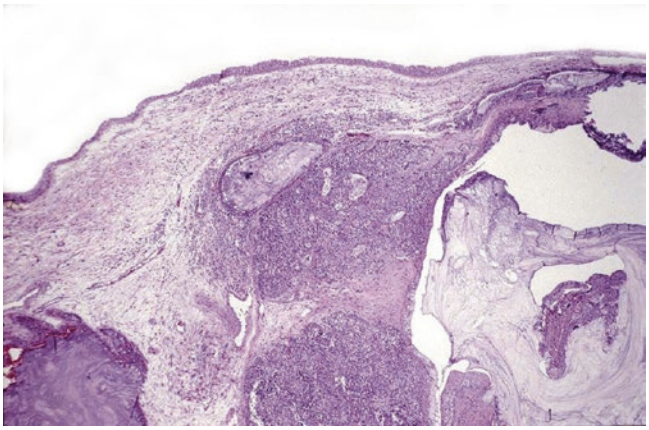

Fig. 2.95 Teratocarcinosarcoma: cystic spaces filled with mucin are partly covered by benign columnar epithelium and surrounded by immature blastematous tissue

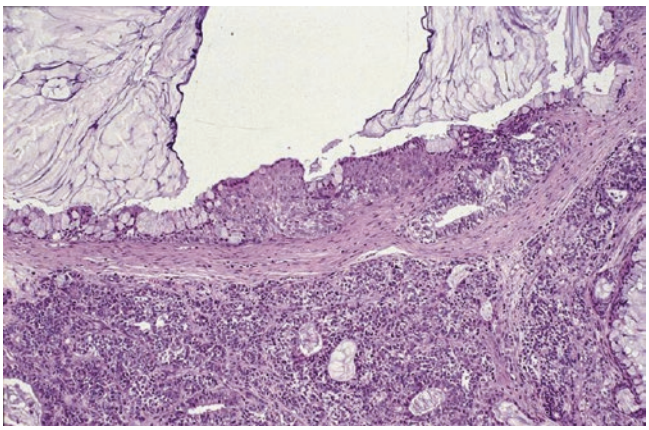

Fig. 2.96 Teratocarcinosarcoma: a mature cystic space covered by benign columnar mucous-secreting epithelium alternating with nests of mature squamous cells is seen in continuity with poorly differentiated glandular structures

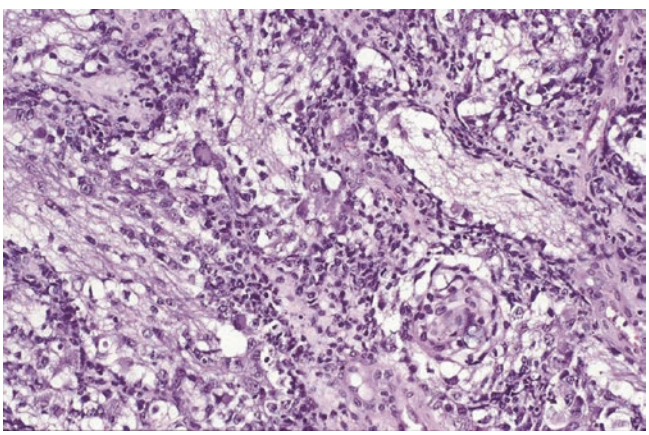

Fig. 2.97 Teratocarcinosarcoma: neuroepithelial elements with ganglion cells and neurofilaments depict neuroblastoma-like areas

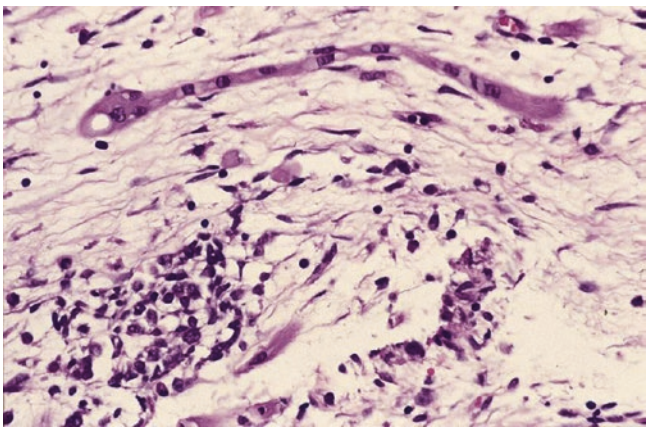

Fig. 2.98 Teratocarcinosarcoma: immature striated muscle cells, one of them displaying a snakelike configuration, are seen amid blastematous elements

sites, such as the lung. Craniospinal dissemination may occur [548]. The average survival of SNTCS is less than 2 years, with 60 % of the patients not surviving beyond 3 years [539]. Improved outcomes have been reported in more recent years [546].

2.12.7 Choriocarcinoma

Definition Choriocarcinoma is a malignant neoplasm composed of syncytiotrophoblast, cytotrophoblast, and intermediate trophoblast [549].

Synonym Non-gestational choriocarcinoma

Epidemiology Sinonasal choriocarcinoma is an extremely rare entity. Of the two cases reported, one originated in the maxillary sinus, and the other affected the nasal cavity, ethmoid and sphenoid sinuses. Both patients were males, one 44 years of age and the other 49 year old [550].

Clinical features One patient presented with epistaxis and the other with nasal obstruction. Both had elevated beta-HCG in serum.

Macroscopy Tumors are soft and hemorrhagic.

Microscopy Tumors are composed of an admixture of small, round to polygonal cytotrophoblastic cells, forming fenestrated sheets or pseudopapillae, surrounded by large multinucleated syncytiotrophoblastic cells.

Immunohistochemistry All choriocarcinoma cells are positive for pancytokeratin and syncytiotrophoblast reacts with beta-HCG.

Differential diagnosis Before establishing a diagnosis of primary sinonasal choriocarcinoma, it is mandatory to rule out metastatic gestational or non-gestational disease [551, 552].

Treatment and prognosis Patients with primary sinonasal choriocarcinoma are tributary of the aggressive chemotherapy regimens for non-gestational choriocarcinoma. Gestational choriocarcinoma requires less aggressive therapy.

2.13 Metastatic Tumors

Definition Sinonasal metastatic tumors are secondary malignancies that derive from a noncontiguous neoplasm. Direct extension from an adjacent neoplasm and leukemia-lymphoma is excluded [553, 554].

Synonym Secondary tumors

Epidemiology Metastases to the nasal cavity and paranasal sinuses are rare [555, 556]. The median age of patients with sinonasal metastatic tumors is 57 years, range 3 months to 76 years, and about 60 % occur in males [557]. The most frequent primary sites of origin of the tumors are the kidney (40 %), lung (9 %), breast (8 %), thyroid (8 %), prostate (7 %), and miscellaneous (28 %) [556]. The most habitual anatomic sites involved by the metastases are maxillary (33 %), sphenoid (22 %), ethmoid (14 %), frontal (9 %), and multiple sinuses (22 %) [556, 558]. In 10–15 % of cases, the metastases are limited to the nasal cavity [553].

Clinical aspects Metastases of tumors to the sinonasal tract are hematogenous and may be solitary or multifocal [554]. Usually symptoms are indistinguishable from those of a primary sinonasal tumor. Epistaxis is particularly common in metastatic renal and thyroid carcinomas; other common symptoms are nasal obstruction, headache, facial pain, visual disturbances, exophthalmos, facial swelling, and cranial nerve deficits. Metastases may be the first manifestation of an otherwise clinically occult carcinoma [553].

Microscopy Often, metastatic tumors to the sinonasal tract reproduce the most common histological features depicted by the primary tumors, which facilitates their recognition. Most renal cell carcinomas are of the clear cell type, while other types are very rarely seen (Fig. 2.99) [556]. Thyroid carcinomas are usually of the papillary and follicular types [559, 560]. Examples of colonic adenocarcinoma and of hepatocellular carcinoma metastatic to the sinonasal tract have been reported (Fig. 2.100) [561–566].

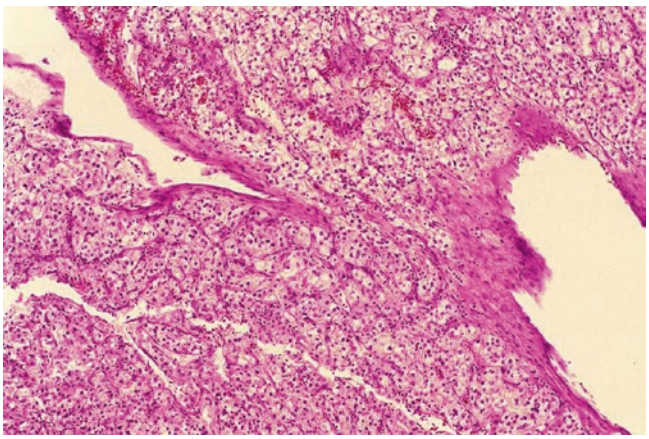

Fig. 2.99 Metastatic clear cell carcinoma of the kidney: the finding in a clear cell carcinoma of a rich network of well-formed mature blood vessels is highly suspicious of renal origin

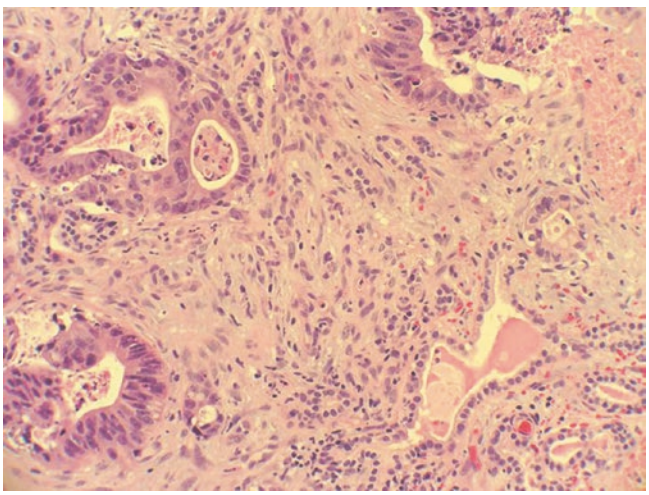

Fig. 2.100 Metastatic adenocarcinoma of the colon and rectum: in the sinonasal tract, the presence of intestinal-type adenocarcinoma with foci of necrosis and preservation of neighboring seromucous glands is quite suggestive of metastasis

Differential diagnosis Metastatic clear cell carcinomas have to be distinguished mainly from primary sinonasal clear carcinomas of minor salivary gland derivation [553]. Metastatic lung cell carcinomas, mainly those of small and intermediate cell type, have to be distinguished from their primary sinonasal counterparts [272]. Metastatic thyroid carcinomas have to be differentiated from primary sinonasal low-grade carcinomas tubulopapillary type [333]. Metastatic intestinal adenocarcinomas require precise distinction from primary ITAC [565]. Diagnostic difficulties usually arise with undifferentiated metastatic tumors, mainly those of unknown primary site of origin; clinical history, immunohistochemistry, and molecular techniques are in these instances of help.

Treatment and prognosis For metastatic sinonasal tumors, palliative therapy is in most instances recommended. However, prognosis may depend on whether the metastasis is isolated or part of widespread disseminated disease. If the sinonasal metastasis is localized and treated aggressively, the average survival following its discovery may be as long as 20–30 months [555].

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Oral Cavity

3

John Wallace Eveson and Miranda Pring

Contents

J.W. Eveson, BSc, BDS, PhD, FDS, FRCPath • M. Pring, BSc(Hons), MFDSRCPS,PhD,FRCPath (✉)
Bristol Dental Hospital, School of Oral and Dental Science, Bristol, UK
e-mail: J.W.Eveson@bris.ac.uk; miranda.pring@bristol.ac.uk

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3.1 Introduction

3.1.1 Embryology

The primitive mouth cavity (stomatodaeum) begins to develop when the embryo is 3–4 weeks old. It forms a narrow slit lined by ectoderm between the rostral brain capsule and the caudal pericardial sac. The sides are limited by the first branchial arches. Posteriorly it is bounded by a thin septum called the buccopharyngeal membrane which separates the ectodermal oral cavity from the endodermal pharynx and foregut. This membrane soon develops multiple fenestrations and breaks down, and the primitive mouth cavity establishes continuity with the pharynx.

By the fifth week in utero, ventral mandibular processes extend forwards and meet in the midline to delineate the lower jaw. The maxillary processes bud off the posterosuperior surfaces of the mandibular processes and grow anteriorly beneath the brain capsule to form the upper boundary of the oral aperture.

The tongue has two developmental origins. The anterior two-thirds that forms the oral tongue develops from the mandibular arches as bilateral eminences in the floor of the mouth cavity and a central swelling called the tuberculum impar at the junction between the first and second branchial arches. These predominantly mesodermal swellings rapidly enlarge and merge to form a coherent mass from which the definitive oral tongue develops. The posterior third of the tongue develops from a midline swelling called the hypobranchial eminence in the third branchial arch. This grows over the second branchial arch to establish continuity with the oral tongue at a junction which becomes the sulcus terminalis.

The palate forms from two inwardly directed linear processes, or shelves, which develop from the maxillary processes. They initially hang down on either side of the developing tongue, which at this stage occupies most of the stomatodaeum. Between the eighth and tenth weeks in utero, there is a relative increase in the vertical growth of the primitive mouth cavity. This results in the tongue descending and allows the palatal shelves to elevate and eventually to fuse with each other and the central developing nasal septum. Bone differentiates from the palatal mesoderm anteriorly to form the hard plate, and the muscle develops posteriorly to form the soft palate.

3.1.2 Anatomy and Histology

The oral cavity extends from the lips to the palatoglossal folds. It has two components: the outer vestibule and the oral mucosa proper. The outer vestibule is a slit-like space bordered by the cheeks and lips which separates it from the teeth and gingivae. It is limited superiorly and inferiorly by mucosal reflections. The oral cavity proper is the space surrounded by the teeth and gingivae. The upper boundary is the palate, and the lower is the floor of the mouth.

The buccal mucosa extends from the labial commissure to the palatoglossal fold and is lined by thick, non-keratinized stratified squamous epithelium. It contains variable numbers of sebaceous glands (Fordyce granules) and minor salivary glands.

The gingival mucosa surrounds the cervical margins of the teeth and merges with the mucosa overlying the alveolar bone at a faintly scalloped line termed the mucogingival junction. Normal gingival mucosa is pink and lightly stippled. It is attached firmly to the underlying mucoperiosteum and the necks of the teeth, apart from a shallow groove adjacent to the teeth in the free marginal area. It is typically keratinized or parakeratinized. The reddish coloured alveolar mucosa is thin, non-keratinized stratified squamous epithelium.

The hard palate extends from the upper alveolar gingival margins to the junction of the soft palate posteriorly. There is a median mucoperiosteal raphe extending from the incisive fossa anteriorly to the soft palate junction. Most of the palatal mucosa is firmly adherent to the underlying periosteum, apart from a narrow band just above the attached gingiva in the premolar/molar regions. The mucosa is orthokeratinized stratified squamous epithelium and posteriorly contains large numbers of minor mucous salivary glands.

The oral tongue is mobile and is separated from the posterior, oropharyngeal component by a V-shaped groove called the sulcus terminalis. The dorsal surface is covered by keratinized stratified squamous epithelium and several different types of papillae. The most numerous are the filiform papillae which are hair-like and have a thick surface layer of keratin which is often covered by dense bacterial aggregates. The less numerous fungiform papillae are relatively evenly distributed on the dorsum and macroscopically appear as small, pink nodules. They may contain taste buds on their superior surface. Taste buds can occasionally be confused with pagetoid melanocytic proliferation. There are 10–12 circumvallate papillae in a row immediately in front of the sulcus terminalis. They are usually 4–5 mm in diameter and are surrounded by a deep groove. There are many taste buds over their whole mucosal area (Fig. 3.1). Minor serous salivary gland ducts open into the base of the groove (von Ebner's glands). At the palatoglossal junction laterally, there are foliate papillae which vary considerably in size and appearance, but typically form leaflike folds. They also contain taste buds. In addition, the core of the papillae contains lymphoid aggregates and is part of Waldeyer's ring (Fig. 3.2). They can become inflamed and enlarged and cause discomfort in the posterolateral region of the tongue. This is called

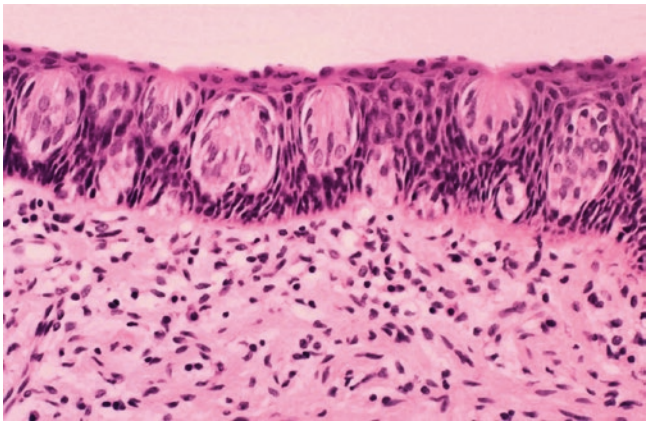

Fig. 3.1 Taste buds in oral surface epithelium of circumvallate papilla

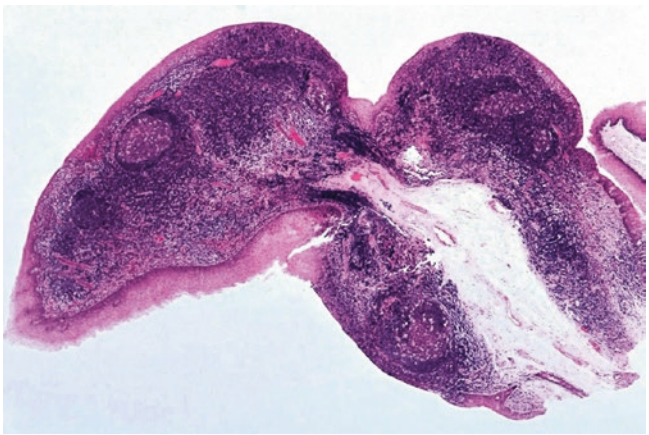

Fig. 3.2 Foliate papilla showing dense lymphoid aggregates with germinal centres

foliate papillitis. In the underlying lingual musculature, there are mixed seromucous salivary glands.

The ventral surface of the tongue is covered by non-keratinized stratified squamous epithelium. Towards the midline frenulum particularly, there are minor salivary glands (glands of Blandin and Nuhn) deep in the sublingual musculature. These are most frequent towards the junction with the floor of the mouth, but they can extend to the tip of the tongue.

The floor of the mouth forms a horseshoe-shaped gutter between the ventrum of the tongue and lower gingivae. The non-keratinized stratified squamous epithelium covers the sublingual glands and the opening of the submandibular duct anteriorly. About 75 % of oral squamous cell carcinomas develop in an area including the floor of the mouth, adjacent ventral lingual mucosa and the retromolar trigone. This region of increased susceptibility forms only 20 % of the total oral mucosal area and has been called the drainage area [1]. Precursor epithelial lesions in this area should therefore be regarded with a high degree of suspicion.

3.2 Embryonic Rests and Heterotopias

3.2.1 Fordyce Granules/Spots

Definition Fordyce granules are 'ectopic' sebaceous glands in the oral mucosa [2].

Epidemiology The main site is the buccal mucosa, but they may also involve the vermilion border and labial mucosa, particularly in the upper lip. In the latter location, some patients believe them to be a disease manifestation or complain that they are cosmetically unsightly. More rarely, the tongue, palatoglossal fold, tonsil and other intraoral sites may be affected, and the condition can then be confused with other lesions.

Clinical aspects They appear as soft, creamy white or yellowish spots or clusters, which are typically a few millimetres in diameter. They are symmetrically distributed and tend to increase in size and number with age.

Microscopy They are typical sebaceous glands opening directly onto the surface by short, keratinized ducts with no associated hair follicles.

Treatment and prognosis Fordyce granules are normal anatomical structures and no treatment other than reassurance is necessary.

3.2.2 Juxtaoral Organ of Chievitz

Definition Chievitz's organ, or the bucco-temporal organ, is a small, juxtaoral mass that is thought to be a vestigial neuroepithelial structure.

Epidemiology It has been demonstrated in neonates and children and can persist into adult life [3]. The organ is usually found between the temporalis muscle and the bucco-temporal fascia or pterygomandibular raphe and is usually present bilaterally. It is typically only a few millimetres in size. Similar appearances to the juxtaoral organ have been reported elsewhere in the mouth, including intraosseous locations [4]. Very rare cases have presented in the infratemporal fossa [5].

Etiology and pathogenesis It has been suggested that the juxtaoral organ is an anlage of the parotid gland or arises from Schwann cells that have undergone squamous metaplasia [6].

Clinical aspects It is usually detected fortuitously, generally in material taken from surgical resections, and is important as

it can be misinterpreted as a squamous cell carcinoma. In addition, it has rarely been reported presenting as a tumor-like mass [5] and as a chance finding radiographically [7].

Microscopy The lesion forms a multilobulated mass of discrete cell nests that resemble squamous epithelium but do not show obvious keratinization. Occasionally, the cells have clear cytoplasm and form duct-like structures that may contain mucin-negative colloid.

Immunohistochemistry The central areas of the epithelial cell nests are positive for cytokeratin 19, and most cell nests are positive for vimentin and weakly positive for epithelial membrane antigen. They are negative for S-100 protein, glial acidic fibrillary protein and neuroendocrine markers such as chromogranin, synaptophysin and neurone-specific enolase [6]. Pacinian corpuscles have been described in association with the juxtaoral organ in the German and English literature, suggesting it has a mechanosensory function [8]. Melanin has been reported in cells in the connective tissue, close to the epithelial component [9]. The majority of these cells were melanophages but some were dendritic and were positive for melanocyte markers such as S-100 and HMB45. These observations would tend to support a neural crest origin for the juxtaoral organ.

Differential diagnosis The cell nests are associated with nerve fibres, particularly at the periphery, and this may be mistakenly interpreted as a squamous carcinoma with perineural involvement or sometimes mucoepidermoid carcinoma and thyroid carcinoma [10].

Treatment and prognosis The lesion is entirely benign.

3.3 Vesiculo-bullous Diseases

3.3.1 Herpes Simplex Infections

Herpes simplex is a common virus that often causes subclinical infections. However, it can be a cause of serious and sometimes fatal illnesses in immunocompromised patients. In the orofacial tissues, clinically apparent infections can be primary or recurrent.

3.3.1.1 Primary Herpes Simplex

Definition Primary herpes infection (primary herpetic gingivostomatitis) is an acute *Herpes simplex* virus infection characterised by widespread vesicular lesions of the oral mucosa [11].

Epidemiology Although in the past primary herpes affected children most frequently, in Western societies, it is seen increasingly in young and middle-aged adults. Any oral site may be involved but the hard palate and the dorsum of the tongue are the most common locations.

Etiology The majority of cases of oral infections are due to *Herpes simplex* type 1, but an increasing proportion is being attributed to *Herpes simplex* type 2 that is typically more closely associated with genital infections. The virus is transmitted by close contact.

Clinical aspects The vesicles quickly rupture to leave shallow, painful, sharply demarcated ulcers which are 1–2 mm in diameter and have an erythematous halo. Ulcers frequently coalesce to form more irregular lesions. Gingivitis is a very characteristic feature of primary herpes. The gingivae are swollen and often strikingly erythematous, even in the absence of frank ulceration. There is often conspicuous cervical lymphadenopathy, together with mild fever and malaise.

Microscopy It is uncommon for herpetic lesions to be biopsied. In the early stages, there is intercellular oedema and ballooning and vacuolisation of keratinocytes due to intracellular oedema. This leads to intraepithelial vesiculation (Fig. 3.3). Nuclei become enlarged, and occasionally basophilic or eosinophilic nuclear inclusions with a clear halo (Lipschütz bodies) can be identified. Cells may fuse to form multinucleated epithelial giant cells. The vesiculation is followed rapidly by epithelial necrosis and breakdown, leading to ulceration and more florid inflammatory infiltration.

Treatment and prognosis Severe cases can be treated with topical and/or systemic aciclovir. Oral lesions usually resolve spontaneously within 1–2 weeks. About a third of patients infected with *Herpes simplex*, either clinically or subclinically, are susceptible to recurrent infections.

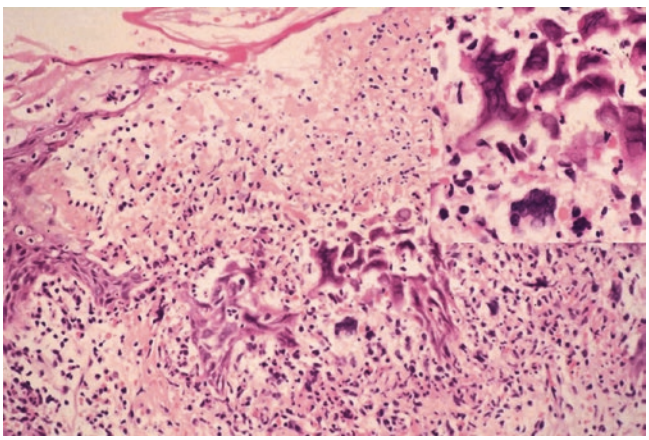

Fig. 3.3 Primary herpetic stomatitis, showing intercellular vacuolation, inflammatory infiltration and multinucleated epithelial cells. *Inset* shows high power of the multinucleated cells

3.3.1.2 Recurrent Herpes Simplex

Definition Recurrent herpes simplex is a recurrent infection due to reactivation of latent *Herpes simplex* virus lying in the trigeminal ganglion.

Epidemiology Lesions are typically seen at the mucocutaneous junctions of the mouth or nasal cavity, and involvement of the lips, the most common site, is called herpes labialis.

Etiology and pathogenesis A variety of apparently disparate factors can trigger reactivation, including the common cold ('fever blister'), exposure to sunlight, menstruation, stress and others.

Clinical aspects There is usually a brief prodromal burning or prickling sensation in the affected area, followed by the formation of a localised cluster of vesicles. These rapidly break down, ulcerate and crust. The lesions usually heal spontaneously in 1–2 weeks. Occasionally there may be intraoral recurrences, particularly in the hard palate. These may be triggered by local anaesthetic injections. Persistently recurrent intraoral herpes, however, should always raise the possibility of immunosuppression. Atypical and often very severe forms of intraoral herpes infections can be seen in patients who are immunocompromised [12].

Treatment and prognosis Topical aciclovir cream can be applied in the prodromal phase to help prevent lesions developing.

3.3.2 Varicella Zoster Infections

3.3.2.1 Chickenpox

Definition Chickenpox is a highly contagious, vesiculating, mucocutaneous infection caused by the herpesvirus *Varicella zoster*.

Epidemiology It is typically seen in children where it causes crops of pruritic cutaneous vesicles. It is usually transmitted by direct contact and has an incubation period of 2–3 weeks.

Clinical aspects The exanthem is frequently preceded by a slight fever, malaise and mild headache. The cutaneous lesions start as an itchy macular rash, which progressively becomes vesicular and pustular before breaking down to form focal crusting lesions. They tend to erupt in crops, but lesions at all stages of evolution are frequently present. The back and chest are often the first sites of involvement, but later lesions appear on the face, neck and limbs. They can involve the nose, ears, conjunctiva and genital areas. In the mouth, they form small, non-specific, scattered ulcers.

Treatment and prognosis Treatment is symptomatic only. The symptoms last from a few days to 2 weeks. In many cases, the virus remains latent in dorsal root ganglia.

3.3.2.2 Herpes Zoster

Definition Herpes zoster (shingles) is a localised, vesiculating, cutaneous and/or mucosal rash due to reactivation of the *Varicella zoster* virus.

Epidemiology It most frequently affects adults of middle age and older, but occasionally also presents in children. In the orofacial region, it is characterised by pain, a vesicular rash and stomatitis in the related dermatome.

Etiology and pathogenesis Occasionally there is an underlying immunodeficiency. Herpes zoster is a hazard in organ transplant patients and can be an early complication of haematolymphoid neoplasms and HIV infections.

Clinical aspects The first signs are often pain, irritation or tenderness in one or more divisions of the trigeminal nerve. The pain may be severe and can be misinterpreted as toothache, leading to inappropriate dental intervention. Malaise and low-grade fever are common constitutional symptoms. There is usually a strikingly unilateral, vesicular exanthem restricted to the affected dermatome. Intraorally, there may also be extensive unilateral ulceration in the distribution of the involved nerves. There is usually tender regional lymphadenopathy.

Treatment and prognosis Herpes zoster is treated by high-dose aciclovir (800 mg 5× day). The acute phase lasts about 7–10 days, but pain may continue until the lesions ulcerate and crust over which may take several weeks, especially if there is suppuration and subsequent scarring. In these circumstances, a significant number of patients develop the most unpleasant consequence of post-herpetic neuralgia. Unlike herpes labialis, repeated recurrences of herpes zoster are very rare.

3.3.3 Hand-Foot-and-Mouth Disease

Definition Hand-foot-and-mouth disease is a common, highly infectious, but usually mild viral infection that often causes local clusters of infections among groups of young children and is characterised by oral ulceration and a vesicular rash on the extremities.

Epidemiology It frequently spreads through classrooms, schools and local communities in an epidemic manner. The incubation period is between 3 and 10 days.

Etiology and pathogenesis It is usually caused by a variety of strains of the Coxsackie A16 virus and enterovirus 71. Sporadic cases associated with Coxsackie A4–7, A9, A10, B1–B3 and B5 have also been reported. It presents clinically as small, scattered oral ulcers that often cause few symptoms.

Clinical aspects Although the initial lesions are vesicular, intact blisters are rarely seen. Unlike primary herpes infections, the gingivae are rarely affected. It is unusual for regional lymph nodes to be involved except in severe cases, and constitutional symptoms tend to be mild or absent. The cutaneous exanthem consists of small vesicles or occasionally larger blisters that form mainly around the base of fingers or toes, but may extend to involve any part of the limb. In some outbreaks, either the mouth or the extremities alone may be affected. Although serological investigations can confirm the diagnosis, due to the relatively mild and transient nature of the disease, this investigation is rarely undertaken.

Treatment and prognosis Typically, the condition resolves spontaneously within a week to 10 days and does not recur. However, in some epidemics, patients have developed severe complications, including interstitial pneumonitis, myocarditis and encephalitis and resulting in death [13].

3.3.4 Herpangina

Definition Herpangina is an acute, vesiculating oral infection caused predominantly by Coxsackie viruses.

Epidemiology It is highly contagious and tends to affect young children in the summer and early autumn period. Like hand-foot-and-mouth disease, it rapidly spreads through close-knit communities, such as schools, and presents with acute pharyngitis, anorexia and dysphagia, with or without cervical lymphadenopathy. Typically, the lesions are restricted to the soft palate, uvula, anterior pillars of the fauces and palatine tonsils.

Etiology and pathogenesis Herpangina is caused by a variety of group A Coxsackie viruses including A1–6, 8, 10 and 22. Other causes include Coxsackie group B (strains 1–4), echoviruses and other enteroviruses [14].

Clinical aspects The lesions consist of multiple, small vesicles that rapidly rupture to form superficial ulcers, which may coalesce. In addition, there is often more generalised oropharyngeal erythema.

Treatment and prognosis The condition usually last 1–2 weeks and is treated symptomatically. Epidemic enterovirus 71 infections can be complicated by encephalomyelitis with neurological sequelae in some children [15, 16].

3.3.5 Pemphigus Vulgaris

Definition Pemphigus vulgaris (PV) is an uncommon but potentially lethal, mucocutaneous blistering disorder associated with circulating autoantibodies to intercellular adhesion molecules of squamous epithelium.

Epidemiology Pemphigus is more common in Asians and Ashkenazi Jews than other races and most patients are in the fourth or fifth decades. The mouth is the most common site of initial involvement and remains the only site affected in about half of patients. The buccal mucosa, gingiva and soft palate are the most common sites. Occasionally the eyes are also involved.

Etiology and pathogenesis Patients with the mucocutaneous form of the disease have anti-desmoglein 1 and 3 autoantibodies [17, 18], whilst those with the mucosal form have only anti-desmoglein 3 autoantibodies [19]. The pathogenic mechanisms of PV are not fully understood, but it appears likely that there is perturbation of the desmosomal network at the transcriptional, translational and interaction level [20, 21]. Associations have been described between pemphigus vulgaris and myasthenia gravis and thymoma, together with a variety of drugs, including penicillamine, rifampicin and captopril. There is also considerable evidence linking *Herpes simplex* virus and PV, which may influence the course of the disease [22]. In addition, some cases are associated with internal malignancies, particularly of the haematolymphoid system, and the condition is then termed paraneoplastic pemphigus.

Clinical aspects The oral features are very variable. It is uncommon to find intact vesicles and most patients present with painful, ragged superficial ulcers and areas of boggy and shredded mucosa. In the tongue, the condition may present as deep, non-healing fissures.

Microscopy Fluid from intact or recently ruptured blisters may contain acantholytic (Tzanck) cells, although this is rarely used as a diagnostic measure. There is suprabasal clefting of the epithelium due to loss of intercellular attachments and acantholysis (Fig. 3.4). A single layer of keratinocytes may remain attached to the corium by their hemidesmosomes, but the cells are separated from each other laterally to form a characteristic 'tombstone' appearance. The acantholytic cells floating in the vesicular fluid are rounded, with condensed cytoplasm surrounding hyperchromatic nuclei. The vesicles may contain acute and chronic inflammatory cells, and eosinophils may be a conspicuous feature. In many cases, the roof of the blister is lost during

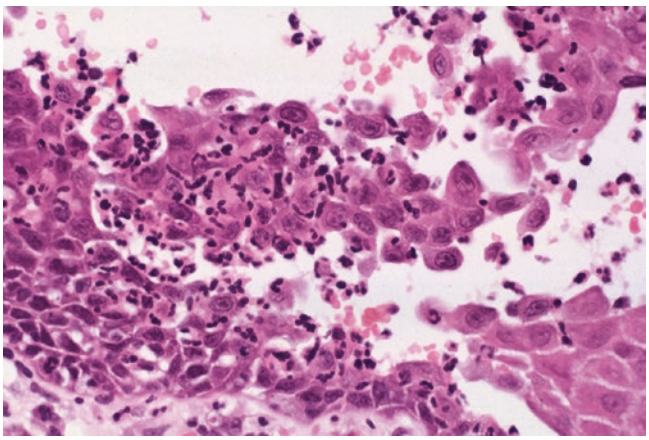

Fig. 3.4 Pemphigus vulgaris showing suprabasal clefting and acantholytic cells in an intraepithelial blister

the biopsy procedure due to a positive Nikolsky phenomenon, but a row of keratinocytes remains adherent to the floor.

Immunohistochemistry Direct immunofluorescence on frozen tissue shows deposits of IgG and less frequently IgM and IgA in the intercellular junctions, producing a characteristic 'chicken wire' appearance.

Treatment and prognosis The disease may be relatively mild or even regress, but some cases, particularly those with extensive cutaneous involvement, may be fulminant, either as a consequence of the disease itself or as a complication of medical treatment. This includes topical or systemic immunosuppressive drugs, often together with adjuvant agents such as dapsone, azathioprine and methotrexate. In severe cases, plasmapheresis and intravenous immunoglobulins may be necessary.

3.3.6 Pemphigus Vegetans

Definition Pemphigus vegetans is a localised form of pemphigus vulgaris characterised by a vegetating, papilliferous response.

Epidemiology It is considerably less common in the mouth than pemphigus vulgaris [23].

Etiology and pathogenesis As in pemphigus vulgaris, drugs, particularly ACE inhibitors, have been invoked as possible causative agents in some cases [24, 25]. A proposed association with intranasal cocaine abuse has also been reported [26].

Clinical aspects It usually presents clinically as serpiginous ulcers that are most frequent on the dorsum of the tongue and lips [27]. The lingual lesions closely resemble those of erythema migrans. The papillomatous, proliferative lesions that characterise cutaneous pemphigus vegetans can sometimes be seen at the angles of the mouth.

Microscopy The epithelium tends to proliferate and become verruciform. Acantholytic cells may not be conspicuous and eosinophil microabscesses are the most typical histological feature.

Immunohistochemistry The immunohistochemical profile is similar to pemphigus vulgaris.

Differential diagnosis The lesions frequently resemble pyostomatitis vegetans and conventional microscopy may not be diagnostic. The presence of typical skin lesions often helps in making the diagnosis and it may be differentiated from pyostomatitis by the clinical picture and appropriate immunocytochemical investigations [28].

3.3.7 Paraneoplastic Pemphigus

Definition Paraneoplastic pemphigus is a rare form of pemphigus which is associated with underlying malignancy.

Epidemiology Although an occasional association between pemphigus and malignancy had been recognised for many years, it was not until 1990 that paraneoplastic pemphigus was recognised as a distinct clinical, histological and immunohistochemical entity [29]. The buccal mucosa and lips are the most common sites, but almost anywhere in the mouth, oropharynx and nasopharynx can be involved. About two-thirds of patients have conjunctival involvement characterised by a frequently severe pseudomembranous conjunctivitis and symblepharon.

Etiology and pathogenesis The condition is seen predominantly in association with B-cell lymphoproliferative disorders, especially non-Hodgkin lymphoma, chronic lymphocytic leukaemia, Castleman disease, thymoma and Waldenström's macroglobulinaemia [30]. Less commonly it is associated with non-lymphoid neoplasms, including some carcinomas of the bronchus, breast and pancreas. In some cases showing otherwise typical features of the disease, no underlying malignancy is found.

Clinical aspects Paraneoplastic pemphigus is characterised by the following features:

- Painful mucocutaneous vesiculo-bullous eruptions
- Histopathologic features of intraepithelial acantholysis and vacuolar interface changes

- Demonstration of intercellular epithelial IgG and C3, with or without granular linear deposition of complement along the basement membrane zone
- Presence of circulating autoantibodies that bind to the surfaces of stratified squamous epithelia as well as simple, columnar and transitional epithelia
- Presence of a characteristic complex of proteins derived from keratinocytes and serum antibodies demonstrated by serum immunoprecipitation. These include desmoplakins I and II, bullous pemphigoid antigen I, envoplakin and periplakin [31].

The mouth is almost always involved, and oral lesions present as a painful, intractable stomatitis that extends into the oropharynx and often beyond the vermilion borders of the lips. It causes blisters and irregular, ragged ulceration.

Microscopy There is intraepithelial acantholysis with suprabasal clefting, dyskeratotic keratinocytes, basal cell liquefaction and epithelial inflammatory cell exocytosis [32]. In many cases, however, the condition cannot be distinguished from conventional pemphigus [33]. The overall appearances suggest that there is an overlap between paraneoplastic pemphigus and erythema multiforme. Indirect immunofluorescence on the transitional epithelium of rat bladder appears to be a highly specific test for paraneoplastic pemphigus [34].

Treatment and prognosis Paraneoplastic pemphigus tends to be extremely refractory to the usual immunosuppressant drugs used to control pemphigus vulgaris. Treatment of the underlying malignancy and intravenous immunoglobulins are essential.

3.3.8 Mucous Membrane Pemphigoid

Definition Mucous membrane pemphigoid (MMP) has been defined as a group of putative autoimmune, chronic inflammatory, subepithelial blistering diseases predominantly affecting mucous membranes and characterised by linear deposition of IgG, IgA and C3 along the epithelial basement membrane [35, 36]. It has also been called benign mucous membrane pemphigoid and cicatricial pemphigoid. However, it can be a severely disabling condition and rarely causes scarring except in the eye and oesophagus/larynx, so these terms are not appropriate.

Epidemiology MMP is more common in women than men and most patients are in the 40–60 year age range. The mouth is often the first and only site of involvement. Lesions are most common on the attached gingiva, usually buccally and labially, and the palatal mucosa. Less frequent oral sites include the labial, lingual and buccal mucosae. Other sites of involvement include the eyes, skin and mucosa of the oropharynx, nasopharynx, larynx, oesophagus and anogenital region [37]. Skin lesions are uncommon and usually involve the scalp and upper torso.

Etiology and pathogenesis The pathogenesis of MMP has not been fully elucidated, but the development of autoantibodies to basement membrane components is a key factor.

Several possible target antigens have been identified in the sera of patients with mucous membrane pemphigoid. These include the hemidesmosomal proteins bullous pemphigoid antigens 1 and 2 [38], laminins 5 and 6, type VII collagen and ß4 integrin subunit [37].

Recent advances in immunohistochemical techniques have allowed the identification of distinct clinical subgroups of MMP. An example is anti-laminin-332 BMP which is associated with the development of solid organ malignancy [35, 38–40].

Clinical aspects Oral lesions can be intact blisters that may contain clear or sero-sanguinous fluid, erythematous patches or superficial ulcers. Ocular lesions are characterised by conjunctival inflammation, ulceration and symblepharon due to fusion of the palpebral and bulbar conjunctivae. There may be severe scarring, entropion and blindness.

Microscopy There is subepithelial blister formation with clean separation of the full thickness of the epithelium from the underlying connective tissue (Fig. 3.5). There is usually a dense mixed inflammatory infiltration of the corium. Due to the strongly positive Nikolsky phenomenon seen in MMP, it is very common to receive a biopsy specimen where most or all of the epithelium has been lost or completely separated from the connective tissue. The specimen then consists of non-specifically inflamed connective tissue which lacks the surface fibrinous slough that

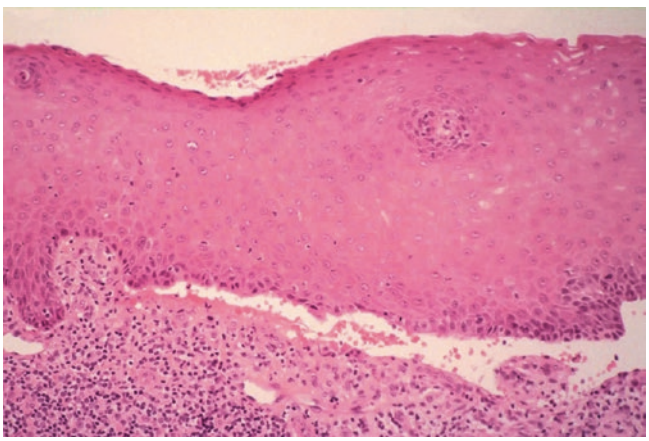

Fig. 3.5 Mucous membrane pemphigoid, showing clean subepithelial blistering

would be more typical of a non-specific oral ulcer. This type of appearance, though not diagnostic, is highly suggestive of MMP.

Immunohistochemistry Direct immunofluorescence on peri-lesional mucosal biopsies shows continuous deposits of IgG, IgA or C3, either singly or in combination, along the basement membrane zone (BMZ) in about 80 % of cases. When present, these deposits help to distinguish MMP from several other common oral mucosal inflammatory disorders.

Differential diagnosis Lichen planus does not have linear immunoglobulin deposits but has linear and shaggy deposits of fibrin in the BMZ, and erythema multiforme has no linear BMZ deposits. However, these deposits do not distinguish MMP from bullous pemphigoid, epidermolysis bullosa acquisita or linear IgA bullous dermatosis. Such distinctions should be made on the basis of clinical findings.

Treatment and prognosis Immunosuppressive agents, including corticosteroids and azathioprine, are the most commonly used therapeutic agents.

3.3.9 Dermatitis Herpetiformis

Definition Dermatitis herpetiformis is an uncommon, intensely pruritic mucocutaneous disorder related to coeliac disease that only occasionally involves the mouth [41].

Epidemiology Dermatitis herpetiformis can involve both keratinized and non-keratinized mucosa, and head and neck cutaneous lesions tend to affect the scalp and periorbital regions. It is seen most frequently in teenagers and young adults, particularly males, and there is a predilection in people of Anglo-Saxon and Scandinavian origin.

Etiology and pathogenesis There is a strong association between dermatitis herpetiformis and gluten-sensitive enteropathy. The class I antigen HLA-B8 is found in the large majority of patients with both dermatitis herpetiformis and coeliac disease, and HLA-DR3 is expressed in nearly 95 % of patients. The leading theory for dermatitis herpetiformis is that a genetic predisposition for gluten sensitivity, coupled with a diet high in gluten, leads to the formation of IgA antibodies to gluten-tissue transglutaminase (t-TG), which is found in the gut. These antibodies cross-react with epidermal transglutaminase (e-TG) [42].

Clinical aspects Oral dermatitis herpetiformis presents as patches of mucosal erythema, clusters of small, friable vesicles, painful herpetiform ulcers or more extensive areas of non-healing ulceration. In conventional gluten-sensitive enteropathy, oral ulcers tend to be of the typical minor aphthous stomatitis type.

Microscopy The lesions of dermatitis herpetiformis show polymorphonuclear leukocyte microabscesses in the tips of the papillary corium (Fig. 3.6). Initially, neutrophils predominate, but as the microabscesses enlarge, eosinophils become more conspicuous. The microabscesses eventually fuse to form visible blisters that frequently rupture leaving superficial ulcers.

Immunohistochemistry Direct immunofluorescence shows granular deposits of IgA in the BMZ of the epithelial papillae, in both affected and adjacent normal mucosa.

Treatment and prognosis The first line of treatment is usually to institute a gluten-free diet. If this is unsuccessful, patients often respond rapidly to dapsone or sulphonamides such as sulfapyridine.

3.3.10 Linear IgA Disease

Definition Linear IgA disease is a rather poorly defined heterogeneous group of mucocutaneous blistering disorders that closely resemble mucous membrane pemphigoid clinically and microscopically [43, 44].

Epidemiology The condition is more common in women than men. Like pemphigoid, the eyes may be involved. Linear IgA disease in adults has been separated from similar conditions in childhood such as bullous dermatosis of childhood and childhood cicatricial pemphigoid.

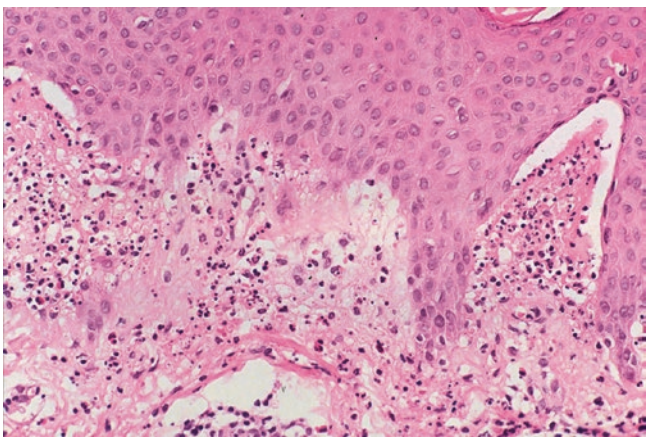

Fig. 3.6 Dermatitis herpetiformis showing polymorphonuclear leukocyte abscesses in the papillary corium

Etiology and pathogenesis Cutaneous linear IgA disease of adults has a strong association with a history of bowel disease. This association is much less clear in patients with oral lesions. However, patients with oral linear IgA disease appear to have a higher risk of severe ocular lesions. Some cases of oral lesions have been associated with drugs [45].

Clinical aspects It usually presents as a desquamative gingivitis with, or without, ulceration.

Microscopy There is subepithelial vesiculation and full thickness blister formation.

Immunohistochemistry Direct immunofluorescence shows linear deposition of IgA along the basement membrane zone and a low titre of circulating IgA to the BMZ. Although small amounts of IgG, IgM and C3 may be seen, if these are present in other than trace amounts, mucous membrane pemphigoid is a much more likely diagnosis.

Treatment and prognosis Linear IgA disease tends to be refractory to systemic steroids, but it may respond to dapsone or sulphonamides.

3.3.11 Erythema Multiforme

Definition Erythema multiforme is an idiopathic, mucocutaneous inflammatory disorder which is due to a hypersensitivity reaction, but sometimes the mouth is the only site of involvement [46, 47]. It can be relatively mild (erythema multiforme minor) or manifest with fever, malaise and extensive skin, mucosal and ocular lesions when it is sometimes called Stevens-Johnson syndrome or erythema multiforme major.

Epidemiology Erythema multiforme is usually seen in young adults (20–40 years) and is more common in males. Although patients may suffer a single episode, it is often recurrent. Oral lesions may be the only feature of the disease or cutaneous involvement may follow several attacks of oral ulceration.

Etiology and pathogenesis The pathophysiology is still not completely understood, but it is probably immunologically mediated and appears to involve a hypersensitivity reaction that can be triggered by a variety of stimuli, particularly bacterial, viral or chemical products (Table 3.1). However, in many cases, no precipitating factor is found. Cell-mediated immunity is responsible for the destruction of epithelial cells. A variety of HLA genotypes have been associated with the condition [48]. Triggering agents that have been implicated include infections with *Herpes simplex* virus [49], *Mycoplasma pneumonia* and many others and a wide range of drugs including sulphonamides, anticonvulsants, non-steroidal anti-inflammatory medications and antibiotics [50].

Clinical aspects The lips are the most frequently involved site and typically show swelling and extensive haemorrhagic crusting (Fig. 3.7a). Within the mouth, there are usually diffuse erythematous areas and superficial ulcers on the buccal mucosa, floor of the mouth, tongue, soft palate and fauces. It is uncommon for the gingiva to be involved, and this sometimes helps to distinguish erythema multiforme from primary herpetic gingivostomatitis, where gingival inflammation is a conspicuous feature. The involved areas of mucosa frequently break down to form painful, shallow, irregular ulcers on a background of more generalised erythema. It is unusual to see intact blisters in the mouth.

The classical cutaneous manifestation of erythema multiforme is the development of so-called target or bull's-eye lesions. These begin as dark red macules, usually 1–3 cm in diameter. They become slightly elevated and develop a characteristic bluish centre. These lesions are seen most frequently on the hands and lower limbs. In erythema multiforme major, there may be ocular and genital involvement, together with constitutional symptoms. A very severe and potentially lethal variant is toxic epidermal necrolysis, when there is widespread cutaneous and mucosal involvement with extensive blistering and epidermal loss, leading to fluid and electrolyte loss and secondary infection.

Table 3.1 Etiological factors associated with erythema multiforme

Genetic predisposition	Associated with a range of HLA alleles, particularly in drug-induced cases
Microorganisms	**Viruses** Herpes simplex (70 % of cases), varicella zoster, Epstein-Barr, enteroviruses, hepatitis viruses, HIV
	Bacteria *Mycoplasma pneumoniae*, chlamydia, β-haemolytic streptococci
	Fungi Histoplasma, coccidioidomycosis
Drugs	Sulphonamides, antiepileptic medications, allopurinol, penicillins, tetracyclines, paracetamol, non-steroidal anti-inflammatory drugs
Food additives	Benzoates, nitrobenzene
Immune related	Graft vs host disease, BCG and hepatitis B vaccines, systemic lupus erythematosus, sarcoidosis, pregnancy, inflammatory bowel disease

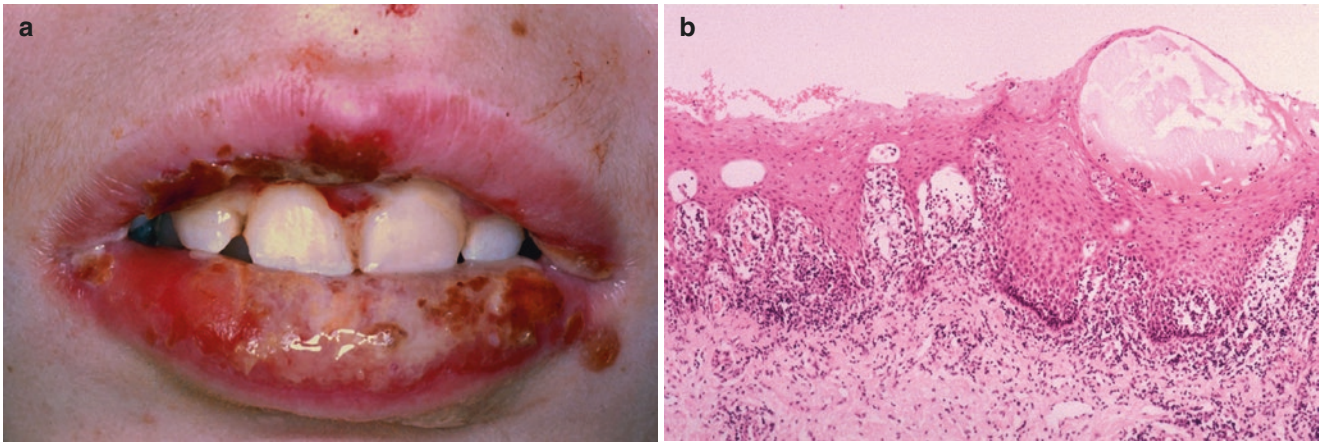

Fig 3.7 (**a**) Erythema multiforme, showing extensive superficial ulceration, and haemorrhagic crusting of the lips. (**b**) Erythema multiforme, showing intraepithelial and subepithelial vesiculation, with underlying inflammatory infiltration

Microscopy This shows variable features and early epithelial breakdown of oral lesions frequently masks any characteristic features [51]. In the early lesions, there is apoptosis and necrosis of keratinocytes, intercellular oedema and inflammatory infiltration of the epithelium. This leads to intra- and subepithelial vesiculation and ultimately loss of the roof of the blister to form an ulcer (Fig. 3.7b). There is lymphohistiocytic and polymorphonuclear infiltration of the superficial corium, and the inflammatory infiltrate can extend more deeply, often in a perivascular distribution. Patchy deposits of C3 and IgM may be found in the walls of blood vessels, but there is no frank vasculitis, and the immune complex deposition appears to be non-specific.

Treatment and prognosis In cases precipitated by *Herpes simplex* virus, aciclovir is often effective. This can be used in early cases or on a longer-term prophylactic basis. Systemic steroids are sometimes effective.

3.4 Ulcerative Lesions

3.4.1 Aphthous Stomatitis (Recurrent Aphthous Ulceration)

Definition Aphthous stomatitis is the most common ulcerative disease of the mouth and is characterised by persistently recurrent, painful oral ulcers.

Epidemiology The incidence can vary from 5 % to 60 % of the population at some time in their lives [52]. There is a female preponderance and a strong familial association. The condition usually starts in early childhood and typically resolves spontaneously in the late teens or early adult life. When the condition develops in older individuals, predisposing causes such as haematinic deficiencies or smoking cessation are more likely to be associated.

Etiology and pathogenesis The etiology of recurrent aphthous stomatitis is uncertain and the majority of cases are idiopathic. Slight trauma is often the ultimate precipitating factor. A minority are caused, or exacerbated, by deficiencies in iron, vitamin B_{12} or folate and as such are potentially curable. Haematinic deficiencies are reported to be twice as common in patients with recurrent aphthous stomatitis compared to controls. The condition is often made worse by emotional or environmental stress [53]. Occasional cases are said to be related to gastrointestinal complaints such as coeliac disease, Crohn's disease and ulcerative colitis, but some of the data are conflicting [54–56]. However, it is likely that in most instances any of the latter associations are secondary to haematinic deficiencies. There is no convincing evidence of a significant association with microbial factors in the etiology of aphthae, including *Helicobacter pylori*, in the general population. However, major aphthae particularly can be a serious problem in patients with HIV infection. These ulcers are seen most commonly in children and are more persistent and painful than those of healthy individuals [57]. They are seen most frequently when the $CD4^+$ lymphocyte count falls below 100/mm^3. Irregular ulcers resembling major aphthae, but usually lacking the more typical marginal erythematous halo, can be caused by the potassium channel blocking, antihypertensive drug, nicorandil [58].

Clinical aspects Traditionally, there are three main clinical forms of the condition (minor, major and herpetiform ulceration), although a minority of patients may show various combinations of these types (Table 3.2). Recently, however, the inclusion of the herpetiform variant in this triad has been questioned [56]. Minor aphthae are by far the most common manifestation (~85 %) and are characterised by the formation of one or several superficial ulcers, usually 2–8 mm in diameter with a yellowish-grey, fibrinous floor and an erythematous halo. The ulcers tend to

Table 3.2 Principal clinical features of recurrent aphthous stomatitis

	Minor	Major	Herpetiform
Site	Non-keratinized mucosa, especially labial and buccal mucosa	Any mucosal surface, including the hard palate	Non-keratinized oral mucosa, especially the lips, lingual ventrum and soft palate
Size	Usually less than 1 cm	Usually more than 1 cm	0.1–0.5 cm
Shape	Round or oval, superficial with variable erythematous margin	Irregular margin and penetrating	Round, usually well-defined ulcer on area of diffuse erythema. Ulcers often coalesce
Number	1–5	1–3	Numerous – there can be over 100
Duration	5–10 days	3–6 weeks depending on size	5–10 days, but some patients are rarely ulcer-free
Healing	No scarring	Scarring	No scarring

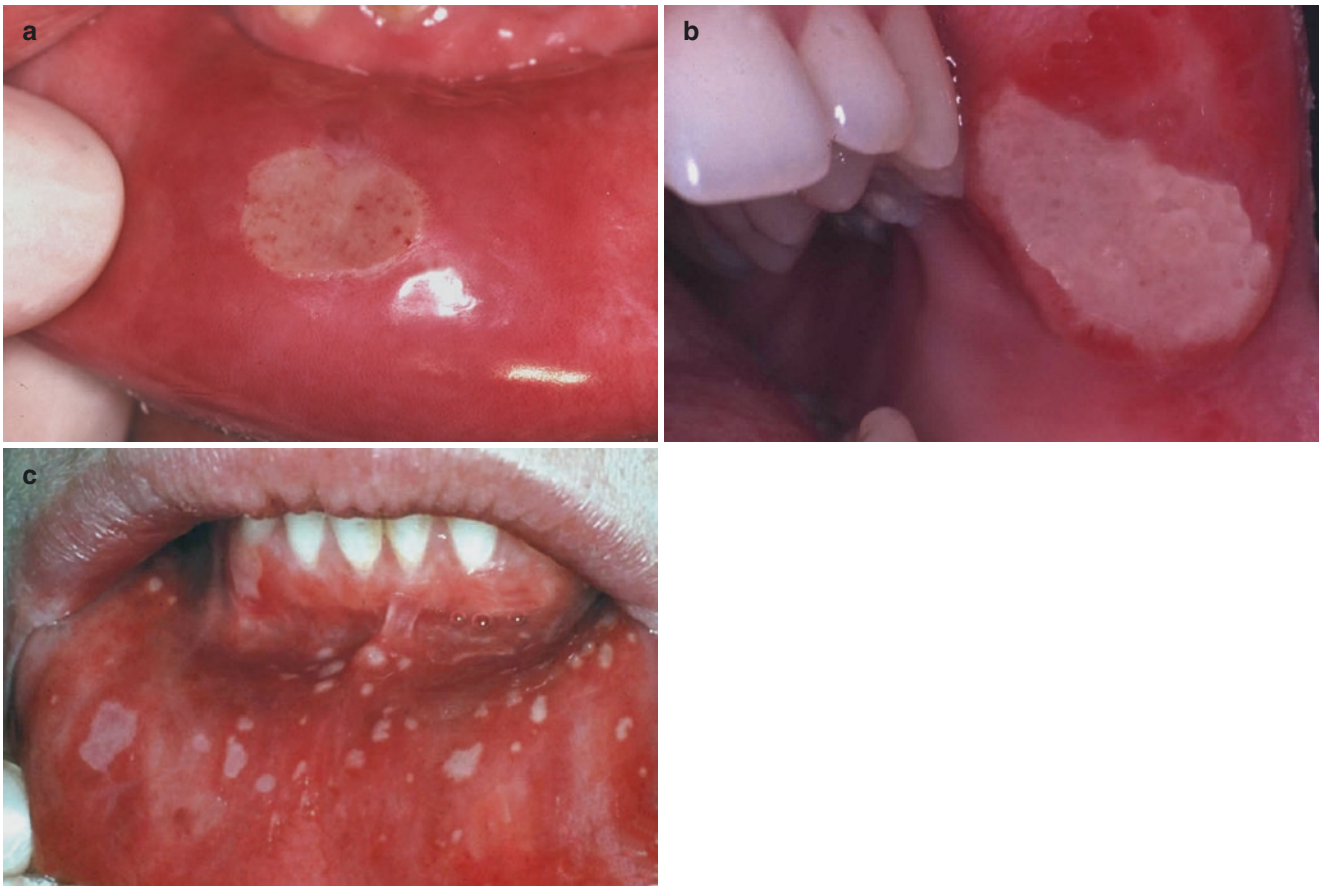

Fig. 3.8 (**a**) Minor aphtha, showing a well-defined, superficial ulcer in the early healing phase. (**b**) Major aphtha forming a large, irregular and penetrating ulcer with a conspicuously erythematous margin. (**c**) Herpetiform aphthous stomatitis with multiple small, superficial ulcers on an erythematous background

involve the non-keratinized mucosa such as the labial and buccal mucosa, ventrum of the tongue and floor of the mouth. They usually heal within 7–10 days by regeneration of the epithelium across the floor of the ulcer and without scarring (Fig. 3.8a). The ulcers frequently recur at regular intervals, typically of 2–3 weeks. Some patients, however, are virtually never ulcer-free, as new crops appear before pre-existing ones have healed. A minority of cases are menstrually related and the ulcers appear monthly in the premenstrual week. Major aphthae are less common (~10 %) and ulcers can form on both keratinized and non-keratinized mucosae. The ulcers are usually single but can be several centimetres in diameter and are penetrating (Fig. 3.8b). Hence, healing is by secondary intention and

characterised by granulation tissue formation and scarring. In severe cases, the scarring following progressive ulceration can be so extensive that it causes trismus and microstomia. Herpetiform aphthae are uncommon (~5 %) and are characterised by the formation of sometimes hundreds of small (~2 mm), superficial ulcers that frequently coalesce and may form on a background of more generalised mucosal erythema (Fig. 3.8c). Any oral location may be involved, but the labial and ventral lingual mucosae are the sites of predilection.

Microscopy It is uncommon for recurrent aphthae to be biopsied, except when a major aphtha simulates malignancy. Reported early changes include infiltration of the epithelium by lymphocytes and histiocytes and focal aggregates of lymphocytes in the superficial corium. This is followed by areas of epithelial cell apoptosis, degeneration and necrosis. The epithelium is lost and the subsequent ulcer is covered by a fibrinous slough, heavily infiltrated by polymorphonuclear leukocytes. More deeply there is a mononuclear cell infiltration and perivascular cuffing is an inconsistent feature. The condition appears to be a T-cell-mediated immunological response [59] and is thought to a response to a keratinocyte-associated antigen that is yet to be identified.

Treatment and prognosis There is a very wide range of treatments, including topical obtundents and steroids, and a range of systemic immunosuppressive and immunomodulatory agents. Many cases spontaneously resolve in early adulthood.

3.4.2 Behçet's Disease

Definition Behçet's disease is a condition comprising recurrent oral ulceration together with genital ulceration and ocular lesions [54, 60]. The ocular lesions include uveitis and retinal vasculitis.

Epidemiology Behçet's disease is a multisystem disorder due to vasculitis of small- and medium-sized vessels and can show a wide range of clinical manifestations. Features of more generalised disease include neurological disorders, arthralgia and vascular, gastrointestinal and renal lesions. The disease is uncommon in the USA and UK but has a much higher prevalence in Southeast Asia, Japan and the Eastern Mediterranean region.

Etiology and pathogenesis The etiology remains uncertain but genetic factors and immunological dysfunction appear to be important. There is a strong association with the presence of HLA-B5 and HLA*B51 [61].

Clinical aspects The oral lesions are clinically identical to recurrent aphthae and are seen in over 90 % of patients. The ulceration may be present more or less continuously and is often major and/or herpetiform in type. Patients also have genital or perigenital cutaneous ulcers, and erythema nodosum is common. A variant of Behçet's disease is termed MAGIC syndrome and is a form of relapsing polychondritis that consists of mouth and genital ulcers, together with inflamed cartilage [62].

Microscopy Like recurrent aphthae, the ulcers associated with Behçet's disease show essentially non-specific features. It has been suggested that perivascular inflammatory infiltration into the deeper corium may be a more characteristic of Behçet's disease, but the significance of this observation is questionable.

Treatment and prognosis The treatment varies according to the systems involved, but includes topical treatment with obtundents and steroids and systemic immunomodulatory therapy with corticosteroids, azathioprine, colchicine, dapsone and others.

3.4.3 Reiter Syndrome

Definition Reiter syndrome (reactive arthritis) comprises non-specific and non-septic urethritis, arthritis and conjunctivitis, although the conjunctivitis is present in less than half of cases.

Epidemiology The disease is typically seen in young males and shows a strong association with HLA-B27 (~70 %) and has been reported in HIV-infected individuals [63].

Etiology and pathogenesis It was initially thought to be only sexually transmitted, often by *Chlamydia* species, but many cases appear to result from enteric infections by a variety of organisms including *Shigella*, *Salmonella* and *Campylobacter* and other atypical infections [64].

Clinical aspects Patients develop painful mono- or polyarticular arthropathy and occasionally the temporomandibular joint is involved. Urethritis and conjunctivitis, although common, can vary considerably in severity. Patients can have fever, weight loss and CNS involvement, and facial nerve palsy has been described. Cutaneous and mucosal lesions are relatively common. The skin lesions include macules, vesicles and pustules on the hands and feet particularly and plaque-like hyperkeratotic lesions of the trunk and scalp. Oral lesions consist of circinate white or yellowish lesions surrounding macular areas that are erythematous or superficially ulcerated. They resemble circinate balanitis and the lesions of geographical tongue and geographical stomatitis [65]. They are painless and transient and are therefore rarely biopsied.

Microscopy This shows features similar to those seen in geographical tongue with spongiform pustules focally and diffusely dispersed in the superficial epithelium, but without evidence of psoriasiform hyperplasia.

Treatment and prognosis If a causative organism can be identified, appropriate antibiotic therapy is the initial treatment. This may be supplemented with non-steroidal anti-inflammatory analgesics or immunosuppressants.

3.4.4 Median Rhomboid Glossitis

Definition Median rhomboid glossitis ('posterior midline atrophic candidosis') is typically a rhomboid shaped area of depapillation in the central area of the posterior lingual dorsum.

Epidemiology It is usually seen in middle-aged or elderly patients and there is a male predominance. Predisposing factors include smoking, wearing dentures, diabetes and steroid inhalers. It can also be seen in HIV positive patients, including children [66].

Etiology and pathogenesis It was originally thought to be due to the persistence of the median developmental eminence called the tuberculum impar, and then most cases were believed to be candidal in origin [67]. However, a recent review of the literature has questioned any direct aetiopathogenic relationship between median rhomboid glossitis and candidal infestation [68].

Clinical aspects It presents as a sharply demarcated, often painless, area of depapillation in the central dorsum anterior to the foramen caecum and sulcus terminalis. In some cases, the area is nodular or grooved. Occasionally there is a 'kissing lesion' in the palate [69].

Microscopy This typically shows atrophy of the filiform and fungiform papillae and elongation, branching and fusion of the rete ridges with mild epithelial atypia (Fig. 3.9). There may be spongiform pustules in the parakeratinized surface layers and evidence of candidal hyphae. Sometimes the epithelial hyperplasia is florid resulting in a pseudoepitheliomatous appearance. Some of these lesions have been misinterpreted as squamous cell carcinomas, with significantly adverse clinical consequences [70]. There is variable chronic inflammatory infiltration in the underlying connective tissue. In addition, in the deeper connective tissue, there may be a dense, band-like zone of hyalinisation that is sometimes mistaken for amyloidosis.

Treatment and prognosis The lesion often responds to antifungal treatment but almost invariably recurs if the patient continues to smoke. There does not appear to be any premalignant potential and the dorsum of the tongue is a very uncommon site for oral cancer.

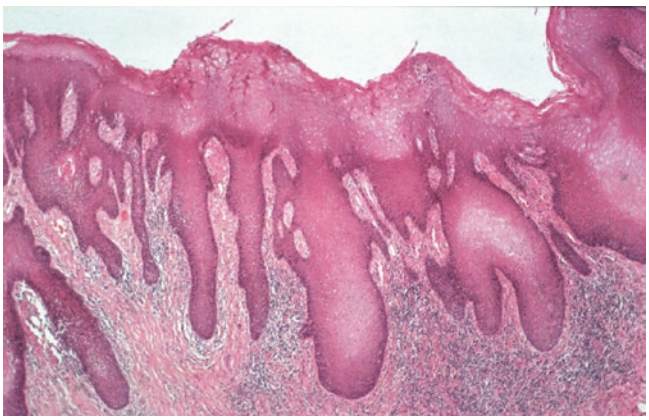

Fig. 3.9 Median rhomboid glossitis showing extensive epithelial hyperplasia and fusing of rete processes

3.4.5 Traumatic Ulcerative Granuloma with Stromal Eosinophilia (Eosinophilic Ulcer)

Definition Traumatic ulcerative granuloma with stromal eosinophilia (TUGSE) is a chronic but self-limiting lesion of a putative traumatic or reactive nature in which there is an intense inflammatory infiltration with a prominent eosinophilic component [71, 72]. Despite their designation, about a third of these lesions do not ulcerate.

Epidemiology TUGSE is most common in children and young adults. Although these lesions can be seen anywhere in the oral mucosa, including the gingiva, buccal mucosa and floor of mouth, they are most common on the tongue (75 %).

Etiology and pathogenesis About a third of patients give a history of trauma, particularly a crush injury of the lingual muscle due to biting [73].

Clinical aspects TUGSE usually presents as solid swellings or ulcers which are 2–5 cm in diameter. Occasionally there may be synchronous or metachronous lesions. They are often painless, but occasionally there can be severe pain. The ulcers have a firm, rounded, elevated margin and a depressed floor covered by fibrinous slough. There is variable erythema in the peri-ulcer mucosa. In infants, particularly, these lesions have been called Riga-Fede disease (Fig. 3.10) [74]. Here, the ulceration is typically sublingual and is due to chronic injury from the lower deciduous incisors. These lesions are usually single and can be several centimetres in diameter.

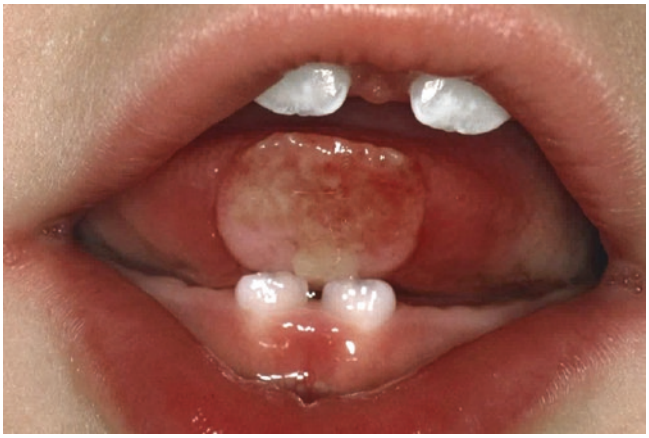

Fig. 3.10 Riga-Fede disease, showing large sublingual ulcer in an infant due to trauma from the lower central deciduous incisors

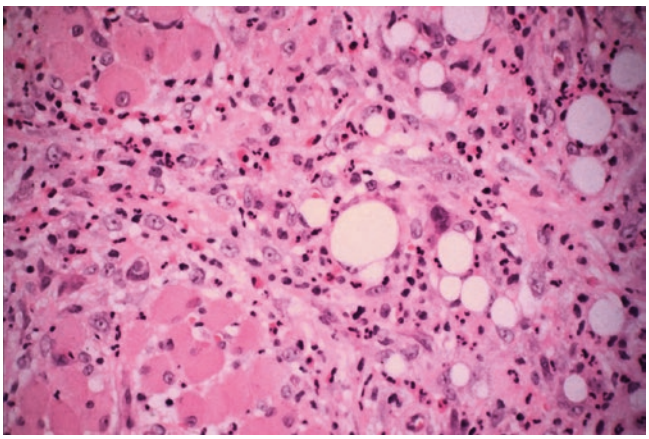

Fig. 3.11 Eosinophilic ulcer, showing plump histiocytic nuclei and eosinophils

Microscopy There is non-specific ulceration with underlying inflamed granulation tissue (Fig. 3.11). There is an associated dense inflammatory infiltrate that extends deeply into the underlying muscle. The infiltrate consists of lymphocytes and plasma cells, atypical mononuclear cells, polymorphonuclear leukocytes and mast cells. Eosinophils are particularly numerous and they may form microabscesses. The atypical mononuclear cells are frequently conspicuous and can form sheets of cells with poorly demarcated cytoplasm but large, vesicular nuclei with prominent nucleoli and a high mitotic frequency.

Differential diagnosis These lesions can be mistaken for malignancy. The atypical cells, together with damaged muscle cells showing sarcolemmal nuclear degeneration and regeneration, can give the erroneous impression of a lymphoma. In addition, the prominent eosinophilic component can lead to a mistaken diagnosis of Langerhans cell histiocytosis. This erroneous diagnosis is particularly likely in lesions involving the gingiva, where there may be associated resorption of the underlying bone. Appropriate immunocytochemical characterisation should avoid this confusion.

Immunohistochemistry The majority of lymphocytes are T cells [71]. Early immunocytochemical studies of the atypical mononuclear cells suggested a myofibroblastic or histiocytic origin [71, 75]. However, some of these cells express CD30 and T-cell lineage antigens and may also be of T-cell origin [76]. In addition, a minority of cases show evidence of T-cell clonality, raising the possibility that they are oral equivalent of primary cutaneous CD30+ lymphoproliferative disorders [77–79]. However, without other supporting evidence, aggressive treatment of such patients is not warranted.

Treatment and prognosis Many cases of TUGSE resolve spontaneously following incisional biopsy. For persistent lesions, the usual treatment is conservative surgical excision. Although recurrence tends to be uncommon, in one report 6 out of 15 cases were recurrent or multiple [73], so patients should be followed up regularly on a prolonged basis.

3.4.6 Acute Necrotising Ulcerative Gingivitis

Definition Acute necrotising ulcerative gingivitis (Vincent disease; trench mouth) is a form of ulcerative periodontal disease with severe inflammation of the gingivae. It is characterised by necrosis of the interdental papillae, ulceration of the gingival margins, pain and halitosis.

Epidemiology It is a common oral disease and is most frequent in young adult men.

Etiology and pathogenesis Although it is generally accepted that bacteria play a pivotal role in the development of the disease, a specific causal agent has not been established. In the past the Gram-negative anaerobes designated as *Treponema vincentii* and *Fusobacterium nucleatum* were strongly implicated, and *Treponema denticola* [80] and *Prevotella intermedia* are some of the current candidate organisms. A wide variety of factors predispose to the development of the disease. The most important local factors are cigarette smoking and poor oral hygiene. General predisposing factors include emotional stress, malnutrition and immunosuppressive disorders. Diabetes has also been implicated in the etiology [81].

Clinical aspects The condition is most common in young adult males, and it starts with painful, punched out and crateriform ulcers developing on and permanently destroying the tips of the interdental papillae. It can spread to the marginal gingiva and progress to involve and erode the

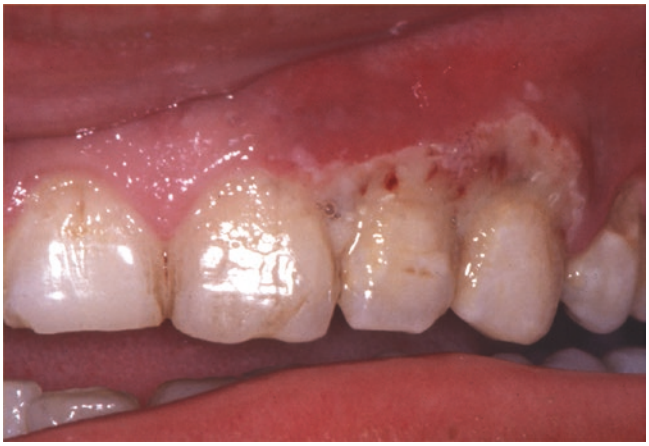

Fig. 3.12 Acute necrotising ulcerative gingivitis, showing loss of interdental papillae and ulceration extending along the adjacent gingival margins

underlying alveolar bone (Fig. 3.12). Lesions sometimes develop in relation to an operculum overlying a partially erupted third molar tooth and occasionally they spread into the adjacent buccal mucosa. There is often severe halitosis and taste disturbances. Regional lymphadenopathy is common, but constitutional symptoms tend to be relatively mild.

Microscopy It is very uncommon to receive a biopsy specimen from patients with active disease, but scrapings from the base of one of the ulcers typically show numerous polymorphs, fibrin and debris, and suitable staining reveals fusiform and spiral organisms.

Treatment and prognosis The acute condition usually responds well to local debridement and oral metronidazole or tinidazole. The tissue loss and consequent scarring often predispose to chronic periodontal disease.

3.4.7 Wegener's Granulomatosis

Definition Wegener's granulomatosis (WG) is a rare but distinctive form of idiopathic vasculitis characterised in its classical form by necrotising granulomatous inflammation and pauci-immune, small vessel vasculitis of the upper and lower respiratory tracts and kidneys [82]. The term 'granulomatosis with polyangiitis' has recently been proposed for this disease [82].

Epidemiology A wide variety of other organs and tissues may be involved. Rarely, a proliferative rather than a destructive response produces tumefactions [83]. Variants of WG include a limited form, which has few extra-pulmonary manifestations, and a protracted superficial form which is characterised by lesions restricted to the upper respiratory tract, mucosa and skin for a prolonged period, although it may eventually progress to renal involvement [84]. A clinical classification of anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitides, including WG into five categories on the basis of disease severity, has been proposed [85].

Etiology and pathogenesis The cause of WG is essentially unknown. A variety of environmental factors have been invoked, including upper respiratory infections and allergies. It is possible that such factors precipitate a humoral autoimmune response in genetically susceptible patients.

Clinical aspects Head and neck manifestations, particularly in the sinonasal complex, are common and can affect as many as 90% of patients at presentation [86]. They include severe rhinorrhoea, sinusitis, epistaxis, otitis media and destruction of the nasal septum and cartilage to produce a saddle-nose deformity. By contrast, oral lesions are less common and affect only about 5% of patients [87]. They include oral ulceration, delayed healing of extraction wounds, tooth mobility and loss of teeth. Perforation of the palate is usually as a direct extension of sinonasal disease [88]. Extraorally, head and neck manifestations include swelling and desquamation of the lips, parotid gland enlargement and cranial nerve palsies.

A rare, but particularly characteristic, oral feature is so-called strawberry gums, which are considered to be virtually pathognomonic of WG [89, 90]. There is a localised or generalised proliferative gingivitis with a mottled, purplish-red granular surface which resembles an overripe strawberry. This may be the presenting feature of the disease [91] and may precede the development of more generalised disease by a protracted period [92]. Disease localised to the gingiva tends to be fairly low grade. Involvement of the underlying bone, however, may cause the related teeth to loosen or exfoliate.

Investigations of patients with suspected oral WG should include sinus and chest radiographs, full blood picture, erythrocyte sedimentation rate, C-reactive protein, autoantibody profile (including rheumatoid factors) and renal function tests. An important investigation is the titre of ANCA, particularly cytoplasmic, or cANCA. It is found in 100% of patients with widespread, active disease, but only 60–70% of patients with limited forms of the disease [93]. Although cANCA has a very high specificity for WG, it is rarely found in other ANCA-associated vasculitides including microscopic polyangiitis and Churg-Strauss syndrome [94, 95], but these do not affect the mouth. The titre of cANCA may be related to the severity of the disease and therefore can be a useful index of prognosis and efficacy of treatment. However, in patients with limited or protracted superficial forms of the disease, the ANCA may be negative for months

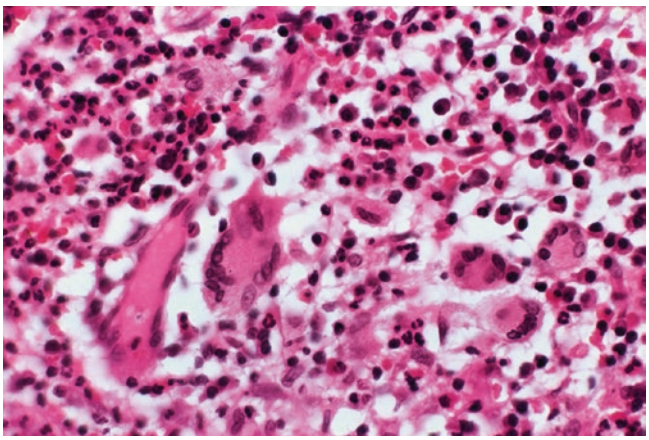

Fig. 3.13 Wegener's granulomatosis, showing intense inflammation, haemorrhage and scattered, small multinucleated giant cells

or even years so that other clinicopathological criteria should not be ignored when making the diagnosis.

Microscopy The gingiva shows irregular epithelial hyperplasia with downgrowths of the rete processes into the underlying connective tissue (Fig. 3.13). The connective tissue shows vascular lakes of extravasated blood and haemosiderin-containing macrophages, and there are neutrophil and eosinophil microabscesses, together with a more diffuse mixed inflammatory infiltration. Small multinucleated giant cells are unevenly distributed in the lesion and, although considered to be characteristic, may be absent in many levels. Vasculitis is not usually seen, possibly because vessels of sufficient size to show this feature are rarely present in gingivectomy specimens. By contrast, biopsies of other oral lesions rarely show microabscesses or necrosis. Also, the granulomatous reaction characteristic of many other sites is uncommon [96].

Treatment and prognosis In the past, WG was almost invariably fatal and it still has a relatively high morbidity and mortality. Current treatments are largely based on immunosuppressive agents [87, 97].

3.4.8 Tuberculosis

Definition Oral tuberculosis is rare but is important as it is usually a complication of advanced open pulmonary disease [98].

Epidemiology Tuberculosis remains a significant problem in the developing world and is becoming increasingly prevalent in more developed countries.

Etiology and pathogenesis The increased incidence is partly due to HIV infections and the fact that multiple drug-resistant mycobacteria are becoming widespread.

Clinical aspects Primary oral tuberculosis is typically seen in children and adolescents. It usually involves the gingiva, mucobuccal reflections and inflammatory foci adjacent to teeth or extraction sites [99]. Regional lymph nodes are frequently enlarged.

Secondary tuberculosis involves a wide age range but is most frequent in the middle aged and elderly. The typical lesion is an ulcer, most commonly on the mid-dorsum of the tongue and gingiva, but other sites may be involved. Occasionally multiple ulcers are present. The ulcer usually has undermined, minimally indurated edges, which may be stellate, and a pale, granular floor. Occasionally it presents as a non-specific area of erythema or a chronic fissure [100]. Other rare presentations include nodules [101], an indurated ulcer resembling squamous cell carcinoma [102], and hemi-macroglossia [103]. Tuberculous ulcers tend to be painless in the early stages but may become painful later. There is usually no regional lymph node involvement in secondary tuberculosis. The clinical features are often entirely non-specific.

Microscopy The diagnosis is often initially suspected when the microscopy shows multiple epithelioid granulomas in the corium underlying an ulcer with undermined margins. The granulomas are usually non-caseating, and it is unusual to demonstrate mycobacteria, even using auramine and rhodamine staining. The organisms may be detected in the sputum (but rarely in the oral lesion), and chest radiographs typically show advanced disease. In patients who are immunosuppressed, the possibility of atypical mycobacterial infection needs to be considered.

3.5 White Lesions

3.5.1 Candidal Infections

3.5.1.1 Pseudomembranous Candidosis (Thrush)

Definition Pseudomembranous candidosis (thrush) is an infection by a species of the yeast Candida causing loosely adhesive white plaques. It may be acute or persistent.

Epidemiology Oral infections with candidal organisms are very common. Thrush, or acute pseudomembranous candidosis, is seen most commonly in neonates whose immune systems are still developing and in debilitated patients at the extremes of life. It is also a feature of patients with xerostomia due to irradiation, Sjögren syndrome and a wide variety of medications, particularly the tricyclic antidepressants. In addition, it has now increasing become a feature of immunosuppressed individuals. Other factors predisposing to the development of thrush include iron deficiency anaemia,

broad-spectrum antibiotics and steroid inhalers used for the control of asthma.

Etiology and pathogenesis The most frequent organism is *Candida albicans*, a yeast-like fungus [68, 104]. It can cause acute and chronic white lesions and atrophic, red lesions. Candidal spores are present as commensal organisms in the mouths of as many as 70% of individuals. The infective phase of the organism is characterised by the presence of hyphae that can directly invade oral keratinocytes. A wide variety of factors predispose to infection by candidal organisms, particularly depressed cellular immunity and inhibition of the normal oral flora by broad-spectrum antibiotics.

Clinical aspects It is characterised clinically by the formation of soft, creamy white, friable plaques that can be easily wiped off to leave underlying erythematous areas of mucosa. The soft palate and areas protected from friction such as the vestibular reflections are the most common sites.

Microscopy The characteristic plaque of thrush is due to invasion of the superficial epithelial layers by candidal hyphae and the subsequent proliferative epithelial response. The surface epithelium is parakeratinized, oedematous and infiltrated by numerous neutrophils. Candidal hyphae penetrate the epithelium vertically and extend downwards as far as the glycogen-rich layer. The hyphae may be inconspicuous in H&E sections unless the microscope condenser is lowered to increase their refractility, but they can be readily visualised with periodic acid Schiff or Grocott's silver stains. The epithelium may show hyperplastic but attenuated rete processes, and there is variable but occasionally florid acute inflammation of the underlying corium.

Treatment and prognosis It often responds to topical antifungal polyenes such as amphotericin and nystatin and miconazole gel. More persistent cases are treated with systemic fluconazole.

3.5.1.2 Candida-Associated Denture Stomatitis

Definition Candida-associated denture stomatitis is a variant of atrophic candidosis in patients who wear a full or partial dental prosthesis.

Epidemiology It is typically seen in the hard palate beneath a full or partial dental prosthesis, particularly one constructed from acrylic.

Etiology and pathogenesis The condition is often related to poor denture hygiene and wearing the prosthesis continually.

Clinical aspects There is a sharply demarcated area of bright red, often boggy erythema limited by the extent of the denture. Occasionally there may be a few flecks of thrush but typically there is no significant plaque formation. Although sometimes referred to as 'denture sore mouth', the condition rarely causes any symptoms unless it is associated with angular stomatitis.

Microscopy There is intercellular oedema and chronic inflammatory infiltration of the corium. Candidal organisms may not be seen in biopsy specimens, as the fungus tends to proliferate within the microscopic interstices of the denture material.

Treatment and prognosis Improved denture hygiene and stopping continuous wearing of the prosthesis are often effective. It often responds to topical antifungal polyenes such as amphotericin and nystatin or miconazole oral gel.

3.5.1.3 Hyperplastic Candidosis (Candidal Leukoplakia)

Definition Hyperplastic candidosis (candidal leukoplakia) is a persistent, adherent, firm white plaque due to candidal infection.

Epidemiology The plaques may be solitary or multiple, particularly in mucocutaneous candidosis syndromes [105]. In the latter, the mouth is often the most severely affected site. Most patients with isolated plaques are men of middle age or older. The most common sites of involvement are the dorsum of the tongue and the post-commissural buccal mucosa.

Etiology and pathogenesis The majority of patients are heavy cigarette smokers.

Clinical aspects The plaques are often thick with a rough, irregular surface that may be nodular. The lesion often forms a variegated red and white patch, producing a speckled appearance.

Microscopy There is a parakeratinized surface infiltrated by neutrophils forming spongiform pustules. The epithelium shows downgrowths of blunt or club-shaped rete ridges with thinning of the suprapapillary areas to produce a psoriasiform appearance (Fig. 3.14). The basement membrane zone may be thickened and prominent, and there is variable but often severe inflammation in the underlying corium. In some cases, there can be conspicuous pericapillary fibrinous exudation, particularly in the papillary corium. Candidal hyphae may be remarkably sparse and not detected unless multiple sections and special stains are used. Electron microscopy shows that the hyphae are intracellular parasites that grow

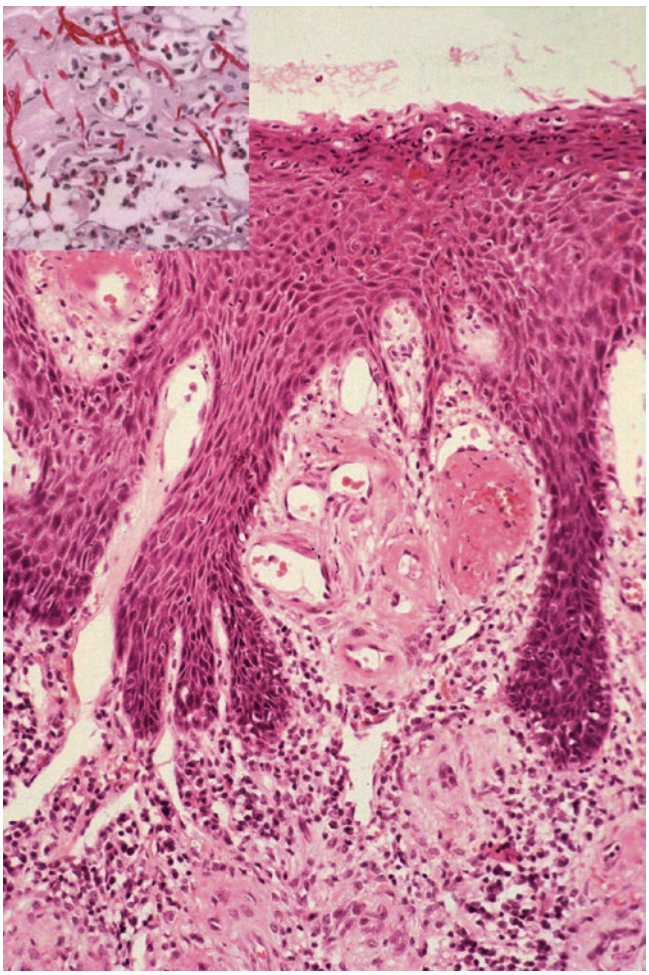

Fig. 3.14 Chronic hyperplastic candidosis, showing irregular epithelial hyperplasia, and conspicuous pericapillary fibrinous exudation. The *inset* shows a PAS-D stain illustrating penetrating candidal hyphae. These may be difficult to find in some cases

within the cytoplasm of the epithelial cells rather than along the intercellular spaces.

Treatment and prognosis Topical or systemic antifungal agents such as miconazole or fluconazole may be effective, but if the patient continues to smoke, relapses are almost inevitable. There is a significant risk of malignant transformation (2–6 %).

3.5.2 Lichen Planus

Definition Lichen planus is a cell-mediated, chronic inflammatory mucocutaneous disease of unknown origin that usually presents as lacy white patches or flat, polygonal plaques. It can also involve the hair and nails [106]. Although the majority of cases of cutaneous involvement resolve spontaneously within 2–3 years, oral lesions can be remarkably persistent, and many cases never resolve. It can give rise to white lesions, atrophic areas or superficial ulcers (erosions).

Epidemiology It is relatively common affecting about 0.5–2 % of the population. Middle-aged or older people are predominantly affected and the disease is rare in children and young adults. Women account for at least 65 % of patients.

Etiology and pathogenesis Lichen planus is a T-cell-mediated autoimmune disease in which the cytotoxic CD8+ T cells trigger apoptosis of the basal cells of the oral epithelium. Several antigen-specific and non-specific inflammatory mechanisms have been postulated to explain the accumulation and homing of CD8+ T cells subepithelially and the subsequent keratinocyte apoptosis. However, the precise etiology remains uncertain and the pathogenesis remains speculative [56, 107]. A lichen planus-like disease can also arise in patients with chronic graft-versus-host disease, autoimmune liver disease, including primary biliary cirrhosis, and chronic active hepatitis. An association between oral lichen planus and hepatitis C virus infection has been proposed in some cohort studies [108]. A wide range of drugs can precipitate or exacerbate the disease.

Clinical aspects Many purely white lesions are asymptomatic or are perceived as a rough sensation from the affected mucosa. However, in some patients, lichen planus can lead to intractable oral ulceration that may persist for decades. The lesions have characteristic clinical appearances and distribution. The most common form is striae which are sharply demarcated and form lace-like (reticular) or annular patterns. These may be interspersed with defined small, elevated papules. The patient may complain that they feel a slight restriction on opening. Less common types of white lesions are confluent plaques which some term homogeneous lichen planus. They are usually well-demarcated, raised plaques and are frequently traversed by intersecting grooves producing a tessellated appearance. The latter appearance is particularly common on the dorsum of the tongue and other sites in long-standing disease. Atrophic areas, with redness due to mucosal thinning, but without ulceration, are usually combined with areas of striation. Erosions are shallow, irregular ulcers usually covered by a slightly raised, yellowish, fibrinous slough. Very rarely bullae form.

Oral lesions of lichen planus are very often symmetrical, sometimes strikingly so, but may be more prominent on one side than another. The most frequently affected sites are the buccal mucosae, particularly posteriorly, but lesions

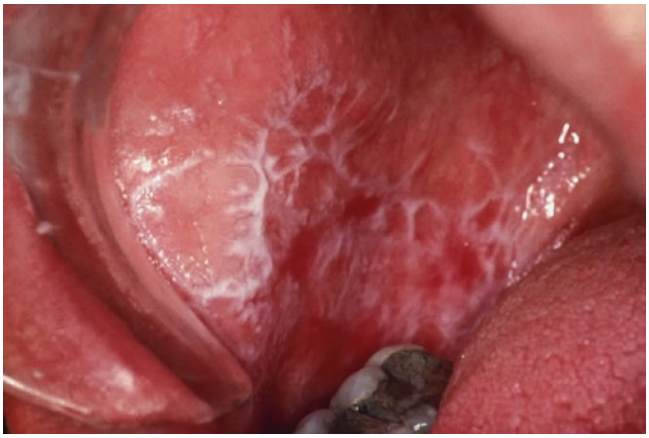

Fig 3.15 Lichen planus of buccal mucosa forming conspicuous, bluish white striations, with focal areas of mucosal erythema

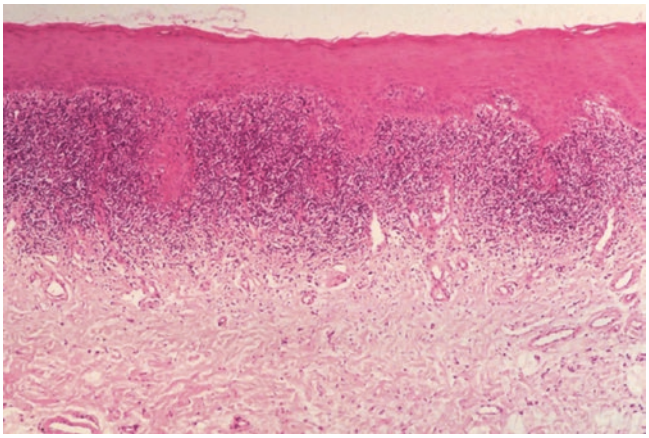

Fig. 3.16 Lichen planus, showing irregular and indistinct rete ridges and a dense, band-like, predominantly lymphocytic infiltrate in the underlying corium

may extend to the commissures (Fig. 3.15). The tongue is the next most commonly affected site. The lesions usually involve the lateral areas of the dorsum bilaterally or less frequently the central area of the dorsum. The ventrum is a relatively uncommon site. Atrophic lichen planus often involves the gingiva, but reticular lesions are relatively uncommon at this site. The lips, sometimes including the vermilion border, may be involved, but the palate is rarely affected, and lesions in the floor of the mouth are exceptional.

Sometimes involvement of the gingiva may be the predominant or only manifestation of lichen planus. As such, it needs to be distinguished from a variety of other inflammatory gingival conditions. The most common appearance is gingival atrophy and the epithelial thinning leads to a shiny, red, smooth appearance. This is known clinically as desquamative gingivitis. It is important to appreciate that desquamative gingivitis is a clinical descriptive term and not a diagnosis. Diseases other than lichen planus that can produce this appearance include mucous membrane pemphigoid, pemphigus and a condition called plasma cell gingivitis that is probably allergy based. Unlike marginal gingivitis, the inflammation can extend onto the alveolar mucosa, but in the absence of secondary plaque accumulation, there is usually sparing of the marginal gingiva and interdental papillae. The condition may be generalised or only patchily distributed. Also, for unknown reasons, it is rare on the lingual and palatal gingiva.

Microscopy Clinically white lesions show parakeratosis or hyperorthokeratosis, sometimes with a prominent granular cell layer [109]. The keratosis may be patchily distributed, as might be expected in reticular or striated lesions. There is a very characteristic band-like lymphohistiocytic infiltrate sharply localised to the superficial corium (Fig. 3.16). Occasionally germinal centres form within the lymphoid infiltrate. There is often conspicuous basal cell damage with apoptosis, ballooning degeneration due to intracellular oedema and the formation of colloid (Civatte) bodies (Fig. 3.17). Fibrinogen deposition along the basement membrane zone is sometimes a conspicuous feature. There may be pigmentary incontinence secondary to the basal cell liquefaction and melanophages in the superficial corium. The rete ridge pattern is variable, but the sawtooth pattern typical of cutaneous lichen planus is relatively uncommon in oral lesions. In lesions from the dorsum of the tongue particularly, the rete processes may be elongated with dense inflammatory infiltrates around their tips.

Atrophic lesions show conspicuous thinning and flattening of the epithelium but the characteristic band-like inflammatory infiltrate is retained. In ulcerated lesions, the inflammatory infiltrate contains polymorphonuclear leukocytes and plasma cells and extends into the deeper corium, often leading to a non-specific appearance.

As lichen planus is often treated with topical steroids, it is not uncommon to find infestation of the superficial epithelial layers by candidal hyphae. These may or may not be associated with spongiform pustules. Some of these lesions may also show reactive cytological atypia.

Differential diagnosis A great variety of drugs can cause diseases resembling or in some cases indistinguishable from lichen planus (lichenoid drug eruptions). There may be a history relating the onset of lesions to the drug administration or exacerbation of previously quiescent disease [110, 111]. The oral lesions are often severely ulcerated, and the dorsum of the tongue and palate appear to be sites of predilection. In some patients, there may be a lichenoid reaction in mucosa in direct contact with dental amalgam fillings and occasionally even composite filling material and gold restorations. There are no absolute diagnostic criteria distinguishing lichen planus from lichenoid drug eruptions [110]. It is

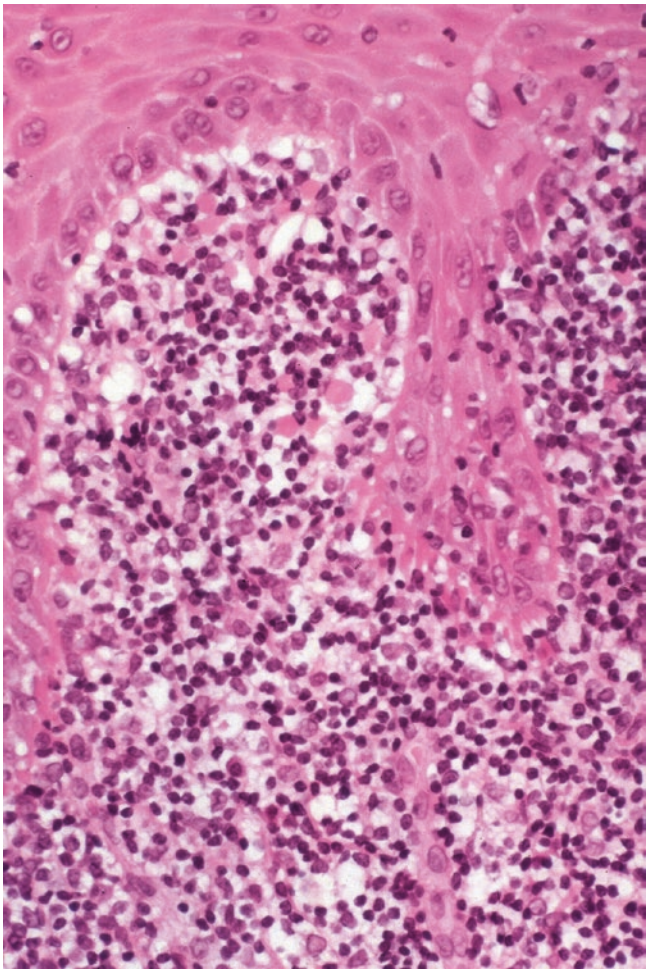

Fig. 3.17 Lichen planus, showing basal cell degeneration and Civatte bodies

reported that in lichenoid reactions the inflammatory infiltrate is denser. In addition, it is said to be more likely to show the presence of plasma cells in the infiltrate, particularly in the leading edge, and a greater likelihood of germinal follicle formation. In addition, the deep layer of the infiltrate is less defined, and perivascular inflammatory infiltration extending into the deeper corium is seen more frequently. However, many of these features may reflect the more severe nature of the disease and be related merely to the effects of ulceration. It is therefore essential to examine areas well away from obvious ulceration when interpreting these biopsies.

Treatment and prognosis The management of lichen planus can be complex. It is usually treated with topical or systemic immunosuppressive or immunomodulatory medications. There is a risk of developing squamous cell carcinoma, but the magnitude of the risk is the subject of ongoing and controversial discussions within the literature. Most reports quote a risk of malignant transformation of 0.5–2 %, but the majority of studies to date have been retrospective, and some lack clarity regarding the criteria for the initial diagnosis [112, 113]. Further prospective and molecular-based studies are required to inform the debate.

3.5.3 Lupus Erythematosus

Definition Lupus erythematosus is a chronic, multisystem autoimmune disease of unknown origin.

Epidemiology The two main forms that affect the mouth are discoid lupus erythematosus (DLE; chronic cutaneous lupus erythematosus) and systemic lupus erythematosus (SLE). Oral lesions are present in over 20 % of patients with SLE.

Clinical aspects The clinical features of oral DLE closely resemble those of lichen planus [114]. They typically show a central area of atrophic, erythematous or granular mucosa with a surrounding radiating, striated white halo. The central area occasionally ulcerates. Lesions are most common in the centre of the palate and on the labial aspect of the upper lip, but they can be seen elsewhere in the mouth. Sometimes there are adjacent 'kissing lesions' on the gingiva opposite labial lesions. The lesions of DLE lack the symmetrical distribution characteristic of oral lichen planus. Patients with SLE may have the classical photosensitive butterfly rash in the midface and show other evidence of a systematised disease. The mucosal lesions may resemble those of DLE or show evidence of more severe mucositis and non-specific ulceration. Shallow linear ulcers running parallel to the palatal gingiva are sometimes a striking feature.

Microscopy This shows many similarities to lichen planus [115, 116]. There is either hyperorthokeratosis or hyperparakeratosis (Fig. 3.18). The follicular plugging characteristic of cutaneous lesions has been described in oral lesions, but it is an inconsistent and frequently poorly defined feature. The rete processes are hyperplastic and can form flame-like downgrowths into the underlying corium. This feature is sometimes so florid that it produces a pseudoepitheliomatous appearance. The slender downgrowths can also show a tendency to fuse with each other producing an appearance simulating an embedding cross-cut artefact. Dyskeratotic cells are an occasional feature. There is apoptosis and liquefaction degeneration of the basal cells, sometimes with Civatte body formation. The BMZ may become hyalinised and thickened and this is sometimes a notable feature. There is a band-like infiltrate of lymphohistiocytic cells in the superficial corium similar to that seen in lichen planus. However, the lower border tends to be less well defined, and the infiltrate often extends into the deeper tissues in a perivascular distribution. Reactive follicles may form in the lymphoid infiltrate. In cases of SLE, there may also be evidence of fibrinoid necrosis in vessels.

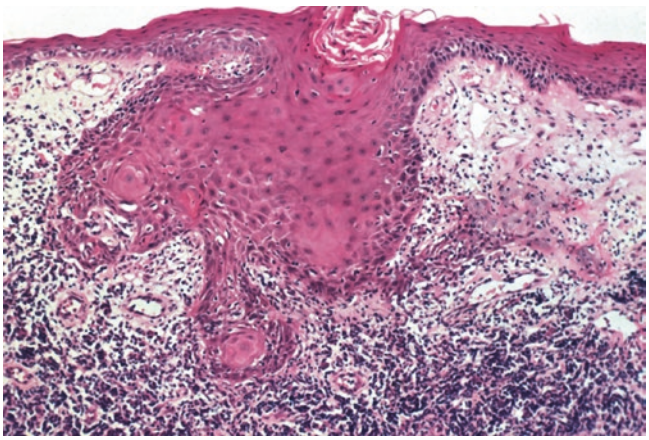

Fig. 3.18 Discoid lupus erythematosus, showing irregular focal epithelial hyperplasia and follicular plugging

Immunohistochemistry Direct immunofluorescence of lesional tissue for IG, IgM or IgA shows a granular deposit in the BMZ in about 75 % of cases of oral DLE and all cases of SLE. A lupus band test in clinically normal skin or mucosa is diagnostic of systemic disease. The presence of C3 or fibrinogen in the BMZ is not specific and is frequently demonstrated in lichen planus.

Treatment and prognosis The treatment of the oral lesions is similar to that of lichen planus.

3.5.4 Oral Epithelial Nevi

Non-pigmented epithelial nevi are rare in the oral cavity. They include white sponge nevus, oral epithelial nevi and nevi associated with nevus unius lateris.

3.5.4.1 White Sponge Nevus

Definition White sponge nevus is an autosomal dominant inherited trait characterised by heaped up, sodden, irregular white plaques.

Epidemiology The plaques can affect any part of the oral mucosa and may involve other mucosae including nasal, anal and vaginal (Cannon's syndrome).

Etiology and pathogenesis It is associated with mutations in the keratin 4 and 13 genes [117, 118].

Microscopy There is acanthosis with vacuolated cells in the stratum spinosum producing a basket-weave appearance and irregular, shaggy parakeratosis.

Treatment and prognosis The lesion is entirely benign.

3.5.4.2 Oral Epithelial Nevus

Definition Oral epithelial nevus is a distinctive white plaque involving the ventral lingual mucosa and floor of the mouth [119].

Clinical aspects The plaques are sharply defined and irregularly butterfly shaped and have a uniformly wrinkled surface.

Microscopy This shows hyperkeratosis, often with epithelial atrophy with no evidence of dysplasia.

Treatment and prognosis A retrospective study of lesions of this type in the floor of the mouth suggested there was a substantial risk of malignant transformation in this lesion and it was renamed sublingual keratosis [120, 121]. Since that time, there have been no other studies substantiating these observations, and the status of these lesions needs to be re-evaluated.

3.5.4.3 Nevus Unius Lateris

Definition Nevus unius lateris typically involves the skin with unilateral linear, papillary or verrucous lesions, usually along the long axis of a limb or across the trunk. It has uncommonly been associated with oral lesions [122].

Clinical aspects Papillary, wart-like proliferations have been described on the lips, tongue, buccal mucosa, palate and gingivae, usually on the left side.

Microscopy There is papilliferous proliferation with non-keratinized, hyperplastic epithelium covering connective tissue cores that may be patchily inflamed. Similar lesions have been described in the absence of cutaneous lesions, usually in the midline of the palate.

Treatment and prognosis The lesions are benign.

3.5.5 Smoker's Keratosis

Definition Smoker's keratosis is a white patch associated with both tobacco smoking and chewing.

Etiology and pathogenesis The lesions appear to be a result of both thermal and chemical irritation.

Clinical aspects The affected mucosa may show diffuse whitening or more focal lesions, and occasionally they are pigmented and have a slate-blue colour.

Microscopy The epithelium can be hyperplastic or atrophic and is not dysplastic [123]. There is very variable parakeratosis or hyperorthokeratosis. Some cases show focal parakeratotic spikes ('chevrons'). Although such chevrons were thought to be particularly characteristic of smokeless

tobacco-induced keratosis, only a minority of such lesions show this feature [124]. Pigmentary incontinence is common and may be florid, especially in darkly pigmented individuals. Inflammatory infiltration is usually minimal.

Treatment and prognosis They rarely show significant dysplasia and appear to have a low premalignant potential. The lesions spontaneously resolve if the habit is stopped.

3.5.6 Stomatitis Nicotina

Definition Stomatitis nicotina is a white patch which is usually seen on the hard palate of pipe or cigar smokers [125]. Minor degrees of the condition may be seen in heavy cigarette smokers.

Epidemiology The overwhelming majority of patients are men.

Etiology and pathogenesis The initial lesion appears to be increased keratosis of the palate exposed to the smoke. This leads to obstruction of the ducts of the underlying minor salivary glands which then become inflamed.

Clinical features The classical clinical appearance is whitening of the palatal mucosa which may show tessellated plaque formation. The involved minor glands become swollen and have red, umbilicated centres. It is usually painless and asymptomatic. Similar lesions are occasionally seen in the mucosal vestibules of tobacco chewers.

Microscopy There is variable hyperkeratosis, acanthosis and duct dilatation. There is usually no evidence of epithelial dysplasia. There is variable submucosal chronic inflammation and there may be evidence of pigmentary incontinence. Keratinization can extend down the salivary ducts, and there is interstitial inflammation of the underlying minor mucous glands.

Differential diagnosis Similar clinical appearances have been reported in patients who regularly drink very hot liquids.

Treatment and prognosis The condition gradually resolves if the habit is discontinued, and there appears to be a minimal risk of malignant transformation. However, affected individuals have an increased risk of developing squamous cell carcinoma in other parts of the mouth, particularly the floor of the mouth and adjacent ventral lingual mucosa, and the retromolar trigone. Conversely, palatal keratosis due to 'reverse smoking' where the lighted end of a cigarette or cigar is held in the mouth is associated with the development of carcinomas of the hard or soft palates in a very high number of patients practising the habit.

3.5.7 Hairy Tongue

Definition Hairy tongue is condition where there is hyperplasia and elongation of the filiform papillae forming hair-like overgrowths on the dorsum.

Epidemiology Hairy tongue is usually seen in older individuals.

Etiology and pathogenesis Smoking, antiseptic mouthwashes, antibiotics and a diet lacking abrasive foodstuffs are the most common predisposing factors. The discoloration is due to proliferation of chromogenic bacteria and fungi.

Clinical aspects The filaments can be several millimetres long. The colour varies from pale brown to intense black. The dorsum of the tongue may also become blackened without elongation of the filiform papillae by antibiotic mouthwashes such as tetracycline and iron compounds. Hairy tongue is rarely biopsied.

Microscopically There are irregular, hyperplastic filiform papillae showing hyperorthokeratosis or hyperparakeratosis with numerous bacterial conglomerates and filamentous organisms in the surface layers and more deeply between fronds of epithelium. It has been shown by immunocytochemical analysis of keratin expression that in black hairy tongue there is defective desquamation of cells in the central column of the filiform papillae. This results in the typical highly elongated, cornified spines that are the characteristic feature of the condition [126].

Treatment and prognosis Tongue scrapers and care with oral hygiene are usually helpful and the condition is entirely benign.

3.5.8 Hairy Leukoplakia

Definition Oral hairy leukoplakia (OHL) is a white patch that typically occurs on the lateral and ventral aspect of the tongue in immunosuppressed patients. The lesion was called hairy leukoplakia, but usually it forms painless, vertical, white corrugations which may or may not have a rough or 'hairy' surface. Some lesions are flat white plaques. Other sites may also be involved, especially the post-commissural buccal mucosa.

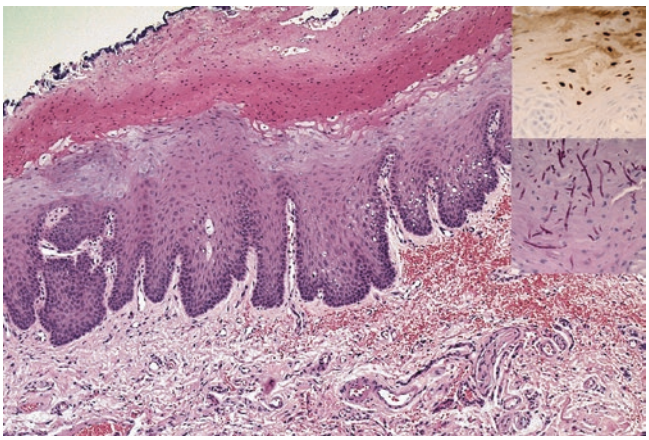

Fig. 3.19 Hairy leukoplakia showing irregular hyperparakeratosis and koilocytes in the upper epithelium. *Upper inset* shows EBV-positive cells. *Lower inset* shows PAS/D-positive candidal hyphae penetrating deeply through the parakeratinized layers

Etiology and pathogenesis It is caused by *Epstein-Barr* virus (EBV) and occurs primarily in patients with human immunodeficiency virus infection/acquired immunodeficiency syndrome (HIV/AIDS). Similar lesions have, however, occasionally been reported in HIV-negative patients in a background of solid organ transplantation, haematological malignancy, chronic graft vs host disease and the use of immunosuppressive medications. OHL has very rarely been reported as an incidental finding in immunocompetent persons [127, 128].

Microscopy There is acanthosis and parakeratosis (Fig. 3.19), usually with verruciform, hair-like surface projections [129]. Invasion of the surface epithelium by candidal hyphae is common. Immediately below the parakeratotic layer, there is a zone of vacuolated and enlarged epithelial cells with intense basophilic, pyknotic nuclei and perinuclear clearing (koilocytes). Epstein-Barr capsid viral antigen and viral particles can be demonstrated in the koilocytic nuclei [130]. There is usually little or no inflammatory infiltration of the epithelium or underlying corium. Similar lesions have occasionally been reported in patients receiving immunosuppressant drugs following organ transplantation. The historically early cases of hairy leukoplakia associated with HIV infection showed a very high rate of progression to full-blown AIDS.

Treatment and prognosis Lesions can resolve spontaneously and usually respond well to antiviral or anti-retroviral drug treatment; they appear to have no premalignant potential.

3.5.9 Geographical Tongue (Benign Migratory Glossitis)

Definition Geographical tongue (benign migratory glossitis) is a relatively common, idiopathic condition typically characterised by migrating areas of depapillation on the dorsum of the tongue [131].

Epidemiology Geographical tongue occurs at all ages. In many cases, it is associated with fissuring.

Etiology and pathogenesis Although geographical tongue is an inflammatory condition histologically, a polygenic mode of inheritance has been suggested because it is seen clustering in families. Associations with human leukocyte antigen (HLA)-DR5, HLA-DRw6 and HLA-Cw6 have also been reported [132, 133].

Clinical aspects There is loss of filiform papillae often surrounded by a slightly raised yellowish-white and crenellated margin. These areas of depapillation tend periodically to heal centrally and spread centrifugally and then to regress. Occasionally, the ventrum is involved and in that site lesions show an area of erythema completely, or partially, surrounded by a circinate, whitish halo. Identical lesions can occasionally be seen elsewhere in the mouth and have been called 'ectopic geographical tongue' although geographical stomatitis or benign migratory stomatitis would be more appropriate terms [134]. The majority of cases of geographical tongue are painless, but some patients complain bitterly of soreness and discomfort, which may or may not be associated with specific foods.

Microscopy Geographical tongue is usually obvious clinically and is rarely biopsied. However, it has very typical microscopical features [135]. There is loss of filiform papillae and usually only a mild chronic inflammatory reaction in the underlying corium. The striking feature is the presence of polymorphonuclear leukocytic microabscesses in the upper stratum spinosum (Fig. 3.20).

Differential diagnosis Spongiform pustules are not pathognomonic of geographical tongue and can be seen in oral psoriasis, acute and chronic candidosis, Reiter syndrome and plasma cell gingivostomatitis. Some reports describe elongation of the rete ridges but this is by no means a consistent observation. However, occasionally, there may be psoriasiform hyperplasia in geographical tongue, and it may be difficult or impossible to distinguish from psoriasis. Indeed, geographical tongue and migratory stomatitis are four to five times more common in patients with psoriasis, and some believe that geographical tongue is the oral homology of psoriasis [136]. The presence of spongiform pustules in oral biopsies should always prompt the search for candidal hyphae with a PAS or Grocott's stain. These are not usually seen in geographical tongue and their presence would make a diagnosis of candidosis much more likely. Chronic hyperplastic candidosis can also show psoriasiform hyperplasia,

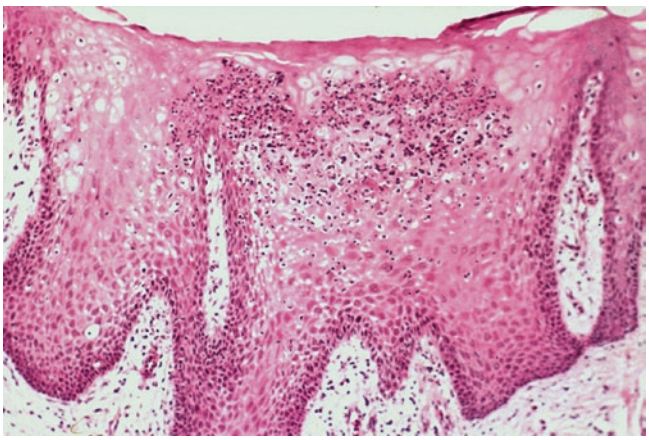

Fig. 3.20 Erythema migrans, showing polymorphonuclear leukocyte microabscesses in the epithelium

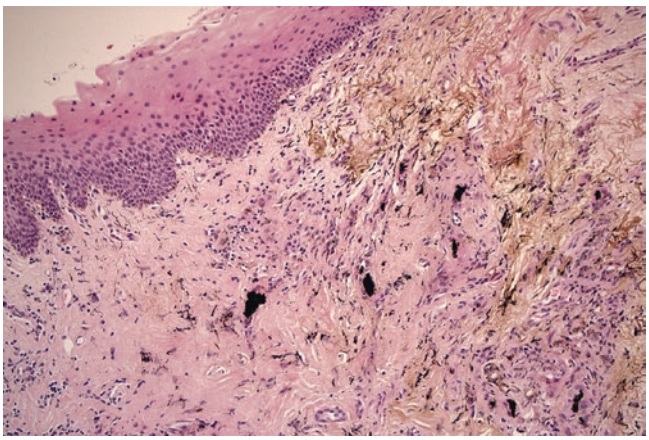

Fig. 3.21 Amalgam tattoo showing solid masses of amalgam and extensive silver staining of fine elastic and reticulin fibres

but typically there is much more florid inflammatory infiltration of the corium and irregular surface parakeratosis.

Treatment and prognosis There are no specific treatments for this condition, but avoiding irritant foods and drinks, and topical benzydamine hydrochloride or chlorhexidine can sometimes be helpful.

3.5.10 Frictional Keratosis

Definition Frictional keratosis of the oral mucosa is common and is a response to low-grade physical irritation.

Etiology and pathogenesis Causes include sharp edges of teeth or restorations, dental prostheses, mucosal chewing, abrasive foods, vigorous tooth brushing and playing wind instruments.

Clinical aspects The lesions tend to form diffuse keratotic plaques. In the early stages, these are pale and translucent and merge imperceptibly into the surrounding normal mucosa. Later, they become more dense and white and may have an irregular, shaggy surface. The habit of cheek, lip and tongue chewing is a usually characteristic variant of frictional keratosis. It results in roughened or shredded, often patchily erythematous, white plaques limited to areas accessible to chewing.

Microscopy In typical frictional keratosis, there is a thick orthokeratinized layer with a prominent granular cell layer. There is no significant dysplasia and inflammation of the underlying corium may be minimal in the absence of ulceration. In keratosis due to habitual chewing there is often acanthosis, and the surface is usually irregular and parakeratinized. It frequently shows a covering of adherent basophilic cocci or more dense bacterial conglomerates.

Treatment and prognosis The condition is benign and removal of the cause leads to resolution.

3.6 Pigmentations

3.6.1 Amalgam Tattoo

Definition Amalgam tattoo is a darkly pigmented area of oral mucosa caused by the submucosal ingress of dental amalgam.

Epidemiology It is the most common localised form of oral mucosal pigmentation [137]. The most frequent sites are the gingiva, alveolar mucosa and floor of the mouth.

Etiology and pathogenesis They are caused by ingress of dental amalgam through a mucosal breach during a restorative procedure or tooth extraction and can also follow an apicectomy with retrograde root filling. A recent case report postulated an association between amalgam tattoo and burning mouth syndrome [138]. In addition, a lichenoid reaction has been reported in association with an amalgam tattoo [139].

Clinical aspects Lesions usually form painless, bluish-black macules, which may be well defined or diffuse.

Microscopy There are dark, refractile particles of amalgam in the corium [140]. These may be coarse but usually form fine, black or brownish granules (Fig. 3.21). These are deposited along collagen and elastic fibres and around small blood vessels, nerves and muscles. About half of cases show a fibrous and chronic inflammatory reaction, with or without

multinucleated foreign body giant cells. Occasionally there is a granulomatous reaction.

Treatment and prognosis When the clinical diagnosis is obvious, most believe that active treatment is not necessary. However, amalgam tattoos contain a wide variety of metals, including silver, mercury, tin, copper, zinc, palladium and other heavy metals. On that basis, some have questioned whether they may be associated with adverse systemic effects, including allergic reactions [141].

3.6.2 Localised Melanotic Pigmentation

3.6.2.1 Oral Melanotic Macules

Definition Oral melanotic macules are benign, ephelis-like pigmented macules.

Epidemiology They are the most common melanocytic lesions of the oral mucosa [142]. They develop during early to middle adult life with a mean age at presentation of 43.7 years [143] and a female predilection of 2:1. Melanotic macules have been described in newborn children where they present as multiple lesions on the dorsum of the tongue. This clinically distinct entity has been termed 'congenital lingual melanotic macules' [144, 145].

Etiology and pathogenesis Rarely, oral melanotic macules have been reported following radiotherapy [146], and in HIV infections, probably related to the administration of retroviral drugs [147].

Clinical aspects They are brownish-blue or black and may be single or multiple. They are usually well defined and rarely exceed 6 mm in diameter. Melanotic macules are most frequent in the anterior part of the mouth affecting the gingiva, buccal mucosa and, most commonly, the labial mucosa. Those involving the vermilion border (labial melanotic macules) often darken in strong sunlight and may cause cosmetic problems.

Microscopy This shows increased melanotic pigmentation in the basal, and occasionally the immediately suprabasal, keratinocytes. There is often pigmentary incontinence and melanophages in the superficial corium (Fig. 3.22).

Treatment and prognosis Melanotic macules are benign and, if necessary, conservative surgical excision is curative.

3.6.2.2 Melanoacanthoma

Definition Melanoacanthoma (melanoacanthosis) is a rare, probably reactive, proliferation of both keratinocytes and melanocytes [148].

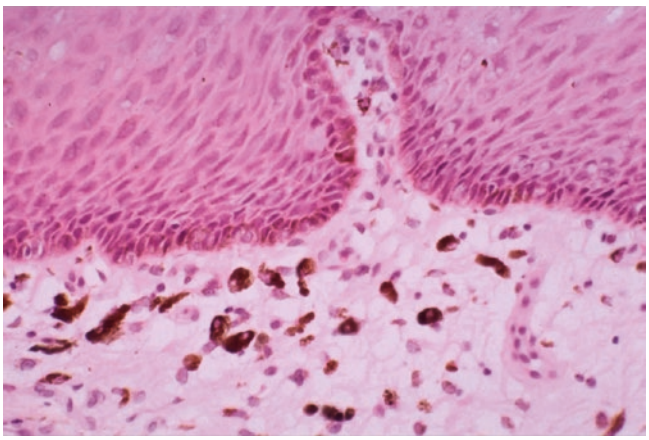

Fig. 3.22 Melanotic macule, showing increased melanocytic pigmentation of basal keratinocytes and melanophages in the superficial corium

Epidemiology It is seen most commonly in black females in the 20–40 year age range, typically involving the buccal mucosa. Other reported intraoral sites include the lips, palate, gingiva, tongue and alveolar mucosa.

Etiology and pathogenesis Trauma, or local irritation, is thought to be the most likely cause.

Clinical aspects Their colour varies from brown to black, and rapid growth is relatively common. They are usually macular; less frequently, they are slightly raised or papilliferous. Lesions may be single or multiple [149, 150]. Multifocal cases present with an equal sex distribution and commonly involve the palate. Rare cases showing more diffuse mucosal involvement have been reported [151].

Microscopy There is acanthosis and frequently spongiosis that can be florid (Fig. 3.23a). The diagnostic feature of melanoacanthoma is the presence of dendritic melanocytes extending throughout the full thickness of the epithelium. These cells are strongly positive with Fontana silver stain (Fig. 3.23b) and are S-100, HMB45 and Melan-A positive [152]. As a consequence of a partial or complete block in pigment transfer, the keratinocytes in melanoacanthoma contain little or no melanin in spite of the abundance of melanocytes. There is variable, usually patchy, chronic inflammatory cell infiltrate in the superficial corium and occasionally there are scattered eosinophils.

Treatment and prognosis Lesions can regress spontaneously or following incisional biopsy. There is no evidence of any malignant potential.

3.6.2.3 Pigmented Nevi

Definition Oral melanocytic nevi are benign, hamartomatous lesions of the oral mucosa caused by a disorder of melanocytes.

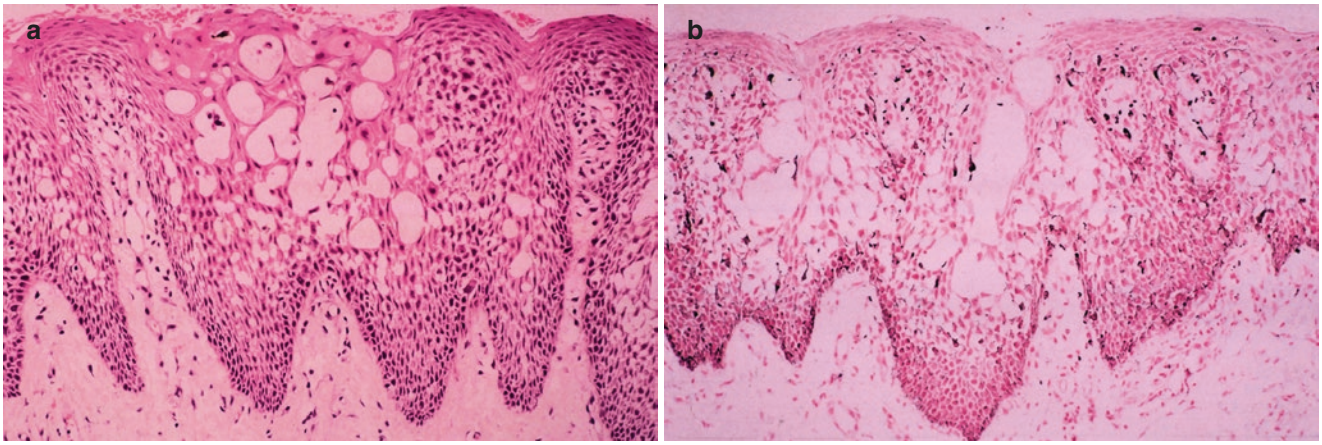

Fig. 3.23 (**a**) Melanoacanthoma, showing acanthotic, spongiotic epithelium with dendritic melanocytes extending through its total thickness. (**b**) Melanoacanthoma. Masson Fontana stain

Epidemiology They much rarer than cutaneous lesions with a prevalence of 0.1 % of the general population [153]. The most common sites are the hard palate, buccal mucosa and labial mucosa. Sixty percent are seen in women and 40 % in men and most cases are seen in the third and fourth decades [143].

Clinical aspects Most are less than 6 mm in diameter [154]. They are usually single and form brown, bluish or black macules or sessile papules. Rare, non-pigmented melanocytic nevi have been reported [155].

Microscopy Intramucosal (intradermal) nevi account for over half of the cases and junctional or compound nevi are uncommon. Blue nevi form 25–35 % of cases and usually present in the palate. The large majority are of the common rather than the cellular type.

Treatment and prognosis There does not appear to be any significant risk of intraoral nevi undergoing malignant transformation [156].

3.6.3 Premalignant Oral Melanoses and Oral Melanoma

Definition Oral mucosal melanoma is a malignant tumor of mucosal melanocytes.

Epidemiology They are rare and account for about 0.5 % of oral malignancies [153, 157]. However, they are more common in Japan, India and Africa [158]. Most arise in adults, with a peak age incidence between 40 and 60 years. Large series show that males account for about 60 % of cases [159, 160]. About 80 % of cases involve the palate and maxillary alveolus and gingivae. The majority arise de novo from clinically normal mucosa, and a third of cases are preceded by long-standing areas of oral hyperpigmentation. They rarely arise from pre-existing melanocytic nevi.

Etiology and pathogenesis The etiology is unknown.

Clinical aspects The majority of cases are painless in the early stages and form irregular, black or brownish flat, raised or nodular areas that are frequently multicentric. Rarely they are amelanotic [161] and may be reddish in colour. Nodular areas are usually a feature of more advanced tumors and may be ulcerated and associated with pain and bleeding. Invasion of the underlying bone is common and involved teeth may loosen or exfoliate. In most cases, there is involvement of the cervical lymph nodes at presentation and half of patients have distant metastases.

Purely nodular melanomas are relatively rare and most tumors have a radial growth element similar to that seen in cutaneous acral lentiginous melanoma together with evidence of upward migration. Oral melanomas have been divided into:

1. In situ oral mucosal melanoma
2. Invasive oral mucosa melanoma
3. Mixed in situ and invasive oral mucosal melanoma

About 15 % of oral mucosal melanomas are in situ, 30 % are invasive, and 55 % have a combined pattern [160]. Borderline lesions have been termed atypical melanocytic proliferations [153].

Microscopy In situ melanomas show an increase in atypical melanocytes. Although these cells have angular and hyperchromatic nuclei, mitoses tend to be sparse. The melanocytes may form aggregates or be irregularly distributed in a junctional location. The characteristic nested pattern commonly seen in cutaneous melanomas is less frequently observed in mucosal lesions. Sometimes the melanocytes are dispersed throughout the epithelium, and this may be combined with a

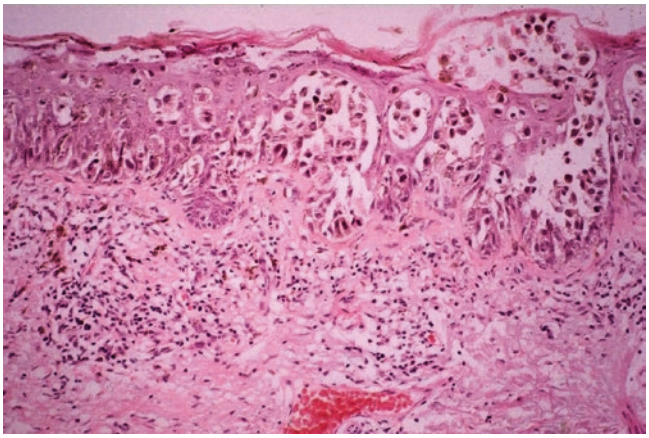

Fig. 3.24 In situ melanoma, showing atypical melanocytes widely dispersed throughout the epithelium

junctional pattern (Fig. 3.24). Sequential biopsies have shown increases in the density of the junctional atypical melanocytes over time. Atypical melanocytes can extend down the excretory ducts of the underlying minor salivary glands. However, there is usually no inflammatory response to in situ lesions.

The melanocytes present in invasive melanomas show a variety of cell types including epithelioid, plasmacytoid, clear and spindle [162]. They typically have large, vesicular nuclei with prominent nucleoli. Mitoses may be present but usually not in large numbers. The melanocytes are usually aggregated into sheets or alveolar groups, and less commonly neurotropic or desmoplastic configurations are seen. There may be pseudoepitheliomatous hyperplasia of the overlying oral epithelium [163]. In some tumors, there is melanin pigment in the underlying salivary gland ducts. This has been termed 'melanogenic metaplasia' [164]. Rarely, tumors show no obvious melanin pigmentation and the diagnosis may not be immediately apparent. Over 95% of melanomas are anti S-100 antigen positive [165], and more specific markers include HMB45, Melan-A and antityrosinase [166].

Atypical melanocytic proliferation or hyperplasia is the term used for lesions with equivocal histopathological features, but the criteria for inclusion in this category are rather ill-defined [167]. These include oral mucosal lesions with melanocytes containing angular or hyperchromatic nuclei with very infrequent mitoses. Melanocytic atypia can vary from mild to severe [153].

Treatment and prognosis Oral melanomas are much more aggressive than their cutaneous counterparts. The prognosis of oral melanomas is poor with a 5-year survival rate of less than 20% [160], and even stage I tumors have a 5-year survival rate of less than 50%. The conventional depth of invasion indicators such as Breslow thickness and Clark's levels tend to be of little value in mucosal melanomas, as many present at an advanced stage and most are deeper than 4 mm [168]. A simple TNM clinical staging system with three stages has been proposed and appears to have some prognostic value [158]. This has been refined to include histopathological microstaging of the stage 1 category (lymph node negative) into three levels of involvement [169]. Histological features associated with a poor prognosis include evidence of vascular invasion, cellular pleomorphism, necrosis and amelanotic tumors [153, 168, 170, 171].

Treatment modalities include surgery, radiotherapy, chemotherapy and immunotherapy [158, 172].

3.6.4 Addison's Disease

Definition Addison's disease is a rare, chronic, endocrine disorder of the adrenal gland due to insufficiency of steroid hormones (glucocorticoids and, often, mineralocorticoids).

Epidemiology There is an estimated annual incidence of 0.8 cases per 100,000 in Western societies.

Etiology and pathogenesis Addison's disease is due to bilateral destruction of the adrenal gland cortex. Formerly, the most common cause was tuberculosis. Most cases now are due to organ-specific autoimmune destruction and opportunistic infections such as histoplasmosis in patients with AIDS. Due to this, it is likely that the number of patients with Addison's disease will increase significantly. Addison's disease may be associated with autoimmune polyglandular deficiency type I (Addison's disease, chronic mucocutaneous candidosis, hypoparathyroidism) and autoimmune polyglandular deficiency type II (Addison's disease, primary hypothyroidism, primary hypogonadism, insulin-dependent diabetes, pernicious anaemia, vitiligo) [173].

Clinical aspects There is usually slowly progressive weakness, lassitude and weight loss. Gastrointestinal symptoms can include diarrhoea or constipation and anorexia, nausea and vomiting. Postural hypotension is a common symptom. An early sign is pigmentation of the skin and oral mucosa secondary to increased ACTH secretion. Sun-exposed parts of the skin and areas subjected to trauma or friction become bronzed [174]. There is also increased pigmentation in skin folds and scars. About 10% of patients show areas of vitiligo.

Oral pigmentation is variable and where present ranges from light brown to densely black [175]. The gingiva, lateral margins of the tongue, buccal mucosa and lips are the sites of predilection.

Microscopy Affected areas of mucosa show increased melanin, predominantly in the basal keratinocytes.

3.6.5 Peutz-Jeghers Syndrome

Definition Peutz-Jeghers syndrome (periorificial lentiginosis) comprises melanotic spots of the face, mouth and, less commonly, the hands and feet, together with gastrointestinal polyposis [176].

Epidemiology There are multiple, small ephelides on the face (Fig. 3.25) and melanotic macules in the mouth involving the labial and buccal mucosa in particular [177]. Lesions are often present at birth.

Etiology and pathogenesis It is inherited as an autosomal dominant trait with nearly complete penetrance [178], but new mutations occur in 40 % of cases. It is caused by germ line mutations of the LKB1/STK11 gene [179, 180].

Clinical aspects The facial pigmentation is around the mouth, nose and eyes and tends to fade progressively after puberty. The mucosal pigmentation persists into adult life.

There are hamartomatous polyps throughout the gastrointestinal tract, but typically these are most numerous in the small intestine [181]. They can give rise to abdominal pain and bleeding and intussusception is a rare complication.

Treatment and prognosis The orofacial lesions are entirely benign. The gastrointestinal polyps, however, have a low malignant potential, with those in the colon carrying the highest risk. Patients also are at increased risk of malignancy at other sites including the uterus, ovary, pancreas, thyroid and breast. Women are at greater risk than men.

3.6.6 Oral Racial Pigmentation

Definition Oral racial pigmentation is seen where parts of the oral mucosa show varying degrees of melanocytic pigmentation in defined ethnic groups.

Epidemiology It is the most common cause of intraoral pigmentation [182]. It is seen predominantly in Blacks, Asians and people of Mediterranean origin, but about 5 % of Caucasians also have significant intraoral pigmentation.

Clinical aspects The degree and extent of racial pigmentation are very variable and do not necessarily correlate with the depth of skin pigmentation. It can vary from light brown to almost black, and the most commonly involved sites are the gingiva, palate and buccal mucosa. Sometimes, when the tongue is involved, the only areas affected are the tips of the fungiform papillae, producing a spotty pigmentation. This appearance is so characteristic it could be called 'fungiform papillary melanosis'.

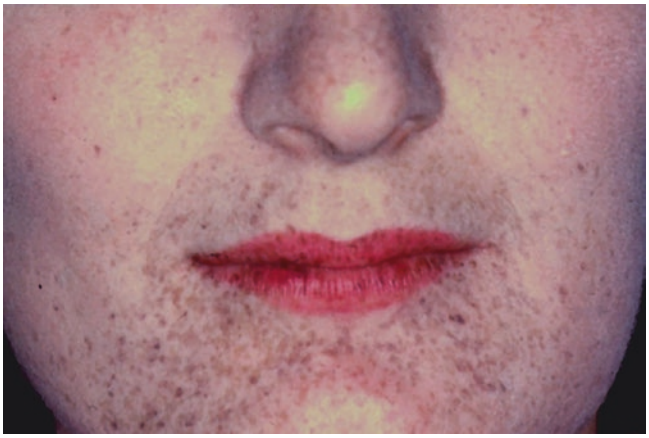

Fig. 3.25 Peutz-Jeghers syndrome, showing multiple labial and perioral ephelides

Microscopy There is increased melanocytic pigmentation of the basal and, to a much lesser degree, the immediately suprabasal keratinocytes. The denser pigmentation is due to increased synthesis of melanin by melanocytes, which are otherwise normal in number and distribution.

3.6.7 Laugier-Hunziker Syndrome

Definition Laugier-Hunziker syndrome (idiopathic lenticular mucocutaneous pigmentation) is a rare, acquired, benign macular hyperpigmentation of the lips and oral mucosa [183, 184].

Epidemiology It typically starts in early to middle adult life and is more common in women than men. The lesions are seen most commonly on the buccal mucosa, lips and the hard and soft palates. Other less frequent sites include the tongue, gingiva and floor of the mouth. Occasionally, the pharynx and oesophagus are also involved [185]. About half of the cases also have nail involvement in the form of longitudinal pigmented bands in one or more fingers or toes. There are no known systemic associations.

Clinical aspects The pigmentation consists of brownish, circular or linear macules that may be sharply circumscribed or more diffuse in nature, and lesions may coalesce.

Microscopy There is increased melanotic pigmentation of basal keratinocytes and melanophages in the superficial corium secondary to pigmentary incontinence. Ultrastructural studies show increased numbers of normal-appearing melanosomes in keratinocytes in the lower epithelial layers [183].

Treatment and prognosis The condition is entirely benign.

3.6.8 Smoker's Melanosis

Definition Smoker's melanosis is areas of oral melanotic hyperpigmentation in heavy smokers.

Epidemiology It is more common in women than men. Although any part of the mouth can be affected, the anterior gingivae and lips are involved most frequently [186].

Etiology and pathogenesis A dose-response relationship has been shown between the level of smoking exposure and lip and gingival pigmentation.

Clinical aspects The lesions vary in colour from light brown to bluish-black and the lesions may be focal or diffuse. Sometimes the overlying mucosa has a somewhat milky-white appearance, particularly in the buccal mucosa. The condition can slowly resolve if smoking is stopped or reduced [187]. Pigmentation of the soft palate has been reported in a significant number of patients with suppurative lung disease and malignancy [188]. Nearly a quarter of patients with confirmed bronchogenic carcinoma can show this feature. Most patients have a long history of cigarette smoking, and it is possible that in many cases these lesions were merely smoker's melanosis rather than being related directly to the pulmonary lesions.

Microscopy There may show slightly increased melanotic pigmentation of the basal keratinocytes, but the most striking feature is usually pigmentary incontinence and accumulation of melanophages in the superficial corium.

Treatment and prognosis The condition is benign.

3.6.9 Drug-Associated Oral Pigmentation

Definition Drug-associated oral pigmentation is where the oral mucosa becomes discoloured due to systemic pharmaceutical agents.

Clinical aspects Gingival pigmentation due to heavy metals such as mercury, lead, bismuth, arsenic and others was not rare in the past due to industrial exposure and in some cases therapeutic administration, particularly for the treatment of syphilis. They caused blue, brown or black lines close to the gingival margins due to the deposition of sulphides as a result of reactions with products of the dental plaque. A wide range of drugs can cause more generalised oral pigmentation including antimalarials, minocycline, phenothiazines and some contraceptive pills [189]. Drugs used in the treatment of HIV infection such as zidovudine and some antifungals such as ketoconazole have also been shown to cause oral pigmentation. Recently, imatinib mesylate, a drug used in the treatment of chronic myeloid leukaemia, has been associated with diffuse hyperpigmentation of the hard palate [190]. The pigmentation was due to submucosal deposits of melanin and iron.

3.7 Hyperplastic Lesions

3.7.1 Fibrous Hyperplasias

Definition The majority of fibrous and fibroblastic oral lesions are reactive rather than neoplastic. They are the most common tumor-like swelling of oral mucosa. Although these lesions are usually considered to be a response to low-grade irritation, the source of the putative irritation is often not apparent.

Clinical aspects Fibroepithelial polyps tend to form smooth nodules or swellings that may be soft or firm and are usually covered by normal, pink mucosa unless ulcerated. The polypoid swellings may be sessile or pedunculated.

Fibrous overgrowths of the gingiva are a type of epulis (Lit – swelling of the gum). They can arise from the interdental papilla or gingival margin and tend to affect the anterior part of the mouth. They can grow to several centimetres in diameter. Fibrous epulides are frequently associated with local irritation from dental calculus, sharp edges of restorations or carious teeth. A very characteristic form of hyperplasia is associated with the edges of loose dentures. Such denture-induced fibrous hyperplasia has been termed denture granuloma and epulis fissuratum. The rocking backwards and forwards of the denture causes extensive overgrowth of fibrous tissue on either side of the edges, or flanges, of the denture. This often leads to the formation of a series of linear folds of hyperplastic tissue, and the base of the grooves so formed is often ulcerated by the denture's edge. Other common sites for fibrous overgrowths are along the occlusal line of the buccal mucosa, lateral border of the tongue and opposite edentulous spaces.

Microscopy Most of these fibrous overgrowths consist of interlacing bundles of sparsely cellular fibrous tissue. The overlying epithelium is often hyperplastic with irregular rete processes extending sometimes deeply into the underlying fibrous tissue. There may be candidal infestation of the superficial epithelium. The degree of inflammatory infiltration is very variable but tends to be mild unless there has been ulceration.

The microscopical appearances of fibrous epulides can differ from fibrous overgrowths seen elsewhere in the mouth. They typically show much more evidence of cellular fibroblastic proliferation. These lesions may consist predominantly or focally of a more vascular stroma containing plump fibroblasts with large, vesicular nuclei and prominent nucleoli. There can be brisk mitotic activity. Ulceration is more frequent

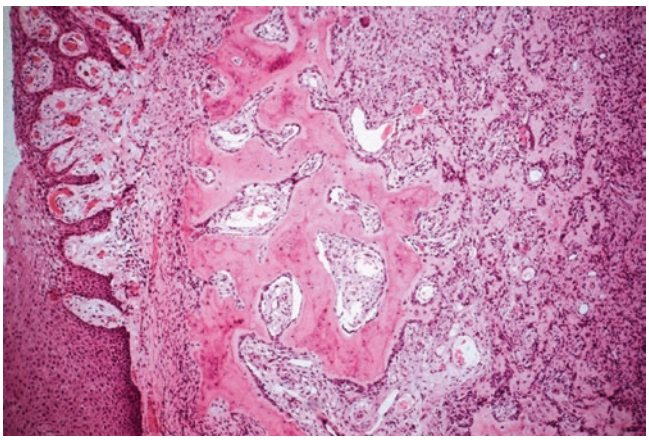

Fig. 3.26 Fibrous epulis, showing osseous metaplasia

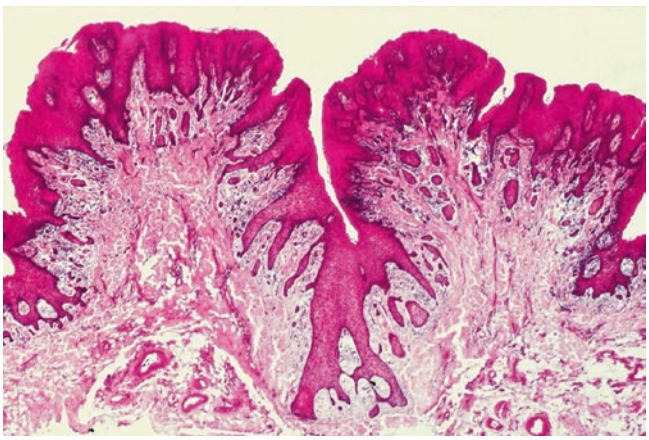

Fig. 3.27 Papillary hyperplasia of the palate, showing long rete processes extending into the underlying connective tissue

and the lesions are often heavily inflamed. Calcifications are common in fibrous epulides, and there may be florid osseous metaplasia (Fig. 3.26) or dystrophic calcification. Sometimes the calcified masses are basophilic and they can also be laminated and resemble cementicles. Such lesions have been termed 'peripheral ossifying fibromas', but there is no evidence they are neoplastic or have any relationship with central cemento-ossifying fibromas. Mineralisation tends to be uncommon in extra-gingival oral fibrous overgrowths.

Treatment and prognosis Most fibrous overgrowths respond to conservative surgical removal, but a minority of fibrous epulides can recur, sometimes repeatedly.

3.7.2 Papillary Hyperplasia

Definition Papillary hyperplasia is a papilliferous lesion of the oral mucosa, typically seen in the hard palate.

Epidemiology The large majority of cases involve the hard palate, particularly when this is high arched, but similar lesions are very occasionally seen on the dorsum of the tongue.

Etiology and pathogenesis In many cases, it is related to dentures as part of the clinical spectrum of denture-induced stomatitis [191]. Although *Candida albicans* is frequently invoked as the causal agent, in a significant number of cases there is no evidence of fungal infection [68].

Clinical aspects The lesions form painless, nodular or papilliferous proliferations. Florid cases have been reported in immunocompromised patients [192].

Microscopy There is nodular, papilliferous hyperplasia of the epithelium and underlying fibrous connective tissue (Fig. 3.27). The surface usually shows parakeratosis or less commonly orthokeratosis. There may be evidence of candidal infestation such as spongiform pustules or obvious hyphae. The underlying hyperplastic rete ridges often extend into the cores of the papillae resulting in a striking pseudoepitheliomatous appearance. The corium often contains a dense chronic inflammatory cell infiltrate. The condition then needs to be distinguished from oral papillary plasmacytosis [193].

Treatment and prognosis Papillary hyperplasia is benign and active treatment is usually unnecessary.

3.7.3 Generalised Gingival Fibrous Hyperplasia

Generalised fibrous hyperplasia/hypertrophy of the gingiva can be familial or drug induced.

3.7.3.1 Hereditary Gingival Fibromatosis

Definition Hereditary gingival fibrous hyperplasia is a rare, inherited condition characterised by widespread gingival enlargement.

Epidemiology It can affect all of the gingiva, often in a symmetrical manner. It may be associated with hypertrichosis, coarsening of the facial features and neurological problems such as epilepsy and mental retardation. The condition usually first affects adolescents but occasionally it can involve the deciduous dentition.

Etiology and pathogenesis It is usually inherited as an autosomal dominant trait [194].

Clinical aspects The enlargement is typically most conspicuous in the interdental areas and affects the palatal and lingual gingiva as well as the labial and buccal aspects. The overgrowths may be so florid that involved teeth are almost

completely buried (pseudo-anodontia). The overgrowths are rounded, smooth, firm and pale coloured.

Microscopy The microscopical features of generalised gingival fibrous hyperplasia tend to be the same irrespective of the cause. There is fibrous hypertrophy of the affected gingiva which often contains myofibroblastic cells. An increase in the amount of myxoid ground substance material is also common. In areas distant from the gingival sulcus, there is usually no significant inflammatory component. There is often elongation and fusing of the rete process of the overlying epithelium. It is unusual for these lesions to become ulcerated.

Treatment and prognosis Treatment is meticulous oral hygiene and surgical removal of the redundant fibrous tissue (gingivectomy) but the condition often recurs.

3.7.3.2 Drug-Associated Gingival Hypertrophy

Definition Drug-associated gingival hypertrophy is a typically widespread gingival enlargement due to systemic medications.

Epidemiology The enlargements may be generalised or more localised. The anterior interdental gingival papillae tend to be the most severely affected areas, particularly on the labial aspect.

Etiology The condition is seen in about half of patients using the antiepileptic drug phenytoin for long-term treatment [195]. Other drugs producing a similar reaction include cyclosporine (~30 % of patients) and calcium channel blockers such as nifedipine, amlodipine and verapamil (~10 % patients).

Clinical aspects The gingivae usually enlarge laterally and this growth pattern may result in the formation of vertical clefts between adjacent overgrowths. The normal gingival stippling may be enhanced producing an orange-peel appearance.

Treatment and prognosis The condition is exacerbated by poor oral hygiene, and meticulous attention to tooth cleaning may help prevent its development or progression. Treatment is surgical removal of the redundant fibrous tissue (gingivectomy), but the condition often recurs.

3.7.4 Crohn's Disease

Definition Crohn's disease is a multisystem disorder which is characterised microscopically by non-caseating, epithelioid granulomas.

Epidemiology Patients are usually children and young adults and there is a male preponderance.

Etiology and pathogenesis Despite extensive investigations, the cause remains unknown. The possibility of a genetic predisposition has been extensively investigated, and among others, a susceptibility gene has been identified on chromosome 16q [196]. Smoking also appears to play a critical role in some patients [197]. Although an infective etiology, particularly mycobacterial, has been long suspected, critical proof is lacking [198]. The most likely candidate organism is *Mycobacterium avium* subspecies paratuberculosis [199]. Granulomatous vasculitis, possibly initiated by the measles virus, has also been proposed as a significant factor in the pathogenesis [200]. However, blood vessel involvement may be a secondary phenomenon rather than a primary event [201]. Associations between alterations in the intestinal microflora are also suspected, and a statistically significant association between Crohn's disease and previous antibiotic use has been proposed [202]. The disease may be exacerbated by cigarette smoking [203].

Clinical aspects Oral lesions are relatively uncommon in patients with Crohn's disease of the lower gastrointestinal tract [204, 205] but may be the presenting symptom. They include swelling of the lips and cheeks (Fig. 3.28a); recurrent aphthae; painful, indolent linear ulcers in the vestibular sulci; cobblestone thickening of the buccal mucosa; mucosal tags; and hyperplastic, oedematous or granular gingivitis [206]. The palate, tongue and pharynx, including the palatine tonsil, are only rarely involved [207]. Extraorally, there may be angular stomatitis and vertical fissuring of the lips and perioral erythema and scaling.

Oral lesions may precede, or accompany, bowel symptoms, but in a significant number of cases, intestinal disease is subclinical. In patients with active bowel disease, there may be atrophic glossitis secondary to malabsorption of the haematinics iron, vitamin B_{12} or folate.

Microscopy Oral lesions typically show oedema of the superficial corium with lymphangiectasia and diffuse and focal aggregates of lymphocytes. Non-caseating epithelioid granulomas, with or without multinucleated giant cells, are present in about 90 % of cases [206]. However, granulomas may be small and poorly formed and may only be present in the underlying muscle so that they can be easily missed, especially if the biopsy is superficial (Fig. 3.28b). Granulomas can also sometimes be seen in the minor salivary glands. Aggregates of mononuclear cells or granulomas may be seen bulging into or within the lumina of lymphatics. This feature has been termed granulomatous lymphangiitis and endovasal granulomatous lymphangiitis

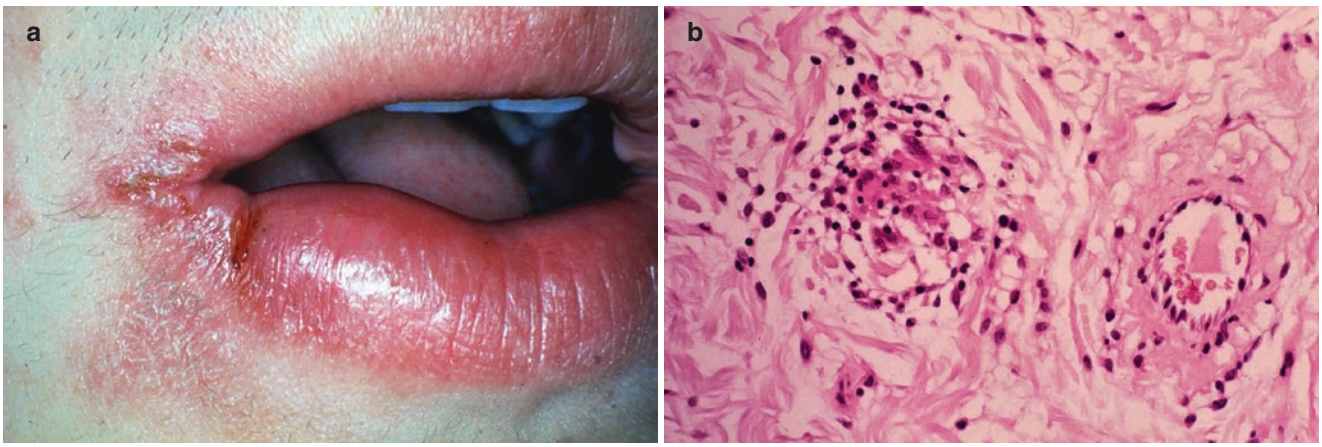

Fig. 3.28 (**a**) Crohn's disease, showing unilateral lip swelling with fissuring and perioral dermatitis. (**b**) Crohn's disease, showing a small, irregular epithelioid granuloma and patchy chronic inflammation

[208]. Dilated lymphatics, with or without associated granulomas, are characteristic of Crohn's disease elsewhere in the alimentary tract [201].

Treatment and prognosis Oral Crohn's disease is usually treated by aminosalicylates and systemic, intralesional or topical steroids. The disease is often intermittent and is associated with spontaneous relapses and remissions.

3.7.5 Orofacial Granulomatosis

Definition As many as 80–90 % of patients with orofacial lesions that closely resemble those of Crohn's disease, both clinically and microscopically, have no gastrointestinal signs or symptoms and do not develop gut disease [209]. The term orofacial granulomatosis was introduced to describe this group of patients [210].

Epidemiology The global incidence of orofacial granulomatosis is rising, whereas the incidence of Crohn's disease in high-prevalence areas such as Northern Europe and the USA is falling [211].

Etiology and pathogenesis Orofacial granulomatosis is a diagnosis based on the exclusion of other causes of granulomatous inflammation, particularly sarcoidosis, tuberculosis and other mycobacterial infections, and Crohn's disease itself. Unlike patients with Crohn's disease, who have no increased prevalence of allergy, up to 60 % of patients with orofacial granulomatosis are atopic [212, 213], and some patients appear to show an idiosyncratic intolerance to a variety of foods or additives, including cinnamaldehyde, carvone, carnosine, sun yellow, benzoates and monosodium glutamate [214], and to metallic compounds containing cobalt [215]. Cases of orofacial granulomatosis have been associated with hypersensitivity to dental amalgam restorations as a contact stomatitis reaction [216].

Differential diagnosis Orofacial granulomatosis may be part of a spectrum of diseases which includes Melkersson-Rosenthal syndrome, cheilitis granulomatosa (Miescher syndrome) and oral allergy syndrome. Melkersson-Rosenthal syndrome, in its complete form, is a triad of fissured tongue, labial or facial swelling due to granulomatous inflammation and facial nerve palsy, which may be the first indication of the disease [217]. Cheilitis granulomatosa is probably merely a focal manifestation of orofacial granulomatosis. Oral allergy syndrome has a clear immunological pathogenesis. Although the signs and symptoms can be similar to those of orofacial granulomatosis, there is no histological evidence of granulomatous inflammation [213].

Treatment and prognosis In many cases, there is a partial or complete resolution of symptoms following withdrawal of the provoking agent.

3.7.6 Chronic Marginal Gingivitis and Localised Gingival Fibrous Hyperplasia

Definition Chronic marginal gingivitis is an inflammation of the gingiva localised to the marginal area and interdental papillae.

Epidemiology Chronic marginal gingivitis of variable degrees is so common as to be almost universal.

Etiology and pathogenesis It represents a response of the gingival tissues to accumulation of dental microbial plaque around the teeth.

Clinical aspects Chronic marginal gingivitis is characterised by moderately enlarged gingivae which are erythematous and friable, leading to bleeding with minimal trauma.

Microscopy Chronic marginal gingivitis is characterised microscopically by mild vascular hyperaemia and dense chronic inflammatory infiltration. The crevicular epithelium is ulcerated and may become hyperplastic with thin, irregular and anastomosing processes extending into the gingival connective tissue. There may be considerable intercellular oedema and infiltration of the spongiform spaces by neutrophils, especially in the presence of gross dental plaque and calculus deposits. Many lymphocytes and plasma cells are present in the inflammatory infiltrate, and dense, basophilic, granular deposits of extracellular immunoglobulin are common. Russell bodies may be a conspicuous feature. There is variable loss of collagen in areas of severe inflammation.

Treatment and prognosis If left untreated, the inflammation can become more severe and extend into the underlying periodontal tissues causing loss of periodontal ligament attachment. A pocket then develops between the tooth and the overlying gingiva exacerbating the tendency for plaque accumulation. Eventually there is progressive resorption of the supporting alveolar bone leading to loosening or loss of the tooth. In younger patients especially, there may be a proliferative response with extensive formation of new fibrous tissue leading to localised areas of fibrous hyperplasia. The enlarged gingiva may prevent effective cleaning of the related tooth, predisposing to further plaque accumulation and progressive inflammation. This type of localised inflammatory gingival hyperplasia is seen much more frequently on the buccal or labial aspects of the gingiva than in the palatal or lingual areas. Such overgrowths, although frequently removed as part of a gingivectomy procedure, are not commonly sent for histological examination.

3.7.7 Peripheral Giant Cell Granuloma (Giant Cell Epulis)

Definition Peripheral giant cell granuloma (giant cell epulis) is a benign hyperplastic lesion of the gingiva or alveolus characterised by the presence of multinucleated giant cells.

Epidemiology It is seen across a wide age range but the peak incidence is between 30 and 50 years. Lesions tend to affect the area anterior to the permanent molars and are slightly more frequent in the mandible.

Etiology and pathogenesis It is thought to originate from elements of the periodontal ligament or periosteum. Some believe it is due to an abnormality in the resorption of deciduous teeth. This would appear unlikely given the age distribution of the lesion.

Clinical aspects Giant cell epulis usually forms a fleshy, bluish-red swelling that may be sessile or broadly pedunculated and the surface is often ulcerated (Fig. 3.29). There may be erosion of the underlying bone or periodontium. They have rarely been reported in association with dental implants [218].

Microscopy There is usually an uninvolved 'Grenz' zone of fibrous tissue between the lesion and the overlying epithelium, but this is lost if there is acute inflammation or ulceration. The lesion consists of a matrix of plump, spindle-shaped cells with interspersed multinucleated and osteoclast-like giant cells. These can be numerous and may be confluent, blurring the distinction between each other and the stromal cells. The multinucleated cells are large and contain about 15–40 nuclei. There are two types: the most common have lightly eosinophilic cytoplasm and large vesicular nuclei with prominent nucleoli. The other type has much more densely stained cytoplasm and pyknotic and densely haematoxyphilic nuclei. The latter are probably a degenerative form of the first type. The multinucleated cells are thought to be formed by fusion of bone marrow-derived mononuclear preosteoclasts [219, 220]. The lesion is usually very vascular and giant cells may be seen within the dilated vascular spaces (Fig. 3.30). Red blood cell extravasation and haemosiderin deposition are common. Mitoses can often be seen in stromal and endothelial cells, but this observation has no bearing on the likely behaviour. Osseous metaplasia and dystrophic calcification may be present, usually in the middle or deeper aspects of the lesion.

Differential diagnosis Giant cell epulis is indistinguishable microscopically from central giant cell granuloma and the brown tumor of hyperparathyroidism, but is otherwise unrelated. Radiographs should be taken to exclude the possibility of a central bone lesion. If such a lesion is detected, hyperparathyroidism should be excluded by assessing serum calcium, phosphate and alkaline phosphatase and measuring parathormone levels if necessary.

Treatment and prognosis Treatment is usually by conservative surgical excision with curettage of the underlying bone, but about 10 % of cases recur, sometimes repeatedly.

3.7.8 Pyogenic Granuloma

Definition Pyogenic granuloma is a polypoid or lobular form of capillary hemangioma involving skin or mucosal surfaces.

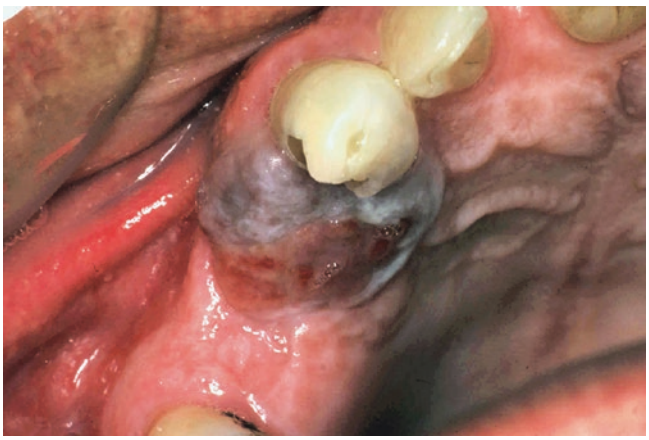

Fig. 3.29 Giant cell epulis showing a purplish-red swelling

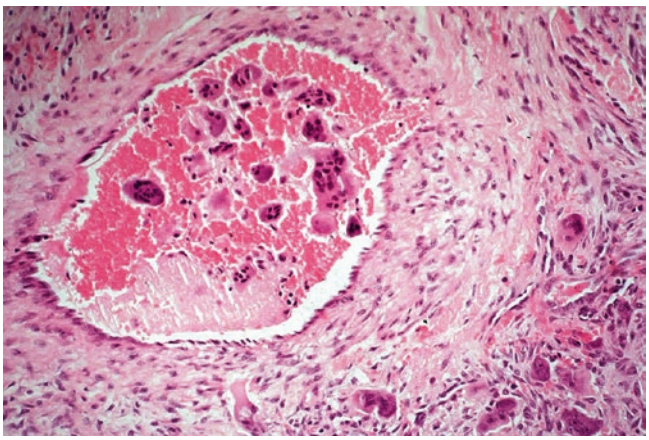

Fig. 3.30 Giant cell epulis showing multinucleated giant cells in a cellular stroma and in a vascular space

Epidemiology They are most common on the gingivae and less frequently in other intraoral sites, particularly the lip and tongue [221]. Occasionally pyogenic granulomas sprout from recently extracted tooth sockets (epulis granulomatosa).

Clinical aspects Pyogenic granulomas form solitary, soft, red and friable nodules that bleed readily. They frequently ulcerate and are covered by a fibrinous slough (Fig. 3.31). Gingival lesions may be seen in pregnancy and appear to be a focal exacerbation of pregnancy gingivitis [222]. Pregnancy epulides ('pregnancy tumor') usually manifest towards the end of the first trimester. They have a strong tendency to recur if removed before parturition and may show partial or complete spontaneous resolution if left following delivery. Occasionally, similar lesions are seen in other parts of the mouth during pregnancy, particularly the dorsum of the tongue, and they are termed granuloma gravidarum.

Microscopy Oral pyogenic granulomas consist of numerous, large, thin-walled, anastomosing blood vessels in a loose, oedematous and moderately cellular stroma. Older lesions may show some fibrosis. Inflammation is very variable and can be minimal or absent. However, if the lesion ulcerates, there may be an intense inflammatory infiltration. Foci of papillary endothelial hyperplasia are an occasional feature.

Treatment and prognosis Although excision is usually curative, rare cases can show repeated recurrences and require more extensive surgery for their eradication.

3.7.9 Pulse (Vegetable) Granuloma

Definition Pulse granuloma (chronic periostitis, giant cell hyaline angiopathy, oral vegetable granuloma and hyaline ring granuloma) is an unusual and uncommon giant cell granulomatous lesion characterised by the presence of eosinophilic, structureless hyaline rings [223].

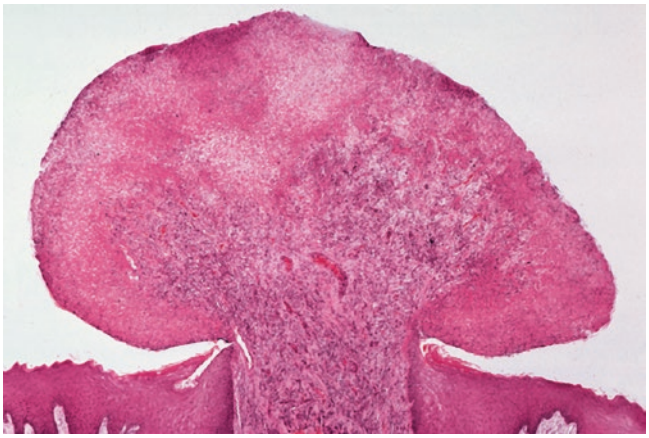

Fig. 3.31 Pyogenic granuloma showing an exophytic mass of vascular granulation tissue covered by fibrinous slough

Epidemiology There is a male predominance of about 2:1. Most cases are seen in the premolar/molar region of the edentulous mandible, and occasionally, the lesion is found within the fibrous wall of an odontogenic or nasopalatine duct cyst [224].

Etiology and pathogenesis The range of terminology has reflected the uncertainty concerning the aetiopathogenesis of this microscopically distinctive lesion. The current consensus is that it is caused by submucosal impaction or ingress of particles or remnants of leguminous foods (pulses). This then results in a foreign body giant cell reaction.

Clinical aspects The most common complaints are recurrent swelling and tenderness. Fifty-three percent of cases are extraosseous (peripheral), and radiographs often show a

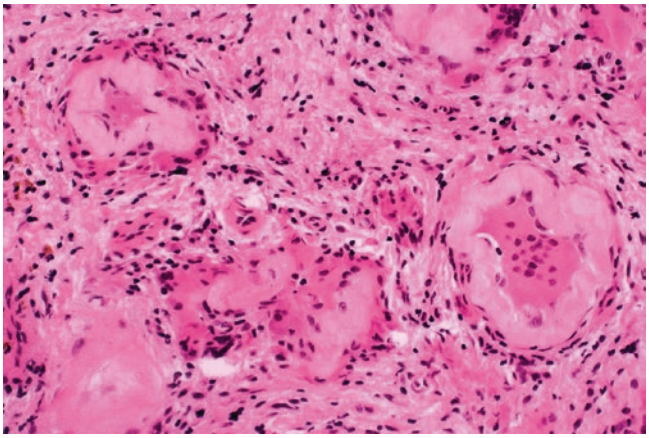

Fig. 3.32 Pulse granuloma, showing chronic inflammation and eosinophilic, hyaline rings, some enclosing multinucleated, foreign body-type giant cells

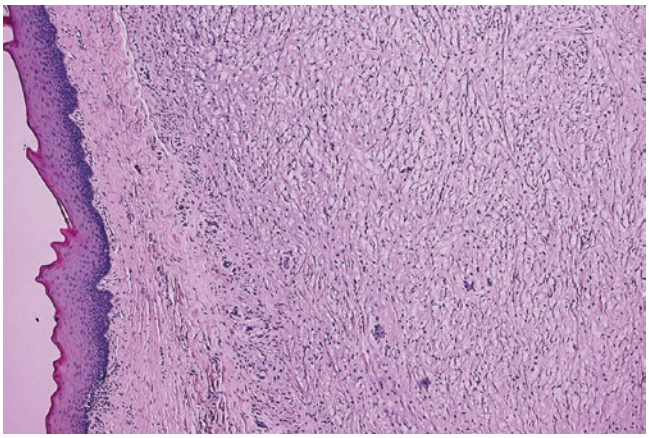

Fig. 3.33 Focal oral mucinosis showing well-circumscribed, but non-encapsulated, myxomatous connective tissue

poorly defined erosion of the underlying alveolar bone. Intraosseous or central lesions (42%) show an irregular radiolucent area that is non-diagnostic.

Microscopy This shows chronic inflammation and eosinophilic, hyaline rings, together with multinucleated foreign body-type giant cells (Fig. 3.32). The rings may be complete or horseshoe shaped and may enclose giant cells, connective tissue and blood vessels. Haemosiderin within the centre of the rings is a frequent finding. The suggestion that the histological appearances are due to thickening and hyalinisation of the walls of blood vessels is not supported by most observers. Light and electron microscopical findings suggest that the rings are the cell walls of vegetable remains, often with collagen attached to their surface [225]. There does not appear to be any evidence that these appearances are exclusively due to pulses.

Treatment and prognosis Complete excision is curative.

3.8 Benign Tumors and Pseudotumours

3.8.1 Focal Oral Mucinosis

Definition Focal oral mucinosis is the oral counterpart of focal cutaneous mucinosis or cutaneous mucoid cyst.

Epidemiology Focal oral mucinosis is uncommon and affects a wide age range (7–74 years) and is most frequent in the fourth and fifth decades. There is a female predominance of nearly 2:1.

Etiology and pathogenesis It results from overproduction of hyaluronic acid by fibroblasts [226]. The majority of cases are idiopathic and a possible role of local trauma is uncertain [227].

Clinical aspects It usually presents as a painless, sessile or pedunculated, fibrous or cyst-like swelling. The gingiva, particularly mandibular, and the hard palate are the sites of predilection. Less common locations include the buccal mucosa, tongue and lips.

Microscopy There is a well-demarcated area of paucicellular myxomatous tissue. Although the tumor is circumscribed, it usually lacks a compressed fibrous tissue periphery (Fig. 3.33). There are elongated fusiform or stellate fibroblasts with sparse cytoplasm in a loose, poorly vascularised stroma of mucinous tissue which is strongly alcianophilic at pH 2.5. The overlying epithelium may be attenuated but is otherwise normal. Occasionally there is scattered, mixed inflammatory infiltration of the lesion.

Differential diagnosis The clinical differential diagnosis includes fibrous overgrowth, neurofibroma, giant cell fibroma, peripheral giant cell granuloma and mucocele. The histological differential diagnosis includes myxomatous degeneration of a fibrous lesion, neurofibroma or nerve sheath tumor, soft tissue myxoma, odontogenic myxoma and extravasation mucocele.

Treatment and prognosis Focal oral mucinosis is usually treated by complete but conservative surgical excision, and there is no evidence of recurrence.

3.8.2 Giant Cell Fibroma

Definition Giant cell fibroma is an unusual but distinctive type of fibrous overgrowth characterised by the presence of large stellate or angular fibroblastic cells.

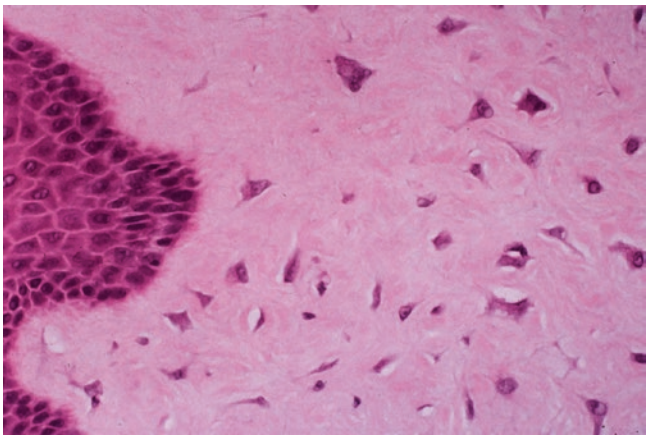

Fig. 3.34 Giant cell fibroma, showing stellate and angular fibroblasts in a collagenous matrix

Epidemiology Most are seen in the first three decades of life. Although they can form anywhere in the oral mucosa, about half of cases are seen on the gingiva [228].

Clinical aspects It is typically less than 5mm and usually presents as a pedunculated polyp with a lobulated surface.

Microscopy Giant cell fibroma consists of interweaving bundles of collagenous connective tissue with a prominent capillary network that surround stellate or angular fibroblastic giant cells with large vesicular nuclei (Fig. 3.34). Occasional cells may have several nuclei. These cells may have conspicuous dendritic processes and some contain melanin pigment.

Immunohistochemistry The giant cells are positive for vimentin, but negative for S-100, cytokeratin, LCA and neurofilament [229].

Treatment and prognosis Conservative surgical excision is usually curative.

3.8.3 Lingual Thyroid

Definition Lingual thyroid is a rare developmental anomaly where ectopic thyroid tissue is present in the posterior tongue.

Epidemiology It is seen in females about four times more frequently than males and usually presents in middle age. The thyroid gland tissue is seen in the base of the tongue, deep to the foramen caecum.

Etiology and pathogenesis It is due to failure of the thyroglossal duct to migrate caudally from the foramen caecum [230].

Clinical aspects It is often asymptomatic but may cause dysphagia, dysphonia or dyspnoea. Symptoms may coincide with puberty, pregnancy or menopause due to hyperplasia secondary to raised levels of thyroid-stimulating hormone. In addition, any of the diseases involving the conventional thyroid gland, including inflammatory conditions, adenomas and carcinomas, can affect the ectopic thyroid tissue.

Microscopy This typically shows normal thyroid tissue.

Treatment and prognosis As many as 70% of patients with lingual thyroid have no other thyroid tissue present, so it is essential that presurgical evaluation includes appropriate imaging and assessment of function using ^{131}I or ^{99m}Tc pertechnetate.

3.8.4 Verruciform Xanthoma

Definition Verruciform xanthoma is warty cutaneous or mucosal lesion characterised by the presence of lipid-laden histiocytes in the papillary corium.

Epidemiology This rare but distinctive lesion, first described in 1971 by Shafer [231], forms most commonly in the oral cavity [232]. Extraoral locations include the male and female genitalia. Verruciform xanthomas are seen at all ages but are most frequent in the fifth to seventh decades. There is an approximately equal sex incidence. The gingival margin accounts for 85% of cases. Other common sites include the hard palate, tongue, buccal mucosa and a variety of other intraoral sites.

Etiology and pathogenesis They do not appear to be related to any local irritating factors and most cases are asymptomatic. There is no association with HPV in the vast majority of cases studied.

Clinical aspects They usually present as solitary, painless, discrete nodules that may be the colour of the surrounding mucosa, reddish or pink. They can be sessile or pedunculated and the surface can be domed or flat and can be keratotic or papilliferous. They usually have sharply defined margins and are typically less than a centimetre in diameter.

Microscopy This typically shows corrugated, hyperplastic epithelium with elongated, broad rete ridges extending to a straight, well-defined lower border. There are deep clefts within the epithelium that often contain keratinized plugs (Fig. 3.35). The surface shows parakeratinized spikes which often stain a deep orange colour. There may be secondary candidal infestation of the surface keratin layers. The char-

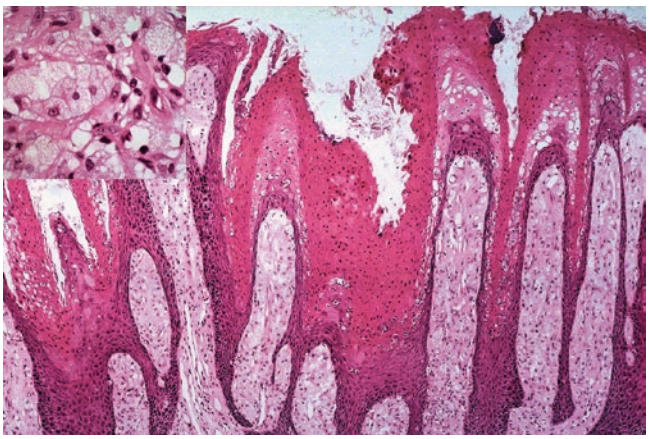

Fig. 3.35 Verruciform xanthoma, showing corrugated, hyperplastic epithelium with a hyperparakeratinized spiky surface and keratinized plugs. The foamy xanthomatous cells in the papillary corium are seen more clearly in the high-power *inset*

acteristic feature is the presence of vacuolated, foamy xanthomatous cells which fill the papillary corium. These xanthoma cells can occasionally extend into the overlying epithelium or into the deeper corium. The xanthoma cells have been shown to be derived from cells of the monocyte/macrophage lineage.

Treatment and prognosis The lesions are entirely benign and recurrence following even conservative surgery is very rare.

3.8.5 Hemangiomas

Definition Hemangiomas are benign vasoformative tumors that closely resemble normal vessels.

Epidemiology They are among the most common soft tissue tumors and about a third of all cases involve the head and neck region. In this location, congenital or neonatal lesions are relatively uncommon and tend to involve the lips and parotid glands. The majority of cases are seen in older individuals, and the most common sites are the lips, postcommissural buccal mucosa and the lateral border of the tongue. There is a male predominance of about 2:1.

Clinical aspects Hemangiomas typically form painless, flat or nodular, soft and purplish-red lesions. They are usually well circumscribed and may blanch on pressure. Congenital lesions behave like hamartomas and increase in size in proportion to general somatic growth and tend to stabilise in size in early adult life. Those presenting in older individuals may show slow but progressive growth over several years.
Their classification and microscopical aspects are discussed among the soft tissue tumors.

3.8.6 Lymphangioma

Definition Lymphangioma is a benign, cavernous or cystic vascular lesion composed of dilated lymphatic channels.

Epidemiology The head and neck region is the most common site of involvement. Many lesions are present at birth or develop within the first few years of life. There appears to be no sex predilection. Oral lesions are seen most commonly in the tongue and lips where they may cause macroglossia and macrocheilia, respectively. Involvement of underlying bone in oral lesions is uncommon [233].

Etiology and pathogenesis Some are associated with Turner syndrome (monosomy X) [234].

Clinical aspects They usually form painless swellings which are frequently superficial, but some extend deeply into the surrounding tissues and are ill-defined. Mucosal lymphangiomas usually have a pale, translucent surface which is nodular or bosselated. It is common for black areas to appear in the lesion due to focal haemorrhage. In the neck, the lesions tend to be larger and show more extensive cystic dilatation. These lymphangiomas are then frequently called cystic lymphangiomas or cystic hygromas [235]. The most common locations for cystic hygroma are the posterior triangle, submandibular region and floor of the mouth. It can extend upwards to involve the cheek and parotid gland, forwards into the anterior triangle or downwards into the mediastinum. Some cystic hygromas are severely disfiguring and they can compromise swallowing or breathing.

The classification and microscopical aspects of oral lymphangiomas are discussed among the soft tissue tumors.

3.8.7 Benign Nerve Sheath Tumors

Neurofibroma and schwannoma are the two most common benign tumors of nerve sheath origin. Although they both appear to be derived from Schwann cells, they have distinctive clinical and microscopical features. They are more extensively discussed among the tumors of the peripheral nervous system.

3.8.7.1 Neurofibroma

Definition Localised (solitary/sporadic) neurofibroma is a benign nerve sheath tumor in the peripheral nervous system.

Etiology and pathogenesis Most tumors are sporadic, but the possibility of neurofibromatosis type 1 should always be considered when dealing with these lesions.

Epidemiology They are relatively uncommon in the orofacial region and tend to affect people in the 20–40 years age group.

Clinical aspects Tumors usually form small, painless, expansile submucosal nodules. The tongue is the most common intraoral site, but occasionally they develop on the inferior alveolar nerve and can appear as a fusiform radiolucent area along the course of the inferior alveolar canal.

Microscopy Neurofibromas are usually discrete but non-encapsulated and may be in close proximity to, or in continuity with, a nerve. They typically consist of variable numbers of spindle-shaped, S-100-positive cells, which may show limited inter-fasciculation. There are frequently many mast cells in the stroma.

Treatment and prognosis Conservative surgical excision is usually curative. There is a very small risk of malignant transformation.

3.8.7.2 Schwannoma

Definition Schwannoma (neurilemmoma; neurinoma) is a benign neoplasm of Schwann cell origin.

Epidemiology Patients are usually in the third or fourth decades and the tumor is more common in women than men.

Clinical aspects Oral lesions tend to form small, painless and slow-growing swellings and the tongue is the most common site. However, occasional cases can be several centimetres in diameter [236].

Microscopy Schwannomas are typically encapsulated and consist of two main components in widely variable proportions (Fig. 3.36). Antoni A tissue consists of spindle-shaped cells, often in an interwoven pattern. Some of these areas may show regimented or palisaded nuclei. Occasionally the nuclei form organoid structures called Verocay bodies. Antoni B tissue is less cellular and can be myxoid in nature. In older tumors, there is a tendency for this tissue to degenerate or become fibrosed, when the tumor has been called an ancient neurilemmoma. The cells in the degenerative areas can sometimes appear pleomorphic and simulate malignancy.

Treatment and prognosis Conservative surgical excision is usually curative.

3.8.7.3 Palisaded Encapsulated Neuroma

Definition Palisaded encapsulated neuroma is a benign neural neoplasm of the skin or mucosa that displays histological features of both a neurofibroma and a schwannoma.

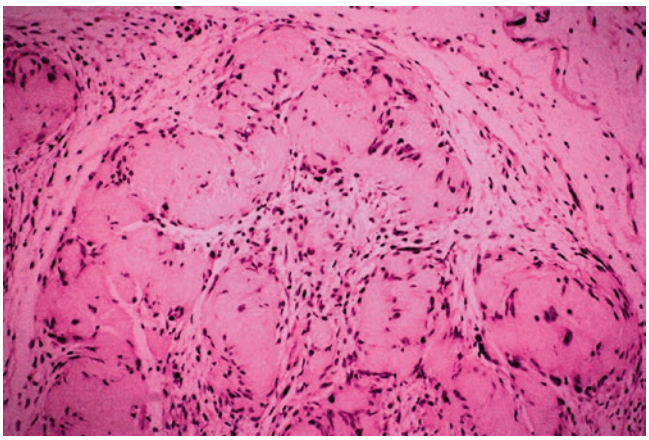

Fig. 3.36 Neurilemmoma, showing whorls of Schwann cells, and focal nuclear palisading

However, unlike these tumors, it lacks the implications for underlying systemic disease or malignancy.

Epidemiology The most common oral sites of involvement are the palate and the maxillary labial mucosa.

Etiology The precise etiology is unknown, but trauma is considered by some to induce or trigger its development.

Clinical aspects It typically presents as a small, painless, solitary papule.

Microscopy There is a generally well-circumscribed and usually encapsulated lesion, although the capsule may be incomplete. It consists of interlacing bundles or fascicles of spindle cells (Schwann cells) with thin, wavy, pointed nuclei and many axons. The cellular fascicles are typically four to six cells thick and are arranged in parallel streams in some areas, and nuclear palisading is seldom pronounced, as it is in neurilemmoma.

Treatment and prognosis The lesion is benign and responds to conservative excision [237].

3.8.7.4 Neurofibromatosis Type 1

Definition Neurofibromatosis type I (von Recklinghausen's disease) is an inherited disease causing multiple neurofibromas.

Epidemiology It is relatively common affecting about 1:4,000 births. It is usually diagnosed before the age of 10 years because of the characteristic cutaneous lesions and the frequent family history.

Etiology and pathogenesis It is an autosomal dominant trait and the gene responsible is located on chromosome 17 [238].

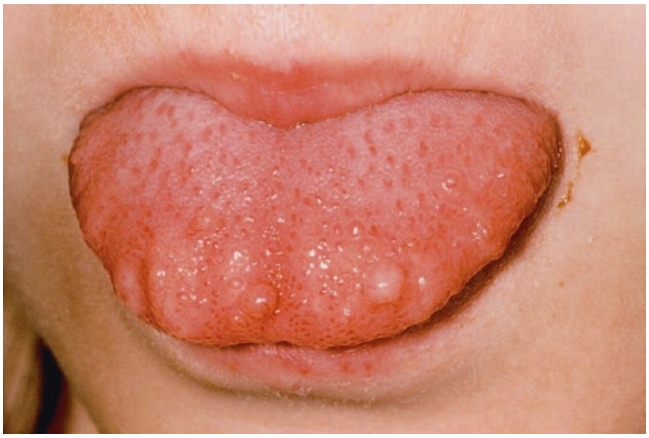

Fig. 3.37 Multiple neuromas on the dorsal lingual surface in a child with endocrine neoplasia syndrome type 2B

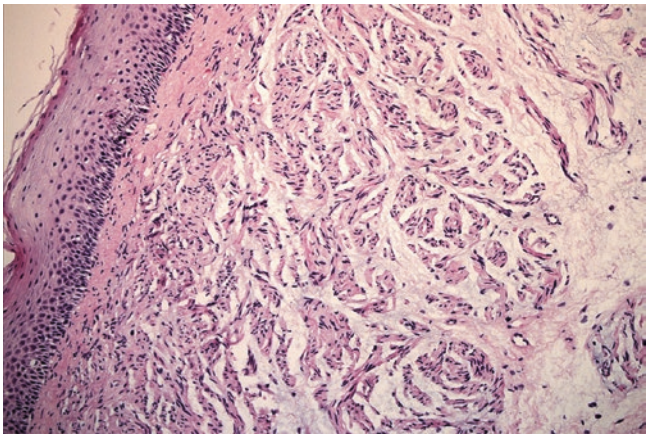

Fig. 3.38 Neuroma in multiple endocrine neoplasia syndrome showing a tangled mass of small nerve fibres

Clinical aspects It is characterised by cutaneous neurofibromas that are usually associated with café au lait pigmentation of the skin. Lesions are focal but sometimes there can be thousands of tumors and the condition is then grossly disfiguring. They are usually painless but itching can be a significant problem. There can be overgrowth of bone and associated soft tissue leading to bizarre localised gigantism. Nearly a quarter of cases involve the head and neck but only about 5 % affect the oral cavity.

Treatment and prognosis Surgery is usually only undertaken on large, painful or obstructive tumors, and recurrence is very common. In addition, there is a risk of tumors undergoing malignant transformation (~2 % of cases), particularly in long-standing cases.

3.8.7.5 Multiple Neuromas in Endocrine Neoplasia Syndrome

Definition Multiple endocrine neoplasia syndrome type 2B is an autosomal dominant condition characterised by the presence of mucosal neuromas, together with medullary carcinoma of the thyroid gland and phaeochromocytoma [239].

Epidemiology The mucosal neuromas appear on the dorsum of the tongue.

Etiology and pathogenesis Nearly 90 % of patients with the condition have point mutations at codon 918 of the *RET* proto-oncogene.

Clinical aspects Patients often have a Marfanoid habitus with arachnodactyly and a narrow face. Mucosal neuromas are the most consistent feature of the disease and may be pathognomonic. They tend to form on the lateral margins and dorsum of the tongue and appear as multiple, small, painless nodules (Fig. 3.37). These nodules may be the first indication of the condition. The lips are sometimes enlarged and blubbery. Mucosal neuromas often affect the palpebral conjunctiva and can also involve the sclera.

Microscopy Neuromas show a partially encapsulated tangled mass of small nerve fibres, often with a thickened perineurium (Fig. 3.38). These nerves lie in a loose fibrous stroma.

Immunohistochemistry The nerves usually stain with S-100 and may stain with epithelial membrane antigen indicating perineurial differentiation.

Treatment and prognosis The mucosal neuromas themselves rarely cause clinical problems but may act as indicators to the more serious aspects of the syndrome.

3.8.8 Granular Cell Tumor (Granular Cell Myoblastoma)

Definition Granular cell tumor (granular cell myoblastoma) is a neural tumor composed of round and/or spindle cells with pink, granular cytoplasm due to abundant intracytoplasmic lysosomes.

Epidemiology It is uncommon but about half of all cases involve the head and neck region. The tongue, particularly the dorsum, is the most frequent site. The peak incidence is in middle life and 10–20 % of cases are multiple. There is a female predominance of about 2:1.

Clinical aspects They usually form nondescript, painless swellings, but occasionally surface candidal infestation causes the lesion to present as a white plaque [240, 241].

See for this lesion also Chap. 7.

3.9 Squamous Cell Carcinoma

3.9.1 Introduction

Squamous cell carcinomas account for about 90% of all malignant neoplasms in the mouth and oropharynx [242]. It is important to consider the site of involvement as the epidemiological factors can vary considerably in tumors at different intraoral locations. There is typically a higher frequency in men than women, and this is attributed to the use of tobacco and alcohol. It has been estimated that as many as 75% of cases of oral squamous cell carcinomas in Western countries and Japan can be ascribed to these factors. Globally, oral cancer accounts for 5% of all malignancies in men and 2% in women. Much higher rates, however, are seen in both men and women in parts of Southeast Asia, where they are usually associated with the habitual use of areca nut and tobacco products. In Western countries, over the last 25 years, significant increases have been reported in the incidence of oral and oropharyngeal carcinomas, particularly in younger men. This has been attributed mainly to the role of oncogenic HPV [243].

3.9.2 Clinical Features

Despite the fact that oral tumors frequently cause symptoms and the mouth can be readily visualised with simple equipment, many oral cancers present at a relatively advanced stage where treatment may be disfiguring and prognosis is poor. This is often because many patients are elderly and frail and frequently wear dental prostheses and are accustomed to minor degrees of oral discomfort. In addition, early lesions may not be regarded as suspicious by the patient or the clinician and may therefore be treated empirically with antibacterial or antifungal preparations.

Any part of the oral mucosa can be the site of development of squamous cell carcinomas. The common oral locations can show wide variations in different geographical areas depending on the prevalent risk factors. The intraoral subsites include the buccal mucosa, tongue, floor of the mouth, upper and lower gingivae and alveolar processes, the hard palate and retromolar trigone. As the clinical presentation can vary according to the specific sites of involvement, these will be discussed separately.

3.9.2.1 Buccal Mucosa

The buccal mucosa extends from the commissure anteriorly to the retromolar trigone posteriorly and from the upper and lower vestibular reflections. The majority of carcinomas arise from the posterior area where they are commonly traumatised by the molar teeth. They soon spread into the underlying buccinator muscle and though insidious initially they may eventually cause trismus (Fig. 3.39). Bone, however, is generally involved only in advanced tumors. Tumors at this site often extend posteriorly into the palatoglossal fold and tonsillar fossa. Metastases are most common in the submandibular, submental, parotid and lateral pharyngeal lymph nodes.

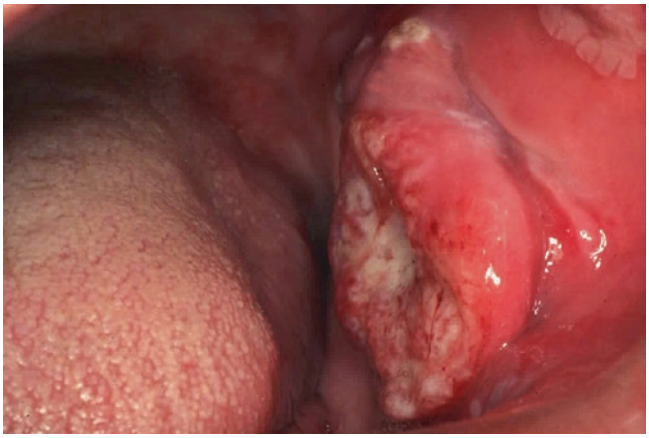

Fig. 3.39 Squamous cell carcinoma of the buccal mucosa, forming a large, exophytic mass with a cratered, central ulcer

3.9.2.2 Tongue

The tongue is the most common oral location of squamous cell carcinoma and can account for half of all cases. The majority affect the middle third of the lateral border and adjacent ventral surface. The dorsum is a very uncommon site, and tumors arising there may be associated with precursor lesions such as lichen planus and candidal leukoplakia. Lingual tumors are often exophytic (Fig. 3.40a) and ulceration is common. Even clinically small tumors can infiltrate deeply into the underlying muscle. With progressive growth, tumors become indurated and frequently develop characteristic rolled, raised, everted margins (Fig. 3.40b). Infiltration of the lingual musculature may cause pain, dysphagia and dysphonia. Half of patients have regional lymph node metastases at presentation. Tumors towards the tip of the tongue drain to the submental and hence to the jugulodigastric lymph node, and those located on the dorsum and lateral borders tend to involve the submandibular and jugulodigastric nodes. Contralateral or bilateral spread is relatively common, particularly in tumors arising anteriorly.

3.9.2.3 Floor of the Mouth

The floor of the mouth is a horseshoe-shaped mucosal trough extending between the lower lingual alveolar mucosa and the ventral lingual mucosa. It is the second most common site for intraoral squamous cell carcinomas and shows the highest frequency of small and symptomless tumors [244]. Tumors are most frequent in the anterior segment, and tumors there tend to spread superficially rather than deeply (Fig. 3.41). Involvement of the submandibular duct can cause obstructive sialadenitis and tumors can also extend down the duct itself. If the tumor extends to involve the mandible,

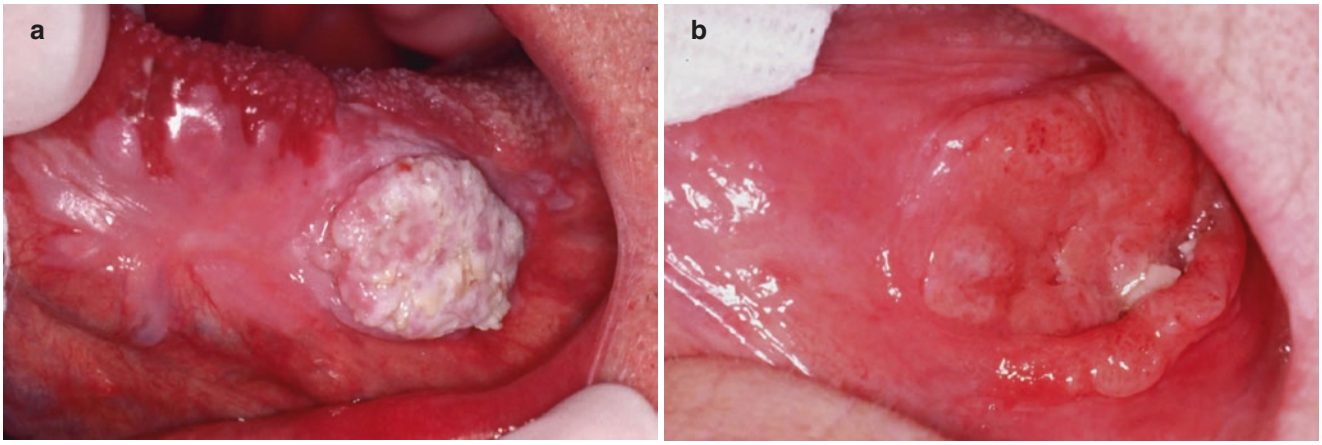

Fig. 3.40 (**a**) Squamous cell carcinoma of the lateral border of the tongue, forming a heavily keratinized, exophytic mass on a background of homogeneous leukoplakia. (**b**) Squamous cell carcinoma of the lateral margin of the tongue forming a large ulcer with a granular floor and a rolled, raised margin

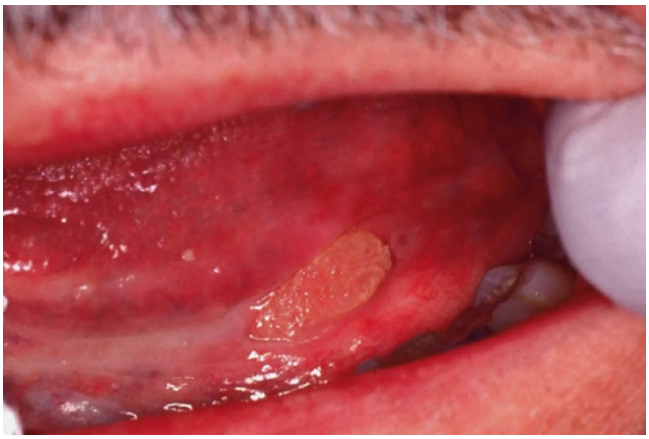

Fig. 3.41 Squamous cell carcinoma of the floor of the mouth, forming an innocuous-looking, slightly granular, well-defined plaque

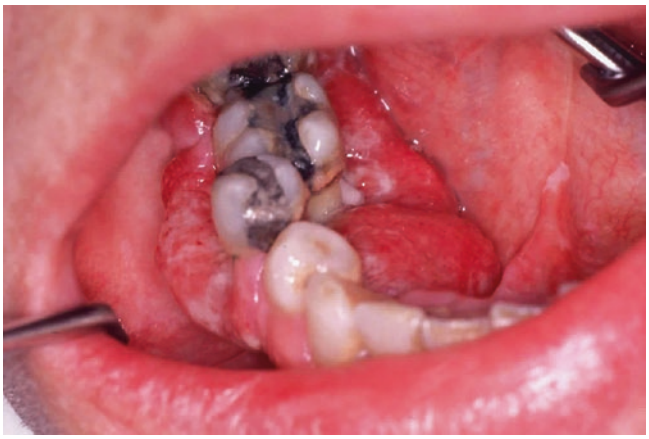

Fig. 3.42 Squamous cell carcinoma of the lower alveolus, forming a large, granular, exophytic mass on both sides of the involved teeth

there can be spread along the periodontal ligament and subperiosteally [245]. Lymphatic involvement is early but less frequent than tumors of the tongue itself.

3.9.2.4 Gingiva and Alveolar Ridge

Tumors at this site can be exophytic resembling dental abscesses or epulides, or are ulcerated and fixed to the underlying bone. They account for about 20% of oral tumors. In parts of the USA, there is a very high frequency in women who practise snuff dipping. Related teeth are often loosened and there is extension along the periodontal ligament (Fig. 3.42). On the alveolus tumors can resemble simple lesions like denture-induced hyperplasia or denture-related ulceration. The underlying bone may be eroded or invaded in 50% of patients and regional metastases are seen in over half the patients at presentation.

3.9.2.5 Hard and Soft Palate

These are relatively uncommon sites of involvement except in societies where reverse smoking is prevalent. Tumors of the hard palate can be exophytic, or ulcerative with raised margins, but initially tend to spread superficially (Fig. 3.43) rather than deeply. Tumors of the soft palate are clinically similar and frequently extend into the parapharyngeal and retromolar regions. There is an increasing role for oncogenic HPV in the development of soft palate squamous cell carcinoma.

3.9.2.6 Retromolar Trigone

Tumors from this site spread to the buccal mucosa laterally and distally involve the tonsillar area. They can penetrate into the parapharyngeal area and may show extensive spread along the lingual and inferior alveolar nerves. In addition, tumors frequently erode or invade the adjacent mandible. However, in some early cases, the diagnosis may not be readily apparent (Fig. 3.44).

3.9.3 Staging

Staging of oral squamous cell carcinomas is undertaken using the current TNM classification (AJCC 7th edn.) (Table 3.3).

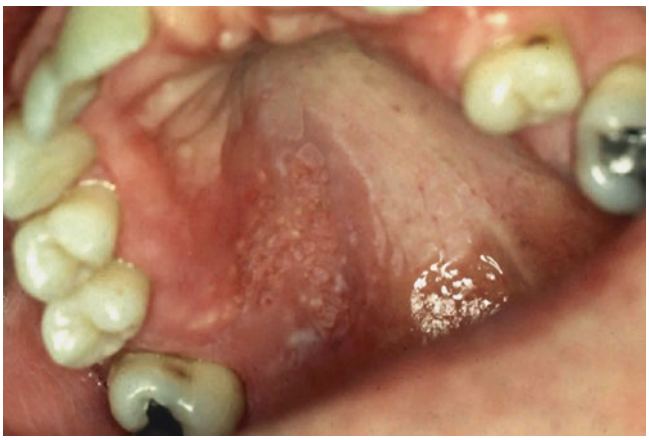

Fig. 3.43 Squamous cell carcinoma of the hard palate, forming a poorly defined, speckled plaque. Despite being relatively small and superficial, this tumor proved to be fatal

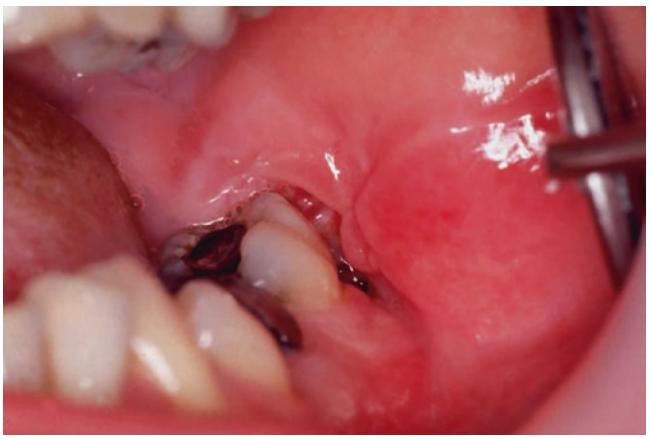

Fig. 3.44 Squamous cell carcinoma of the retromolar region, appearing as an inflamed operculum around an unerupted lower third molar. The tumor originally presented as a secondary deposit in the jugulodigastric lymph node, and initially there was no obvious primary site

Table 3.3 TNM staging for squamous cell carcinoma of the oral cavity – AJCC, 7th edition

Primary tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor 2 cm or less in greatest dimension
T2	Tumor >2 cm but not more than 4 cm in greatest dimension
T3	Tumor >4 cm
T4a	Moderately advanced local disease
	Lip – tumor invades through cortical bone, inferior alveolar nerve, floor of the mouth or skin of the face
	Oral cavity – invasion of adjacent structures (through cortical bone into extrinsic muscles, maxillary sinus or skin of the face)
T4b	Very advanced local disease
	Tumor invades masticator space, pterygoid plates or skull base and/or encases the internal carotid artery
Regional lymph nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node $\leq$3 cm
N2	Metastasis in an ipsilateral node >3 cm but not more than 6 cm in greatest dimension; or in multiple ipsilateral lymph nodes, none >6 cm in greatest dimension; or in bilateral or contralateral lymph nodes, none >6 cm
N2a	Metastasis in a single ipsilateral lymph node >3 cm but not more than 6 cm in greatest dimension
N2b	Metastasis in multiple ipsilateral nodes, none >6 cm in greatest dimension
N2C	Metastasis in bilateral or contralateral lymph nodes, none >6 cm in greatest dimension
N3	Metastasis in a lymph node >6 cm in greatest dimension
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis

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Maxillofacial Skeleton and Teeth

4

Eugenio Maiorano and Pieter J. Slootweg

Contents

E. Maiorano, MD, MSc
Head and Neck Pathology Unit, Division of Pathological Anatomy, Department of Emergencies and Organ Transplantation (DETO), Università degli Studi di Bari Aldo Moro, Policlinico, Piazza G. Cesare 11, Bari 70124, Italy
e-mail: eugenio.maiorano@uniba.it

P.J. Slootweg, MD, DMD, PhD (✉)
Department of Pathology, Radboud University Nijmegen Medical Center, Po Box 9101, 6500 HB Nijmegen, The Netherlands
e-mail: Piet.Slootweg@radboudumc.nl

4.1 Introduction

4.1.1 Embryology

The maxillofacial skeleton is partly derived from migrated cranial neural crest cells. These cells give rise to most connective tissues in the craniofacial region including the bones of the calvarium, face, and jaws. So, the diseases to be discussed in this chapter occur in bones that are formed by mesenchymal cells having an ectodermal/neuroectodermal ancestry – that is why they are also known as ectomesenchyme – and therefore are different from bone and cartilage elsewhere in the body that has a mesodermal origin [1, 2].

4.1.2 Tooth Development

Teeth develop from epithelial cells from the mucosal lining of the oral cavity and cranial neural crest-derived ectomesenchymal cells. Under the influence of reciprocal inductive events, these cells develop into enamel-forming ameloblasts and dentin-producing odontoblasts (Fig. 4.1) [3, 4].

While ameloblasts and odontoblasts are depositing enamel and dentin, the epithelium proliferates in a downward way thus creating a cuff that maps out the form and size of the root of the teeth. This epithelial cuff is known as the sheath of Hertwig. Its remnants form a permanent component of the periodontal ligament; they are known as rests of Malassez and are the source of some cystic jaw lesions (Fig. 4.2). Other epithelial reminiscences to the tooth development lie more superficially in the jaw tissues; they are the epithelial rests of Serres that have their origin from the dental lamina, the epithelial rim from which the tooth germs originate.

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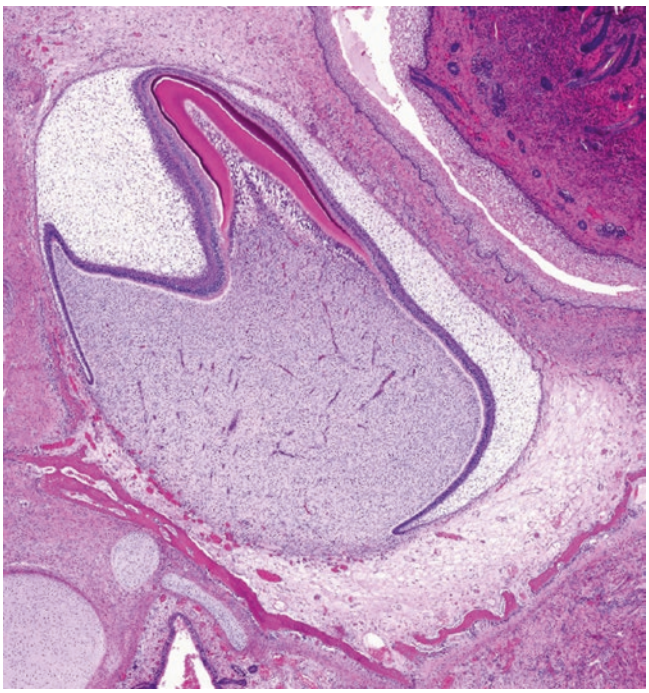

Fig. 4.1 Overview of normal tooth germ lying in its bony crypt. The loose myxoid tissue of the dental papilla is covered by the enamel organ composed of inner enamel epithelium facing the dental papilla and outer dental enamel epithelium facing the fibrous dental follicle that invests the entire tooth germ; the loose epithelial tissue between both layers is called the stellate reticulum. At the interface of dental papilla and inner enamel epithelium, there is deposition of dentin (*pink*) and enamel matrix (*deep purple*)

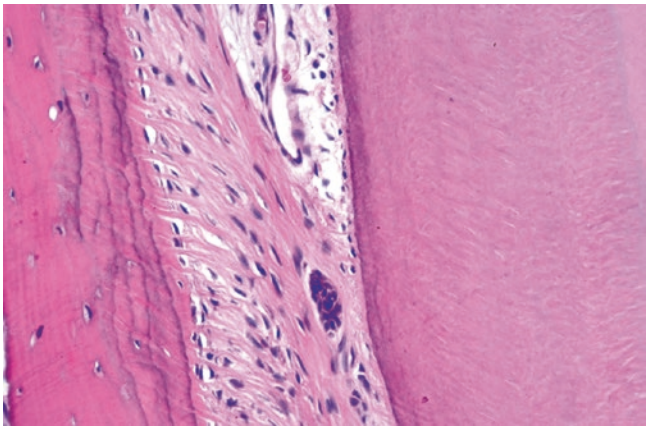

Fig. 4.2 The periodontal ligament connects the root surface (*right side*) with the bony socket (*left side*). An epithelial rest of Malassez is clearly visible

4.2 Cysts of the Jaws

Cysts of the jaws are classified in distinct categories depending on their histogenesis and etiology. Those that arise from odontogenic epithelium are called odontogenic; those that have their source in other epithelial structures are known as non-odontogenic. Within the group of odontogenic cysts, one discerns between inflammatory and developmental [5], as listed in Table 4.1. By definition, cysts are lined by epithelium, but there are also cavities in the jaws that lack such an epithelial investment that are also discussed under this heading.

Table 4.1 Cysts of the jaws [5]

(a) Odontogenic cysts – inflammatory	Radicular cyst/residual cyst
	Paradental cyst
(b) Odontogenic cysts – developmental	Dentigerous cyst
	Lateral periodontal cyst
	Glandular odontogenic cyst
	Odontogenic keratocyst (Keratinizing cystic odontogenic tumor)
	Gingival cyst
(c) Non-odontogenic cysts	Nasopalatine duct cyst
	Nasolabial cyst
	Surgical ciliated cyst
(d) Pseudocysts	Solitary bone cyst
	Focal bone marrow defect

4.2.1 Odontogenic Cysts: Inflammatory

4.2.1.1 Radicular Cyst

Definition Radicular cysts are cystic lesions located at the root tips of teeth (Fig. 4.3) and that arise from the epithelial rests of Malassez.

Epidemiology It is the most frequent odontogenic cyst [6, 7] and mostly occurs during adulthood.

Etiology and pathogenesis A radicular cyst develops from the root apex, following inflammation or trauma.

Clinical aspects Radicular cysts usually are asymptomatic and fortuitously detected by radiographic examination of the dentition. However, when complicated by inflammation, radicular cysts may cause pain and swelling.

Macroscopy Intact radicular cysts exhibit a thick whitish wall and contain brownish fluid.

Microscopy They are lined by non-keratinizing stratified squamous epithelium that may be thin and atrophic or show elongated rete processes. In many cysts, cholesterol clefts with adjacent giant cells occur. Within the cyst epithelium, hyaline bodies (Rushton bodies) of various size and shape may be present (Fig. 4.4), the specific nature of which remains unclear [8]. Occasionally, the lining squamous cells are admixed with mucous cells or ciliated cells (Fig. 4.5). Sometimes, the histologic pattern of the radicular cyst is complicated by extensive intramural proliferation of

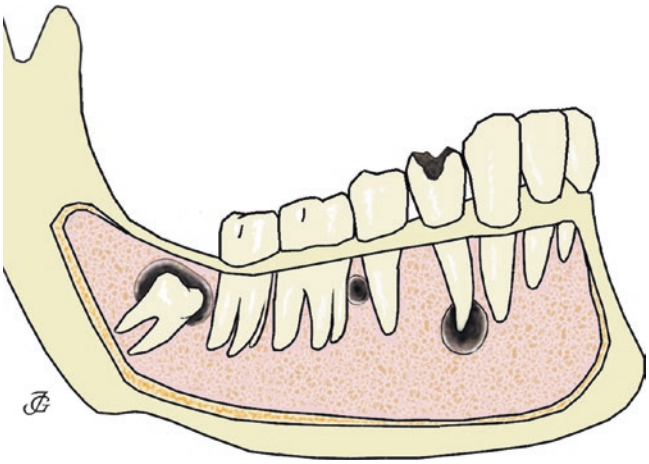

Fig. 4.3 Schematic drawing showing a bisected mandible in which from *left* to *right* an example of a dentigerous cyst, a lateral periodontal cyst, and a radicular cyst (drawing by John de Groot) can be seen

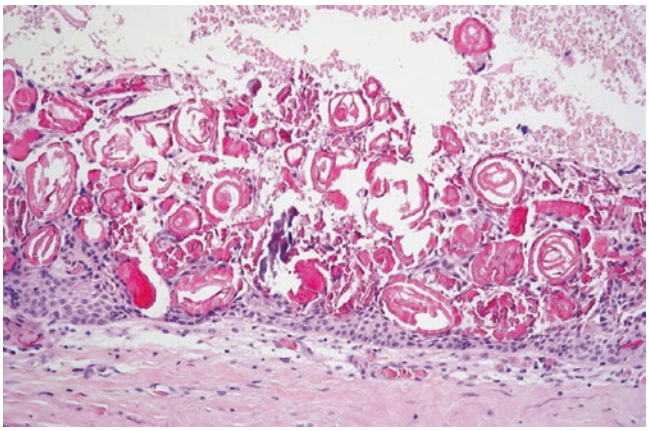

Fig. 4.4 Epithelial lining of a radicular cyst containing many Rushton bodies

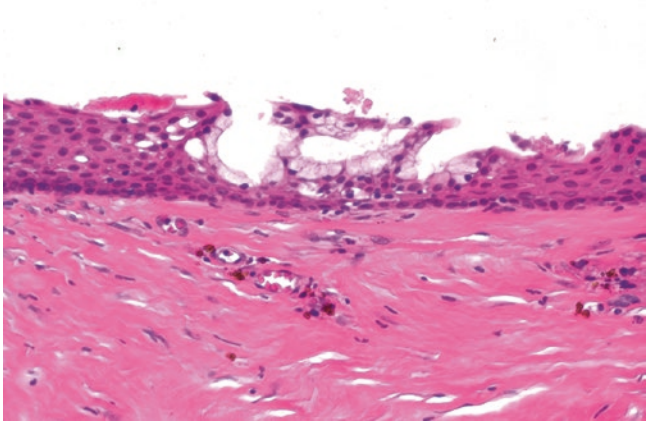

Fig. 4.5 Cyst lining composed of squamous as well as mucous epithelium. This can be found in radicular cysts as well as in dentigerous or residual cysts

squamous epithelial nests of varying size, thus mimicking a squamous odontogenic tumor (see Sect. 4.3.1.4) [9]. The same histology may be shown by other jaw cysts, in particular when there are extensive inflammatory changes. When a radicular cyst is retained in the jaws after removal of the associated tooth, the lesion is called *residual cyst*.

Treatment and prognosis Curettage or simple enucleation usually are curative.

4.2.1.2 Paradental Cyst

Definition The paradental cyst is a cystic lesion located at the lateral side of the tooth at the border between the enamel and root cementum. Less frequently used synonyms are inflammatory collateral or mandibular infected buccal cyst [5].

Epidemiology This is a rather infrequent odontogenic cyst and mostly occurs during adulthood although cases also have been encountered at younger age.

Etiology and pathogenesis This cyst is secondary to an inflammatory process in the adjacent periodontal tissues that induces proliferation of neighboring odontogenic epithelial rests, similar to the pathogenesis of the radicular cyst [10, 11].

Clinical aspects Radiological investigations are essential to assess the location of paradental cysts at the lateral aspect of the tooth root.

Macroscopy Intact cysts have a fibrous wall and contain brownish fluid.

Microscopy Histologically, it resembles other inflammatory odontogenic cysts, the distinction being made by the specific clinical and radiologic presentation.

Differential diagnosis In view of the rather nonspecific histological features of inflammatory cysts as a group, clinicoradiological correlations are frequently necessary to achieve the correct diagnosis.

Treatment and prognosis Treatment consists of excision with or without concomitant extraction of the involved tooth [12].

4.2.2 Odontogenic Cysts: Developmental

4.2.2.1 Dentigerous Cyst

Definition Dentigerous (follicular) cysts surround the crown of an unerupted tooth, mostly the maxillary canine or the mandibular third molar tooth (Fig. 4.3).

Epidemiology It is the most common developmental cyst and frequently is detected in young adults.

Etiology and pathogenesis This cyst arises as a consequence of fluid accumulation between the reduced enamel epithelium and the tooth crown.

Clinical aspects In most instances, dentigerous cysts are a fortuitous finding on oral radiograms, but, when excessively large, they may cause swelling of the involved part of the jaw that may be associated with pain if inflammation also occurs. They lie in close association with an impacted tooth, especially the mandibular third molar.

Microscopy The cyst wall has a thin epithelial lining with a thickness of only two to three cell layers. In case of inflammation, the epithelium becomes thicker and may resemble that of a radicular cyst. Also, mucous-producing cells as well as ciliated cells may be observed (Fig. 4.6). The connective tissue component of the cyst wall may be fibrous or fibromyxomatous and may also contain varying amounts of epithelial nests representing remnants of the dental lamina.

Differential diagnosis Dentigerous cysts share their radiological features with a lot of other jaw diseases that are associated with unerupted teeth, and histologic examination will be decisive in ruling out these alternative possibilities among which the odontogenic keratocyst and the unicystic ameloblastoma (see Sects. 4.2.2.4 and 4.3.1.1) are the most prevalent. Moreover, the radiologic picture of a dentigerous cyst may mimic that of dental follicle hyperplasia, an increase in the connective tissue capsule that surrounds an unerupted tooth [13].

Fibromyxomatous areas in the connective tissue wall of a dentigerous cyst may resemble odontogenic myxoma (see Sect. 4.3.2.1), and the presence of odontogenic epithelial rests may lead to the erroneous diagnosis of an epithelial odontogenic tumor [14]. However, identification of the epithelial cyst lining will rule out these alternatives.

The *eruption cyst* is a specific type of dentigerous cyst located in the gingival soft tissues overlying the crown of an erupting tooth. Mostly, these cysts have a short life, rupturing with progressive eruption of the associated tooth. They are lined by squamous epithelium that is thickened due to inflammatory changes in the underlying connective tissue and thus similar to the lining of a radicular cyst.

Treatment and prognosis Removal of the cyst wall and the involved tooth will yield a permanent cure.

4.2.2.2 Lateral Periodontal Cyst

Definition Lateral periodontal cysts are rare lesions, derived from odontogenic epithelial remnants, and occurring on the lateral aspect or between the roots of vital teeth (Fig. 4.3) [15].

Clinical aspects Lateral periodontal cysts usually are asymptomatic and fortuitous findings on radiograms, where they present as well-demarcated radiolucencies on the lateral surface of a tooth root.

Microscopy They are lined by thin, non-keratinizing squamous or cuboidal epithelium with focal, plaque-like thickenings consisting of clear cells that may contain glycogen (Fig. 4.7) [16].

Treatment and prognosis Simple enucleation is adequate treatment.

The *botryoid odontogenic cyst* represents a multilocular form of the lateral periodontal cyst [17]. Treatment by curettage is the most appropriate treatment but recurrences may occur [18].

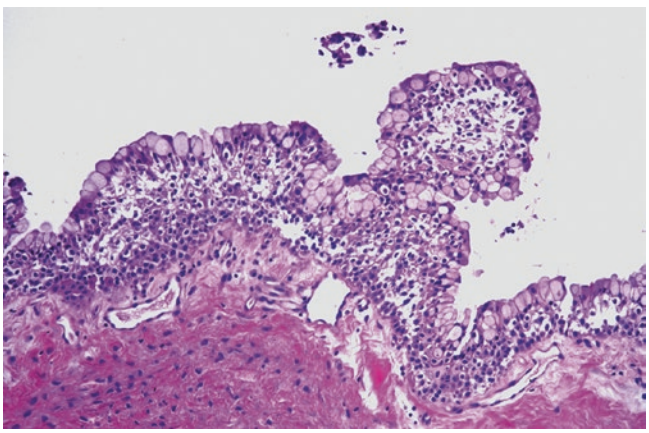

Fig. 4.6 Lining of a dentigerous cyst mainly composed of mucous cells

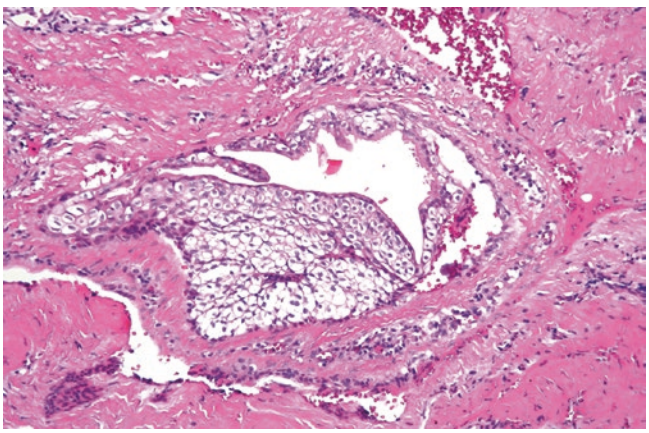

Fig. 4.7 Lateral periodontal cyst. The epithelial lining forms plaques consisting of clear cells

4.2.2.3 Glandular Odontogenic Cyst

Definition The glandular odontogenic cyst, also called *sialo-odontogenic cyst*, is a cystic lesion characterized by an epithelial lining with cuboidal or columnar cells both at the surface and surrounding intraepithelial crypts or cyst-like spaces [5, 6].

Epidemiology This is a rare lesion of adulthood.

Clinical aspects The glandular odontogenic cyst most commonly affects the body of the mandible, particularly the anterior part, and the most prominent symptom is painless swelling.

Microscopy The lining epithelium is partly non-keratinizing squamous with focal thickenings similar to the plaques in the lateral periodontal cyst and the botryoid odontogenic cyst. There may be a surface layer of eosinophilic cuboidal or columnar cells that may be ciliated and form papillary projections. Some superficial cells may assume an apocrine appearance and mucous-producing cells may also be present. The epithelium focally forms areas of increased thickness in which glandular spaces are formed. Moreover, the epithelial cells may lie in spherical structures with a whorled appearance (Fig. 4.8).

Differential diagnosis Mucous cells and ciliated cuboidal cells may also occur in other jaw cysts, but these lack the other epithelial features as described above. Furthermore, mucous cells and non-keratinizing squamous epithelium also occur in mucoepidermoid carcinoma [19–21]. However, epithelial plaques consisting of clear cells are not a feature of this latter lesion. In fact, clear cells in mucoepidermoid carcinoma tend to be more haphazardly distributed throughout the tumor, are larger in size, and do not show a close association with cystic lumina.

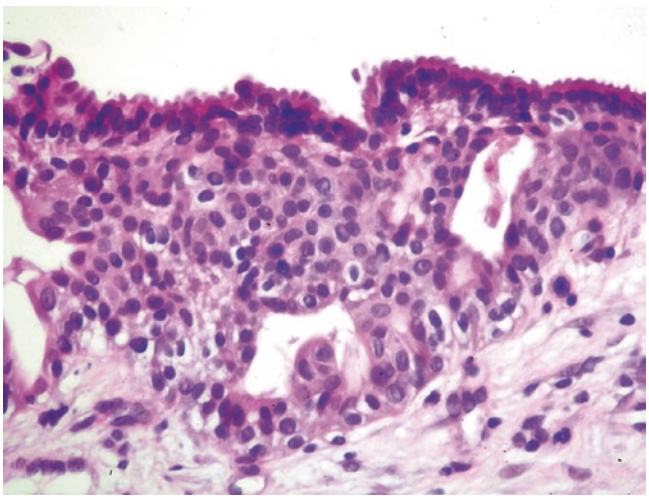

Fig. 4.8 Epithelial lining of glandular odontogenic cyst showing intraepithelial duct formation and apocrine differentiation at the surface

Treatment and prognosis Treatment may be conservative but recurrence may occur in up to 30 % of the cases [22].

4.2.2.4 Odontogenic Keratocyst

Definition Odontogenic keratocyst (formerly primordial cyst) is a developmental cyst with a distinctive epithelial lining. This lesion was renamed as *keratocystic odontogenic tumor* in the current WHO classification of odontogenic tumors [23] in view of its neoplastic nature, but this designation was not universally accepted and will not be maintained in the next WHO classification that is in preparation at the time of writing this text.

Epidemiology Odontogenic keratocysts are common lesions [24–26] that may occur at a wide age range, with a peak prevalence in the second and third decades; they are more common in males than in females.

Clinical aspects They are twice as frequent in the mandible than in the maxilla and involvement of the gingival soft tissues has also been reported [27]. They may occur in the context of the nevoid basal cell carcinoma (Gorlin–Goltz) syndrome [28–30].

Odontogenic keratocysts are asymptomatic unless concomitant inflammation causes pain and swelling; sometimes they are associated with impacted teeth. Radiographs may reveal extensive uni- or multilocular radiolucent lesions that occupy the major part of the jaw without appreciable cortical expansion.

Macroscopy This is a thin-walled lesion containing clear fluid that may become friable especially when inflamed and that may be multilocular.

Microscopy The odontogenic keratocyst shows a thin connective tissue wall lined by stratified squamous epithelium with a well-defined basal layer of palisading columnar or cuboidal cells and with a superficial corrugated layer of parakeratin (Fig. 4.9). Mitotic figures can be identified in parabasal and midspinous areas [31], and Rushton bodies, similar to those seen in radicular cysts, may also be present. The underlying cyst wall may contain tiny daughter cysts and solid epithelial nests. Also solid epithelial proliferations similar to ameloblastoma have been reported. Both daughter cysts and intramural epithelial nests are more common in cysts associated with the nevoid basal cell carcinoma

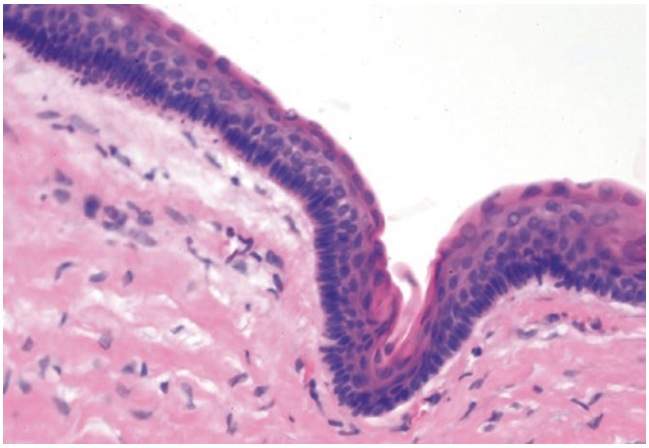

Fig. 4.9 Epithelial lining of an odontogenic keratocyst. The basal palisading and the corrugated parakeratinized surface are unique for this lesion. Moreover, there is a striking parallelism between the basal layer and the inner surface of the cyst

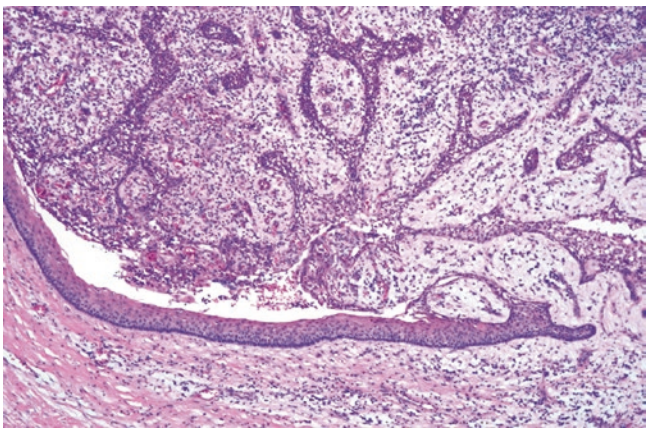

Fig. 4.10 In the event of inflammation, the epithelial lining of an odontogenic keratocyst loses its typical pattern to transform into a lattice of spongiotic squamous epithelium

syndrome [32]. When inflamed, the odontogenic keratocyst loses in part its typical histologic features and shows a non-keratinizing stratified epithelium exhibiting spongiosis and elongated rete pegs supported by a connective tissue containing a mixed inflammatory infiltrate (Fig. 4.10). Rarely, the odontogenic keratocysts show development of epithelial dysplasia and squamous cell carcinoma [33]. Immunohistochemical studies have not yielded data of diagnostic or prognostic significance [26].

Odontogenic keratocysts may also contain mucous cells, melanin-producing cells, dentinoid. and intramural cartilage [31, 34–36]. Ciliated cells may be seen but in maxillary cases, they could be the result from a communication with the maxillary sinus [37]. In addition, the cyst wall may contain intramural odontogenic epithelial remnants. Occasionally, intraosseous cysts are lined by orthokeratinized epithelium, thus having the appearance of an epidermoid cyst. Such cysts are known as orthokeratinized odontogenic cyst and their differentiation from the odontogenic keratocyst that by definition shows parakeratinization is clinically relevant as recurrence of the orthokeratinized cysts is rare [38].

Differential diagnosis The differential diagnosis with unicystic ameloblastoma (see Sect. 4.3.1.1) may be difficult: odontogenic keratocyst exhibits a compact spinous layer and a corrugated superficial parakeratin layer, while ameloblastoma shows a spinous layer with intercellular edema.

When the odontogenic keratocyst forms part of the nevoid basal cell carcinoma syndrome, patients may show any of the other features of this syndrome [39].

Treatment and prognosis Odontogenic keratocysts tend to recur after enucleation [24–26] and a resection offers the highest chance of permanent cure [40]. If associated with the nevoid basal cell carcinoma syndrome, the chance to recur is even higher [40], while peripheral (gingival) lesions usually are less aggressive [41].

4.2.2.5 Gingival Cyst

Definition Gingival cysts may occur in adults or in infants and are located in the gingival tissues.

Clinical aspects *Gingival cysts of adults* are rarely larger than 1 cm and may be multiple. They typically affect the canine–premolar region of the mandible. *Gingival cysts of infants* occur as a single lesion or as multiple ones at the edentulous alveolar ridge of newborn infants. When occurring at the midline of the palate, they are known as *palatal cysts of infants*. These tiny lesions, usually not larger than 3 mm, disappear spontaneously within short time.

Microscopy Gingival cysts of adults are lined by either thin epithelium of one to three cell layers or thicker and exhibiting keratinization. Plaques similar to those occurring in the lateral periodontal cyst (see Sect. 4.2.2.2) may be seen [42]. Gingival cysts of infants histologically resemble epidermoid cysts [43, 44]. Historically, they have been known as *Epstein's pearls* and *Bohn's nodules*.

Treatment and prognosis Gingival cysts of adults may be treated by simple enucleation, while those arising in infants may spontaneously disappear.

4.2.3 Non-odontogenic Cysts

4.2.3.1 Nasopalatine Duct Cyst

Definition Nasopalatine duct cysts arise within the nasopalatine canal from epithelial remnants of the nasopalatine duct.

Clinical aspects Radiologically, they present as radiolucent lesions located between the roots of maxillary central incisor teeth.

Microscopy The cyst lining may be pseudostratified columnar ciliated epithelium, stratified squamous epithelium, columnar or cuboidal epithelium, or combinations of these. As surgical treatment comprises emptying the nasopalatine canal, the specimen always includes the artery and nerve that run in this anatomic structure. These are seen within the fibrous cyst wall and form the most convincing diagnostic feature, as the specific epithelial structures may be obscured by inflammatory changes.

Treatment and prognosis These cysts are treated by simple enucleation and recurrences are rarely seen [45].

4.2.3.2 Nasolabial Cyst

Definition Nasolabial cysts are located in the soft tissue just lateral to the nose, at the buccal aspect of the maxillary alveolar process and are thought to arise from the nasolacrimal duct.

Microscopy Non-ciliated pseudostratified columnar epithelium with interspersed mucous cells form their epithelial lining. These features may be lost through squamous metaplasia [46]. Apocrine metaplasia of the cyst lining has also been reported [47].

Treatment and prognosis Treatment consists of enucleation and there are no recurrences.

4.2.3.3 Surgical Ciliated Cyst

Definition Surgical ciliated cysts arise from detached portions of the mucosa that line the maxillary antrum that are buried within the maxillary bone. This may occur after trauma or surgical intervention in this area [48]. See also Chap. 2, Sect. 2.5.7.

Clinical aspects Mostly, the cyst is an incidental radiographic finding, observed as a well-defined unilocular radiolucency adjacent to the maxillary antrum.

Microscopy The cyst lining is similar to the normal mucosal surface of the paranasal cavities: pseudostratified ciliated columnar epithelium with interspersed mucous cells. Usually, the cyst ruptures during removal, yielding a specimen with a picture similar to normal maxillary sinus mucosa. Therefore, histology in this context is more useful in ruling out any other condition than suited to confirm a preoperative radiological diagnosis of surgical ciliated cyst.

Treatment and prognosis Treatment consists of simple enucleation.

4.2.4 Pseudocysts

4.2.4.1 Solitary Bone Cyst

Definition The solitary bone cyst, also known as *traumatic bone cyst* or *simple bone cyst*, is a unilocular cyst confined to the mandibular body.

Etiology and pathogenesis Its pathogenesis still is poorly understood, a remnant of intraosseous hemorrhage being the most favored hypothesis.

Epidemiology Such lesions most frequently occur during the second decade with no gender predilection.

Clinical aspects Radiographs show a cavity that varies from less than 1 cm in diameter to one that occupies the entire mandibular body and ramus. The margins may extend between the roots of the teeth and usually are scalloped. At surgical exploration, one encounters a fluid-filled cavity.

Microscopy Material for histologic examination may be difficult to obtain as a soft tissue lining of the bony cavity may be entirely absent or very thin. If present, it usually consists only of loose fibrovascular tissue, although it may also contain granulation tissue with signs of previous hemorrhage such as cholesterol clefts and macrophages loaded with iron pigment (Fig. 4.11) [49]. Incidentally, thread-like calcifications also can be found. Sometimes, this cyst develops simultaneously with a variety of fibro-osseous lesions [50].

Treatment and prognosis Lesions are treated with curettage but sometimes the lesion may recur. In a series of 132 cases of simple bone cyst, the recurrence rate was high for cases with multiple cysts and osseous dysplasia, respectively [51].

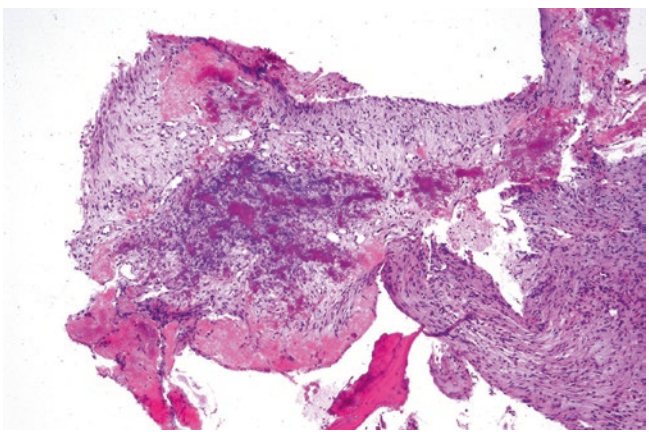

Fig. 4.11 Loose fibrous tissue, fibrin deposits, and thornlike calcifications are typical for a solitary bone cyst

4.2.4.2 Focal Bone Marrow Defect

Definition A localized area of osteoporotic bone, sometimes following tooth extraction.

Clinical aspects Focal bone marrow defect represents an asymptomatic radiolucent lesion of the jaws, which contains normal hematopoietic and fatty bone marrow. It is also called *osteoporotic bone marrow defect*. This condition is mostly seen at the angle of the mandible as a radiolucent lesion with more or less well-defined borders.

Microscopy Histologic examination only reveals the presence of normal hematopoietic marrow [52].

Treatment and prognosis No specific treatment is required following diagnosis.

4.3 Odontogenic Tumors

Odontogenic tumors encompass a group of lesions that share a common origin from odontogenic tissues. They may arise from the epithelial part of the tooth germ, the ectomesenchymal part, or from both. Their behavior varies from frankly neoplastic including metastatic potential to nonneoplastic hamartomatous. Some of them may recapitulate normal tooth development including the formation of dental hard tissues such as enamel, dentin, and cementum [53]. Table 4.2 gives an overview of the various entities listed under this heading.

Table 4.2 Odontogenic tumors [53]

Epithelial	Ameloblastoma
	Calcifying epithelial odontogenic tumor
	Adenomatoid odontogenic tumor
	Squamous odontogenic tumor
Mesenchymal	Odontogenic myxoma
	Odontogenic fibroma
	Cementoblastoma
Mixed epithelial and mesenchymal	Adenomatoid odontogenic hamartoma
	Ameloblastic fibroma
	Ameloblastic fibro-odontoma
	Odontoma – complex type
	Odontoma – compound type
	Odontoameloblastoma
	Calcifying cystic odontogenic tumor/ dentinogenic ghost cell tumor (calcifying odontogenic cyst)
Malignant	Malignant ameloblastoma
	Ameloblastic carcinoma
	Primary intraosseous carcinoma
	Clear cell odontogenic carcinoma
	Malignant epithelial odontogenic ghost cell tumor
	Sclerosing odontogenic carcinoma
	Odontogenic sarcoma

4.3.1 Odontogenic Tumors: Epithelial

Epithelial odontogenic tumors supposedly are derived from the odontogenic epithelium: dental lamina, enamel organ, and Hertwig's root sheath. As there is no contribution, either proliferative or inductive, from the odontogenic mesenchyme, these lesions do not contain dental hard tissues or myxoid tissue resembling the dental pulp.

4.3.1.1 Ameloblastoma

Definition Ameloblastoma, the most common odontogenic tumor [53], closely resembles the epithelial part of the tooth germ, may be locally aggressive, but does not metastasize.

Epidemiology It may occur at any age, but cases in the first decade are rare and most of them are diagnosed between the ages of 30–60 years.

Clinical aspects Maxillary cases are outnumbered by mandibular ones, and in rare instances the sinonasal cavities may be involved [54, 55]. The posterior segments of the jawbones are the elective location. Painless swelling is the most prominent symptom of ameloblastoma, but those arising in the maxilla may show growth into the paranasal sinuses to attain a considerable size without causing any external deformity. Radiographically, ameloblastoma is a radiolucent lesion that usually is multilocular, the so-called soap-bubble appearance, or unilocular with scalloped outlines [54]. Intraosseous lesions (central ameloblastomas) may be either solid, solid with cystic parts, multicystic, or unicystic. In the gingiva (peripheral ameloblastoma), the tumor shows a white fibrous appearance on cut surface, due to the preponderance of fibrous stroma at this site.

Macroscopy Ameloblastomas usually are partly solid, partly cystic tumors that spread intraosseously and cause cortical expansion leading to deformity of the jaw.

Microscopy Ameloblastomas consist of either anastomosing epithelial strands and fields or discrete epithelial islands. The former pattern is referred to as *plexiform* type, the latter as *follicular* type (Figs. 4.12 and 4.13). Both patterns may occur within one and the same lesion [53]. The peripheral cells at the border with the adjacent fibrous stroma are columnar with nuclei usually in the apical halve of the cell body away from the basement membrane. The cells lying more centrally are fusiform to polyhedral and loosely connected to each other through cytoplasmic extensions (so-called stellate reticulum). Especially in the follicular type, increased intercellular edema may produce cysts that coalesce to form the

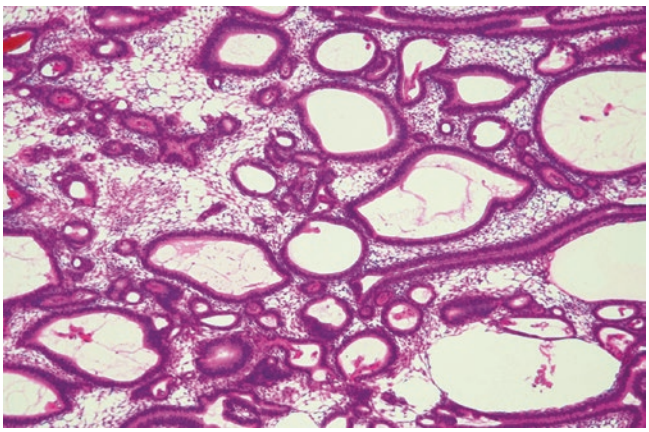

Fig. 4.12 Large epithelial areas of loosely structured spindle epithelium, enclosing liquefacting stromal areas, are typical of a plexiform ameloblastoma. The epithelial cells facing the stroma show nuclear palisading

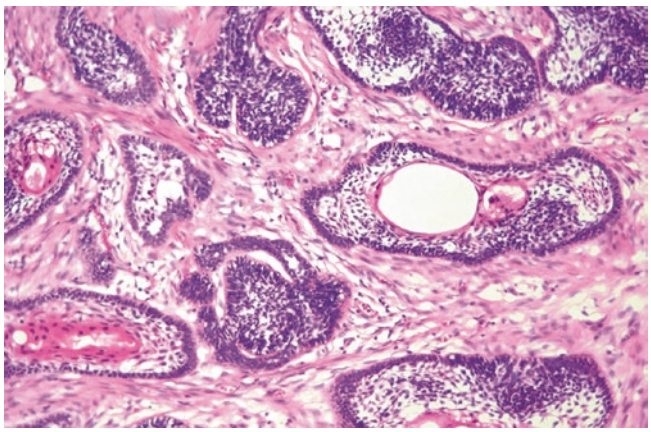

Fig. 4.13 In cases of follicular ameloblastoma, the tumor consists of epithelial islands with a loose edematous center and a peripheral rim of palisading cells. Liquefaction of their center results in cyst formation

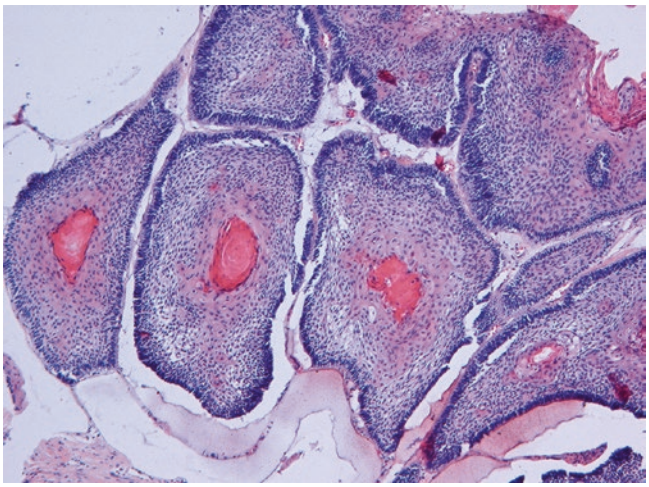

Fig. 4.14 Ameloblastoma showing distinct squamous metaplasia with whorling and keratin formation. Typical peripheral palisading and stellate reticulum are still evident at the border of a neoplastic follicle

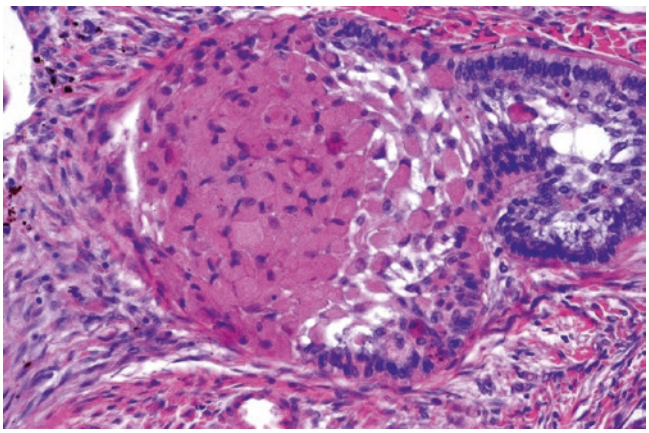

Fig. 4.15 Ameloblastoma with granular cells showing evident cytoplasmic granularity, along with typical peripheral palisading at the periphery

large cavities, responsible for the multicystic gross appearance ameloblastomas may show. In the plexiform type, cyst formation usually is the result of stromal degeneration. Condensation of collagenous fibers may cause juxta-epithelial eosinophilic hyaline bands. At the periphery of the lesion, the tumor infiltrates the adjacent cancellous bone, causing expansion of the lower cortical border of the mandible and of the periosteum. Nevertheless, perforation of the cortical plate is exceptional, the periosteum in particular forming a barrier [56]. Consequently, spread into the adjacent soft tissues is highly unusual and, when observed, ameloblastic carcinoma should be considered in the differential diagnosis (see Sect. 4.3.4.2). Mitotic figures may occur within the peripheral columnar as well as in the stellate reticulum-like cells. In the absence of concomitant cytonuclear atypia and with a normal configuration, they lack any prognostic significance.

Acanthomatous and *granular cell*-type ameloblastoma are variants of follicular ameloblastoma with squamous metaplasia (Fig. 4.14) and granular cells (Fig. 4.15), respectively. If keratinization is abundant, leading to large cavities filled with keratin, lesions are called *keratoameloblastoma* [57]. In these tumors acantholysis may lead to a pseudopapillary lining that characterizes the variant called *papilliferous keratoameloblastoma*.

The *basal cell* (*basaloid*) *ameloblastoma* is composed of nests of basaloid cells with a peripheral rim of cuboidal cells and does not display a well-developed loose edematous center.

Desmoplastic ameloblastoma occurs more often in the anterior parts of both maxilla and mandible and shows a dense collagenous stroma, the epithelial component being reduced to narrow, compressed strands of epithelium. When these strands broaden to form larger islands, a peripheral rim

of darkly staining cuboidal cells and a compact center in which spindle-shaped epithelial cells assume a whorling pattern may be discerned (Fig. 4.16). Within the stromal component, active bone formation can be observed [58].

Unicystic ameloblastoma occurs at a lower mean age than the other types and often has a radiographic appearance similar to a dentigerous cyst because of its association with an impacted tooth [59]. It consists of a single cystic cavity lined by ameloblastomatous epithelium (Fig. 4.17). This epithelium may proliferate to form intraluminal nodules with the architecture of plexiform ameloblastoma. Downward proliferation of this epithelium may lead to infiltration of the fibrous cyst wall by ameloblastoma nests. Sometimes, the cyst lining itself lacks any features indicative of ameloblastoma, these being confined to intramural epithelial nests [60]. Inflammatory alterations may obscure the specific histologic details to such an extent that none are left. Furthermore, multinucleated giant cells may be occasionally detected, particularly in the proximity of foci of inflammation or bone resorption.

Ameloblastomas may also contain clear cells as well as mucous cells [61, 62].

Immunohistochemistry Interestingly, ameloblastomas show distinct nucleocytoplasmic calretinin immunoreactivity (Fig. 4.18) that is particularly intense in the stellate reticulum-like epithelial cells and in areas of squamous metaplasia [63]. This feature may be particularly useful in the differential diagnosis with other odontogenic cysts and tumors [64].

Differential diagnosis Epithelial nests resembling ameloblastoma may be found in calcifying cystic odontogenic tumor and ameloblastic fibroma, lesions to be discussed under the appropriate headings (Sects. 4.3.3.2 and 4.3.3.3). Also, epithelial nests in the dental follicle that surrounds an impacted tooth and in the wall of odontogenic cysts may mimic ameloblastoma. Maxillary ameloblastomas may be mistaken for solid-type adenoid cystic carcinoma (see Chap. 5). Also, *peripheral ameloblastoma* should not be confused with intraosseous ameloblastomas that spread from within the jaw into the overlying gingiva [65]. In the past, these lesions have also been described as *odontogenic gingival epithelial hamartoma* [66].

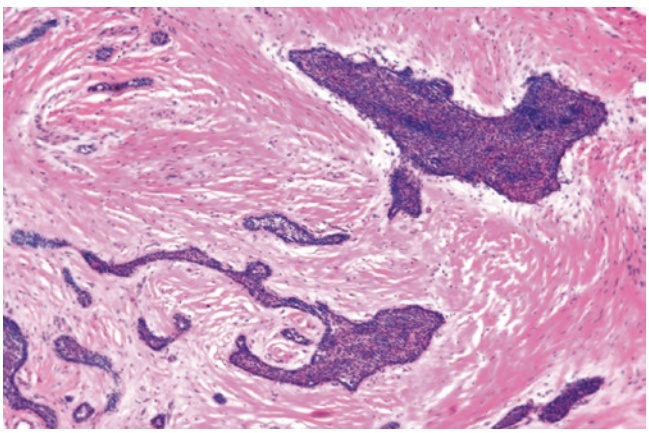

Fig. 4.16 Desmoplastic ameloblastoma consists of densely packed spindle cells lying in a fibrous stroma. Palisading of peripheral cells is not a conspicuous feature in this type of ameloblastoma

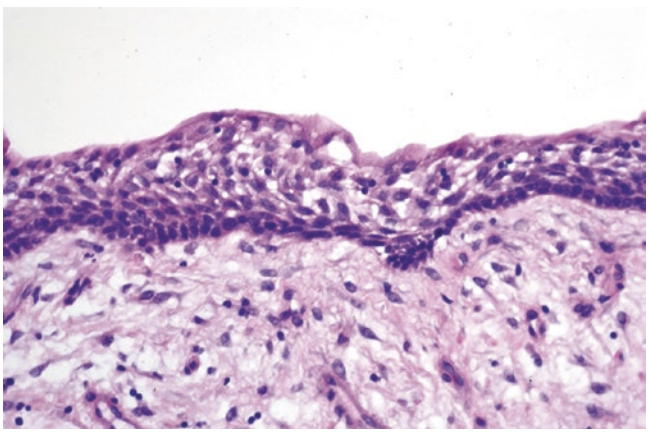

Fig. 4.17 In unicystic ameloblastoma, the tumor consists of cyst-lining epithelium that still shows the typical features of ameloblastoma: loose epithelium and a rim of palisading cells facing the stroma

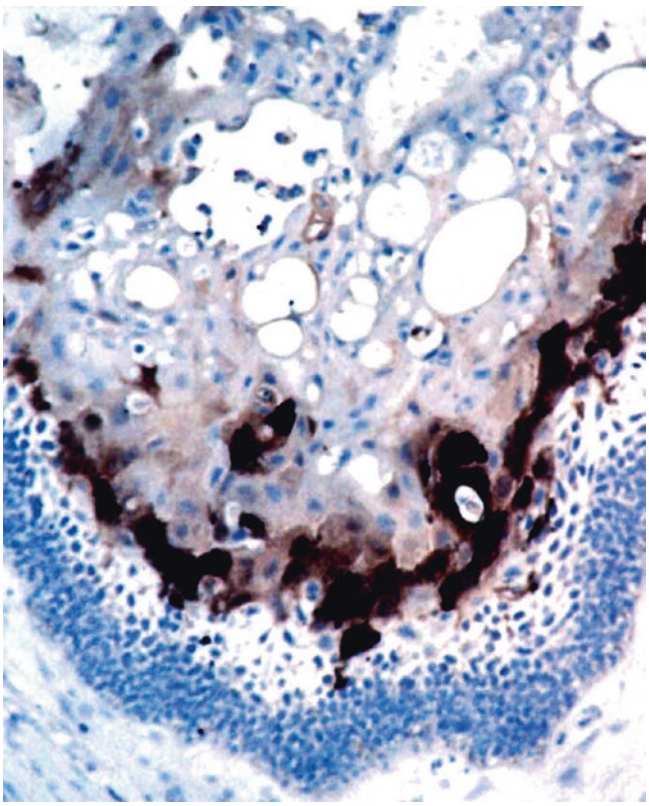

Fig. 4.18 Calretinin immunoreactivity is clearly evident in the tumor cells in suprabasal position in ameloblastoma

Treatment and prognosis Treatment of ameloblastoma consists of adequate tumor removal including a margin of uninvolved tissue. For peripheral ameloblastoma simple excision is considered curative [60, 65]. For unicystic ameloblastoma with the ameloblastomatous epithelium confined to the cyst lining, enucleation would be adequate, but in cases showing intramural proliferation, the treatment should be the same as for conventional ameloblastoma types [67]. When adequately treated, ameloblastomas are not expected to recur. Adequate removal, however, may be difficult to realize in maxillary cases that grow postero-cranially. In such cases, extension into the cranial cavity may be fatal [68]. New treatment opportunities may become available in the future as the finding that a hyperactive RAS–RAF–MAPK pathway is closely associated with ameloblastoma pathogenesis, either through EGFR-mediated signaling or through activating mutations in the *BRAF* gene, may offer opportunities for designing targeted therapies [69–73].

In rare instances, metastatic deposits, mainly to the lung, have been observed. Such lesions, showing typical morphologic features of conventional ameloblastoma and developing distant metastases, are called malignant (metastasizing) ameloblastomas (see Sect. 4.3.4.1).

4.3.1.2 Calcifying Epithelial Odontogenic Tumor

Definition The calcifying epithelial odontogenic tumor, also named Pindborg tumor, is a benign tumor with peculiar morphologic features of the epithelial tumor cells.

Epidemiology It is far less frequent than ameloblastoma, occurs between the second and sixth decade without gender predilection, and mainly involves the posterior jaw area. Also, peripheral (gingival) cases may be seen [74].

Clinical aspects Swelling is the most common clinical symptom of this tumor. Radiographically, the tumor is characterized by a diffuse mixed, radiodense, and radiolucent appearance with an unerupted tooth frequently lying buried in the tumor mass.

Microscopy The tumor consists of sheets of polygonal cells with plump eosinophilic cytoplasm, distinct cell borders, and very conspicuous intercellular bridges. Nuclei are pleomorphic with prominent nucleoli and cells with giant nuclei and multiple nuclei may be present (Fig. 4.19), as well as cells showing cytoplasmic clearing [75]. Mitotic figures, however, are absent.

The epithelial tumor islands as well as the surrounding stroma frequently contain concentrically lamellated calcifications (Fig. 4.20). The stroma contains eosinophilic material that stains like amyloid (Fig. 4.21) and displays green-yellow birefringence under polarized light [53]; the

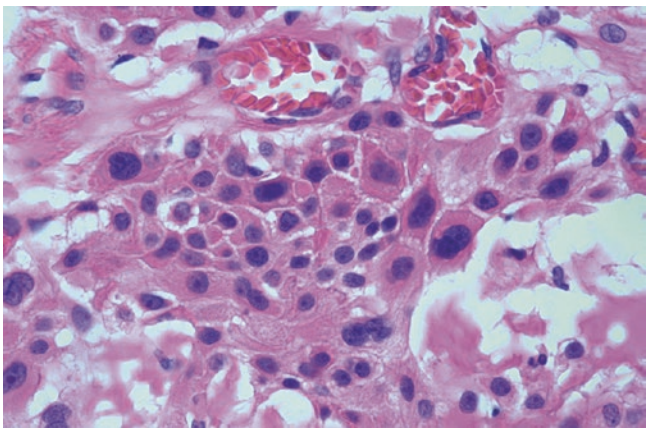

Fig. 4.19 Nuclear atypia, ample cytoplasm, and pronounced intercellular bridging are typical of a calcifying epithelial odontogenic tumor

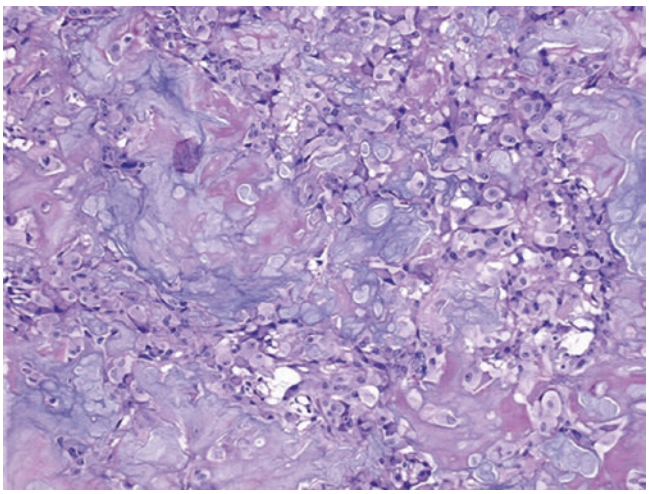

Fig. 4.20 Calcifying epithelial odontogenic tumor may show prominent intra-tumoral calcifications resembling osteoid or dentinoid. Cells with mummified nuclei (ghost cells) are also evident

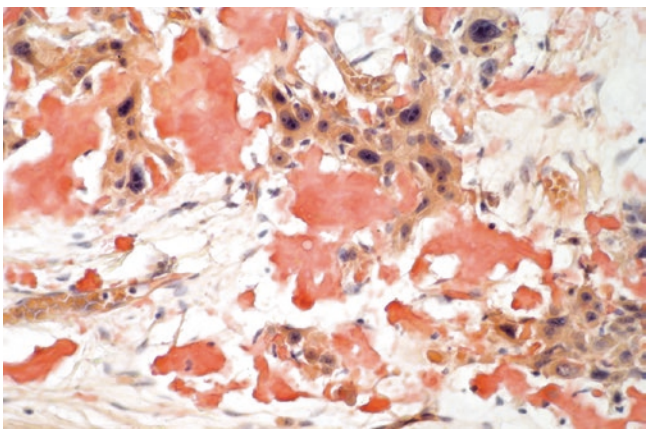

Fig. 4.21 Extracellular material staining for amyloid with Congo Red is another characteristic feature of a calcifying epithelial odontogenic tumor

presence of bone and cementum (Fig. 4.20) has also been reported [76]. The tumor usually is unencapsulated and grows into the cancellous spaces of the adjacent jawbone while causing expansion and thinning of the cortical bone.

Differential diagnosis Due to pronounced nuclear pleomorphism, the tumor may be mistaken for a high-grade malignant carcinoma, but the absence of mitotic figures should prevent this diagnostic error. Occasionally, the tumor may be confused with the so-called multinodular epithelioid osteoblastoma (see Sect. 4.4.5.1).

Treatment and prognosis Appropriate treatment is conservative surgery, consisting in tumor removal with a margin uninvolved tissue, while cases occurring in the gingival tissues can be treated by simple excision as they are less aggressive than the intraosseous ones [74]. Recurrences are occasionally seen, in particular with the clear cell variant [75].

Metastatic disease is only seen in cases that combine the appearance of calcifying epithelial odontogenic tumor with the presence of mitotic activity, suggesting malignant transformation [77]. Mitotic activity has also been seen in combination with perforation of cortical plates and blood vessel invasion, both features being highly unusual for calcifying epithelial odontogenic tumor [78]. Consequently, the detection of mitotic figures in calcifying epithelial odontogenic tumor apparently indicates malignancy.

4.3.1.3 Adenomatoid Odontogenic Tumor

Definition Adenomatoid odontogenic tumor is an unusual odontogenic lesion probably representing an odontogenic hamartoma rather than a neoplasm [53].

Epidemiology This lesion is mostly seen in people in their second decade, the anterior maxilla being the favored site, and the lesion often being associated with an impacted tooth [79].

Clinical aspects Adenomatoid odontogenic tumor usually presents as a swelling at the site of a missing tooth. Radiographically, the missing (impacted) tooth is surrounded by a radiolucent area that may contain multiple microcalcifications.

Macroscopy Grossly, adenomatoid odontogenic tumor is a cystic lesion embracing the crown of the involved tooth.

Microscopy It consists of two different cell populations: spindle shaped and columnar. The former cells form whorled nodules that may contain droplets of eosinophilic material. A lattice of thin epithelial strands may connect these nodules to each other. The columnar cells line duct-like spaces with a lumen either empty or containing eosinophilic material and may form curvilinear opposing rows with interposed eosinophilic material (Figs. 4.22 and 4.23). In the stroma, there are large aggregates of eosinophilic hyaline material, which is considered a dysplastic form of dentin, or cementum or a metaplastic stromal reaction [53, 79]. Also, concentrically laminated calcified bodies, similar to those seen in calcifying epithelial odontogenic tumor, may occur. In some adenomatoid odontogenic tumors, areas of eosinophilic cells with well-defined cell boundaries and prominent intercellular bridges similar to those observed in the calcifying epithelial odontogenic tumor may be seen [80]. They do not influence the biologic behavior of this tumor and are considered to be part of its histologic spectrum, as is the presence of melanin pigment [81, 82].

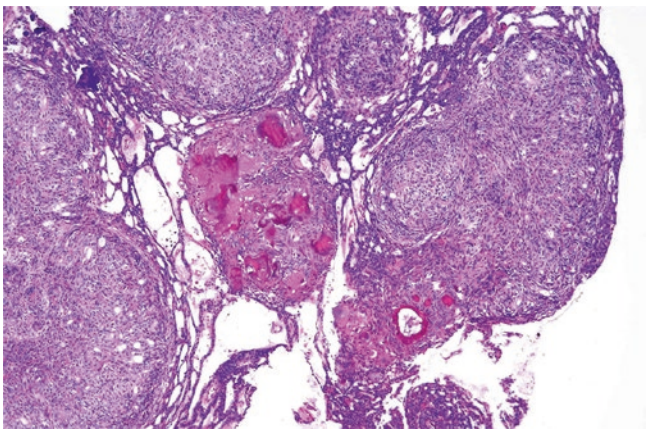

Fig. 4.22 Cellular nodules composed of spindle-shaped epithelial cells arranged in whorls, connected to each other by bilayered epithelial strands are characteristic for adenomatoid odontogenic tumor. Moreover, matrix deposition may occur

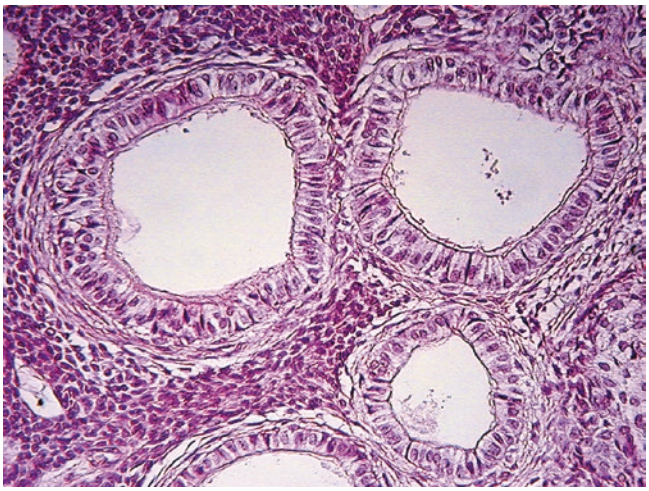

Fig. 4.23 Adenomatoid odontogenic tumor is composed by pseudoglandular structures, lined by columnar cells with nuclear polarization, lying in a highly cellular stroma. The lack of calcified (osteoid, dentin) mesenchymal tissues help to separate this lesion form adenomatoid odontogenic hamartoma

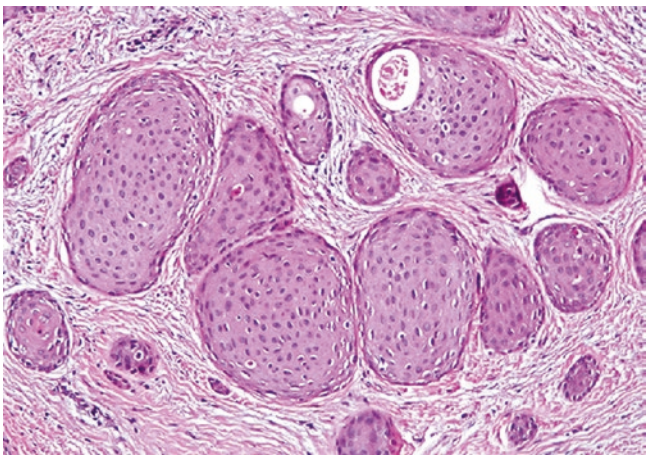

Fig. 4.24 Squamous odontogenic tumor is composed by round aggregates of squamous cells, without nuclear atypia or frank keratinization, dispersed in a collagenous stroma

Treatment and prognosis This tumor follows an indolent clinical course and the treatment consists in simple enucleation.

4.3.1.4 Squamous Odontogenic Tumor

Definition A very uncommon epithelial neoplasm showing squamous differentiation with little or no keratinization or cytonuclear atypia.

Epidemiology Squamous odontogenic tumor mainly involves the periodontal tissues, with no preference for either gender or jaw area and with occasionally a multicentric presentation [53, 83].

Clinical aspects The lesion may cause loosening of involved teeth and radiographically it appears as a radiolucent area.

Microscopy The lesion is composed of islands of well-differentiated squamous epithelium without cellular atypia surrounded by mature fibrous connective tissue (Fig. 4.24). The epithelial component displays spinous differentiation with well-defined intercellular bridges but keratinization is unusual. Cystic degeneration, as well as calcification, may occur in the epithelial islands, and invasion of the cancellous bone may be present.

Differential diagnosis The absence of cytonuclear atypia rules out well-differentiated squamous cell carcinoma, and the lack of peripheral palisading of columnar cells excludes ameloblastoma as an alternative diagnosis. Sometimes, intramural epithelial proliferation in jaw cysts (e.g., follicular cysts), possibly stimulated by inflammation (Fig. 4.25), may simulate squamous odontogenic tumor [9].

Treatment and prognosis Treatment consists of conservative removal of the tumor tissue. Occasionally, more extensive local spread may necessitate surgical excision with wider margins [84].

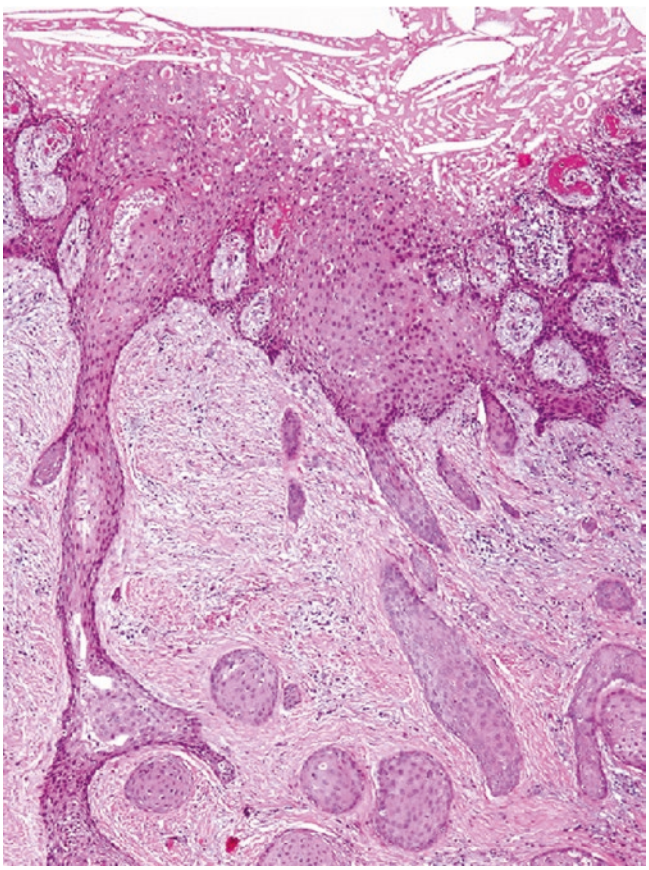

Fig. 4.25 Clusters of squamous cells, resembling those of squamous odontogenic tumor, are occasionally detectable in jaw cysts (e.g., dentigerous cyst in the present case) as a result of cellular response to inflammation

4.3.2 Odontogenic Tumors: Mesenchymal

Mesenchymal odontogenic tumors are derived from the ectomesenchymal part of the tissues that participate in development of teeth and periodontal tissues. Odontogenic epithelial rests may be part of the histologic picture they show but represent merely preexistent normal tissue components of the jaw without any diagnostic significance that are fortuitously engulfed by tumor. They have no neoplastic or inductive potential.

4.3.2.1 Odontogenic Myxoma

Definition A primary intraosseous lesion exclusive for the jawbones and composed of myxoid tissue.

Epidemiology Odontogenic myxoma is one of the more common odontogenic tumors [85, 86] that usually occurs in the second or third decade of life, although cases occurring at very young or old age also have been reported.

Clinical aspects Odontogenic myxomas occur in the maxilla as well as in the mandible and both in anterior and posterior parts. Swelling may be the presenting sign as well as disturbances in tooth eruption or changes in position of teeth already erupted. In maxillary cases, nasal stuffiness may be the presenting sign due to tumor growth in nasal and paranasal cavities. Radiographically, the lesion shows a unilocular or soap-bubble appearance.

Macroscopy It usually appears as a whitish translucent mass of gelatinous consistency.

Microscopy Myxomas consist of rather monotonous cells with multipolar or bipolar slender cytoplasmic extensions that lie in a myxoid stroma. Nuclei vary from round to fusiform in appearance, and binucleated cells and mitotic figures may be present but are scarce (Fig. 4.26). Occasionally, the lesion contains odontogenic epithelial rests; however, they are a fortuitous finding without any diagnostic or prognostic significance.

Immunohistochemistry Myxoma cells are positive for vimentin and muscle-specific actin, whereas positivity for S100 is controversial [87–89].

Differential diagnosis Myxoma may be mimicked by dental follicle and dental papilla as both may contain myxoid areas [14, 90–92]. Dental papilla tissue can be distinguished from myxoma by the presence of a peripheral layer of columnar odontoblasts. For both dental papilla and dental follicle, clinical and radiographic data are decisive in avoiding misinterpretation of myxomatous tissue in jaw specimens: in the first case, a tooth germ lies in the jaw area from which the submitted tissue has been taken whereas in the second case, the tissue sampled surrounded the crown part of an impacted tooth.

Treatment and prognosis As the lesion lacks encapsulation, treatment usually consists of excision with a margin of uninvolved tissue [93]. Incidentally, cases with an extremely aggressive local growth have been reported [94].

4.3.2.2 Odontogenic Fibroma

Definition Odontogenic fibroma is a relatively rare proliferation of fibrous tissue presumed to be derived from mesenchymal odontogenic tissues. Uncertainty still exists about the broadness of histologic spectrum these lesions may show and about its distinction from other fibrous jaw lesions [53].

Epidemiology Odontogenic fibroma has an age distribution of 9–80 years and occurs predominantly in females [95]: lesions are reported both within the jaws and in the gingiva [96].

Clinical aspects Central odontogenic fibromas may present as a local bony expansion of the involved jaw area. Quite often, they are incidental findings on radiographs performed for other diagnostic purposes: demarcated unilocular radiolucencies located adjacent to the roots of the neighboring teeth or surrounding impacted tooth. Peripheral odontogenic fibromas are firm–elastic gingival swellings.

Microscopy Odontogenic fibroma consists of fibroblasts lying in a background of myxoid material intermingled with collagen fibers that may vary from delicate to coarse. Odontogenic epithelium, either scarce or abundant, may occur (Fig. 4.27), but only rarely the epithelial component may be so conspicuous that its differentiation from ameloblastoma may be difficult [97]. This histologic spectrum may expand to include cell-rich myxoid areas, a greater epithelial component, and varying amounts of amorphous calcified globules or mineralized collagenous matrix. Tumors with

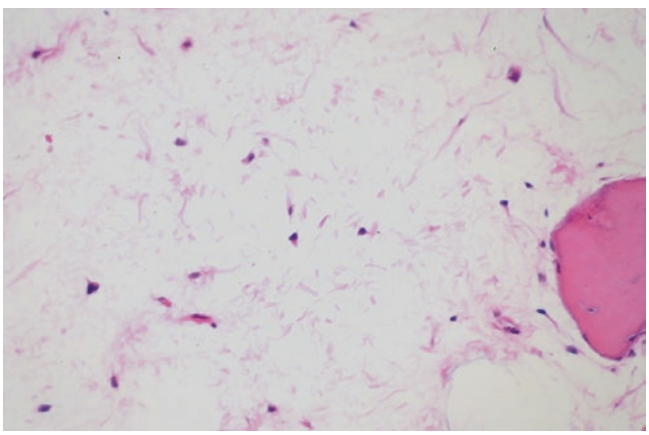

Fig. 4.26 Odontogenic myxoma is composed of poorly cellular myxoid material that surrounds preexistent jaw bone

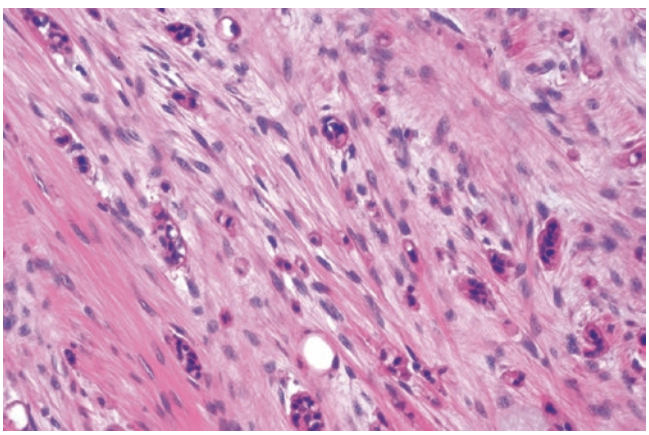

Fig. 4.27 Odontogenic fibroma consists of fibrous areas containing epithelial odontogenic nests

this more complex histology have been referred to as *complex odontogenic fibroma* or *WHO-type odontogenic fibroma* [53]. Odontogenic fibroma may also contain granular cells: such lesions have been called *granular cell odontogenic fibroma* or, alternatively, *granular cell ameloblastic fibroma* (see Sect. 4.3.3.3). This tumor, however, could also represent a unique entity: *central odontogenic granular cell tumor* [98, 99]. The granular cells are negative for epithelial markers and S100 protein, whereas positivity for CD68 suggests a possible histiocytic nature. Rarely, this tumor may show atypical histologic features including mitotic activity and aggressive behavior [100].

Also, lesions combining histologic features of giant cell granuloma and central odontogenic fibroma have been reported [101], and their aggressive nature suggests that the giant cell granuloma (see Sect. 4.4.4.1) component determines the clinical behavior.

Differential diagnosis When odontogenic fibromas show a preponderance of myxoid material, distinguishing them from odontogenic myxoma may become problematic. It is probably best to consider such cases to be myxomas and to treat them accordingly.

Another significant diagnostic problem is the distinction between odontogenic fibroma and desmoplastic fibroma (see Sect. 4.4.5.4), which is clinically very important as the former is benign whereas the latter shows aggressive behavior [102]. Lesions with features of both odontogenic fibroma and desmoplastic fibroma may occur in patients with tuberous sclerosis [103].

Gingival lesions assumed to be odontogenic fibroma pose their own diagnostic problems. In the past, authors have at this site discerned between peripheral odontogenic fibroma and *peripheral ossifying fibroma*, the latter being a gingival soft tissue lesion characterized by the presence of mineralized material of various appearances but, in contrast with the former, lacking odontogenic epithelium [104, 105]. This distinction however becomes less important when realizing that most cases of gingival lesions assumed to be either peripheral odontogenic fibroma or peripheral ossifying fibroma actually represent reactive gingival growths with metaplastic changes of varying nature that better are called fibrous epulis, a lesion discussed in Chap. 3 (see Sect. 3.7.1). For the sake of convenience, it is the best way to ignore any possibility of a gingival (peripheral) odontogenic fibroma as they can never reliably be distinguished from a fibrous epulis unless the lesion shows the more complex histology of the WHO-type odontogenic fibroma [96].

All histologic features shown by odontogenic fibroma also may be displayed by the dental follicle [90, 92, 106, 107]. In these cases, the radiographic appearance of the lesion, a small radiolucent rim surrounding the crown of a tooth buried within the jaw, will make the distinction.

Treatment and prognosis Treatment consists of enucleation. Peripheral cases, however, may recur after excision [96].

4.3.2.3 Cementoblastoma

Definition Cementoblastomas are heavily mineralized cementum masses connected to the apical root part of a tooth (Fig. 4.28) [53].

Epidemiology These tumors are most often seen in young adults with a predilection for males [108].

Clinical aspects Pain is the most common presenting symptom and the posterior jaw areas are the predilection site. Sometimes, the lesion is attached to multiple neighboring teeth [109]. Radiographically, the lesion is demarcated with a mixed lucent and dense appearance and is continuous with the partially resorbed root of a tooth.

Macroscopy It appears as a hard tissue mass very similar to osteoma.

Microscopy Cementoblastoma is composed by a vascular, loose-textured fibrous tissue that surrounds coarse trabeculae of basophilic mineralized material, bordered by plump cells with ample cytoplasm and large but not atypical nuclei.

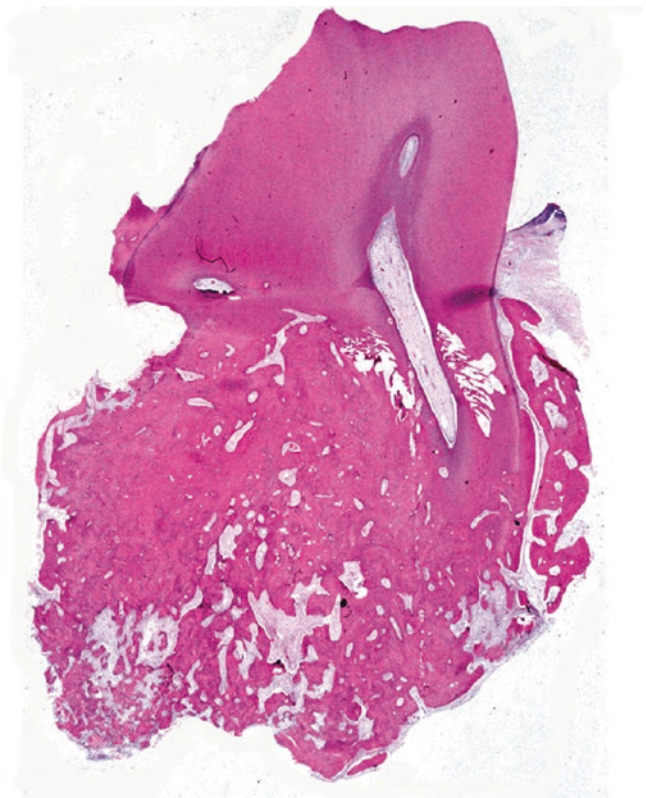

Fig. 4.28 A hard tissue mass firmly connected to the root surface of the tooth involved is diagnostic for cementoblastoma

Mitotic figures are rare. At the periphery, the mineralized material may form radiating spikes. Also, osteoclastic giant cells form part of the histologic spectrum. The hard tissue component is connected with the root of the involved tooth, which usually shows signs of external resorption. The sharp border between the tubular dentin of the root and the hard tissue component is the hallmark of cementoblastomas (Fig. 4.29).

Differential diagnosis All features of cementoblastoma may also be present in osteoblastoma, except the connection with the tooth root. Therefore, cases in which this connection cannot be demonstrated should be diagnosed as osteoblastoma and not cementoblastoma [109].

Treatment and prognosis As recurrence and continued growth are possible, treatment should consist of removal of the lesion along with the affected tooth or teeth and should also include some adjacent jaw bone [109].

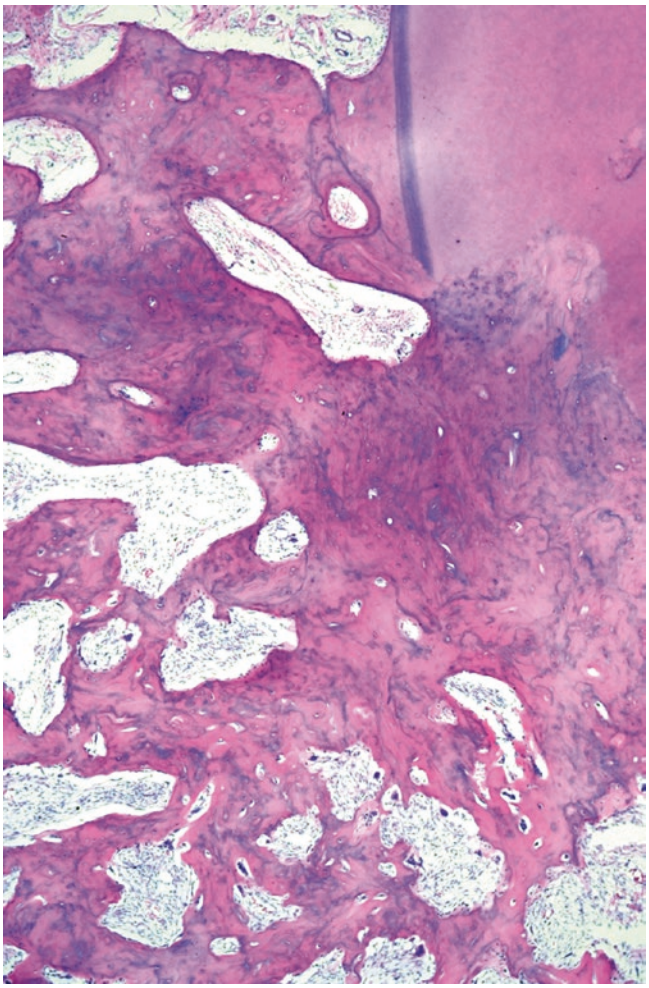

Fig. 4.29 Higher magnification of Fig. 4.28 shows the continuity between the root forming tubular dentin (*right side*) and the cemental masses of the cementoblastoma

4.3.3 Odontogenic Tumors: Mixed Epithelial and Mesenchymal

Mixed odontogenic tumors are composed of both epithelial-derived and mesenchymal-derived tissues. These tumors recapitulate proliferation and differentiation as seen in the developing teeth. Deposition of the dental hard tissues – enamel and dentin – may also occur [53, 110]. Lesions with an identical histology can show neoplastic as well as hamartomatous behavior [111, 112].

4.3.3.1 Adenomatoid Odontogenic Hamartoma

Definition An uncommon odontogenic lesion [113, 114], formerly known as *adenomatoid dentinoma*, closely related to adenomatoid odontogenic tumor and containing large amounts of mineralized tissues.

Epidemiology It mostly occurs in adolescents and young adults.

Clinical aspects This lesion affects the posterior (third molar area) mandible and appears as a well-defined, unilocular radiolucency with scattered intralesional calcifications.

Microscopy Adenomatoid odontogenic hamartoma is composed of an admixture of odontogenic hard (dentin, cementum, enamel) and soft (myxoid) tissues including epithelial structures in a gland-like (adenomatoid) arrangement (Fig. 4.30), thus resembling adenomatoid odontogenic tumor (see Sect. 4.3.1.3).

Treatment and prognosis The clinical course is indolent and conservative (curettage) treatment is usually curative.

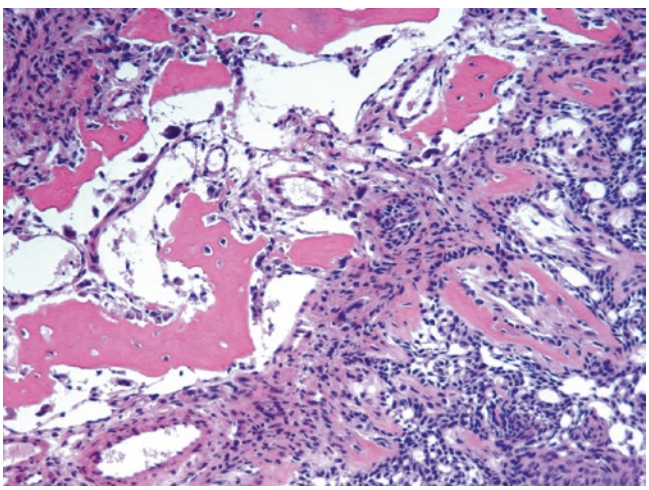

Fig. 4.30 Adenomatoid odontogenic hamartoma is composed by large amounts of mesenchymal tissues, including osteoid, dentin, and cellular collagenous stroma, and by clusters of epithelial cells forming pseudo-luminal structures, resembling those of adenomatoid odontogenic tumor

4.3.3.2 Calcifying Cystic Odontogenic Tumor/ Dentinogenic Ghost Cell Tumor

Definition A lesion showing an ameloblastoma-like epithelial component with ghost cells and a fibrous stroma including dentinoid material, formerly also known as calcifying odontogenic cyst, but in view of its neoplastic nature renamed in the current WHO classification of odontogenic tumors [53].

Epidemiology This lesion is most commonly found in the second and third decades of life [115–117]. It most commonly lies intraosseously but peripheral (gingival) cases have been reported as well.

Clinical aspects Lesions occur both in maxilla and in the mandible in equal proportions. They cause bony expansion when occurring in intraosseous location or gingival soft tissue swelling when peripherally located. Radiographically, central (intraosseous) lesions manifest a lucent appearance with variable amounts of radiopacities.

Microscopy In its most simple form, the lesion is a cavity with a fibrous wall and an epithelial lining. The latter closely mimics the one that is seen in unicystic ameloblastoma, but, in addition, there are intraepithelial eosinophilic ghost cells, in which nuclei are very faintly stained or not evident at all, and that may undergo calcification/mummification. Ghost cell masses also may herniate through the basal lamina to reach the underlying stroma, where they can act as foreign material and evoke a giant cell reaction (Fig. 4.31). Homogenous eosinophilic material, resembling dentin, may be found in varying amounts in the fibrous stroma adjacent to the basal epithelial cells, and dentinoid material and ghost cells together may form mixed aggregates. Sometimes, they may show some melanin pigment [118, 119].

Different subtypes of this lesion have been described [117], including the *proliferative calcifying odontogenic cyst*, showing multiple intramural daughter cysts with an epithelial lining similar to the main cyst cavity, and the *solid* (*neoplastic*) *calcifying odontogenic cyst* (differently named as: *dentinogenic ghost cell tumor*, *epithelial odontogenic ghost cell tumor*, *calcifying ghost cell odontogenic tumor*, and *cystic calcifying odontogenic tumor*) [120]. The most recent WHO classification [53] has attempted to create some clarity in this semantic abundance by proposing the diagnostic designations *calcifying cystic odontogenic tumor* and *dentinogenic ghost cell tumor* to discern between the cystic and the solid variants of lesions that combine an epithelial component resembling ameloblastoma with ghost cells and dentinoid. The former represents the classical calcifying odontogenic cyst as initially defined, the latter combines intraepithelial and stromal ghost cells and accumulations of dentin-like material with the typical morphological features of an ameloblastoma. Whether this proposal will withstand the test of time remains to be seen, and it has already been stated that ghost cell odontogenic tumors comprise a heterogeneous group of neoplasms requiring further study [121].

Differential diagnosis The distinction between a calcifying cystic odontogenic tumor and other odontogenic lesions sometimes is arbitrary as ghost cells may be displayed by several other odontogenic lesions. Occasionally, a lesion may even combine the features of a calcifying cystic odontogenic tumor with those of an ameloblastoma and an odontoma together which makes the distinction from odontoameloblastoma (see Sect. 4.3.3.7) difficult or even impossible (Fig. 4.32).

Finally, it should be noted that the morphology of a calcifying cystic odontogenic tumor is identical with craniopha-

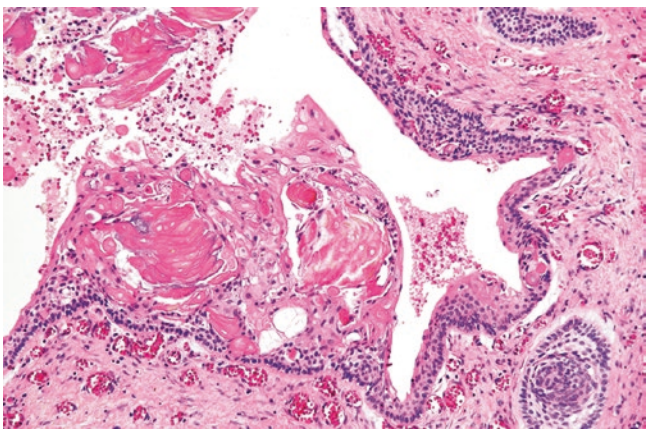

Fig. 4.31 Calcifying cystic odontogenic tumor closely resembles ameloblastoma but the presence of large intraepithelial aggregates of ghost cells rules out the latter diagnosis

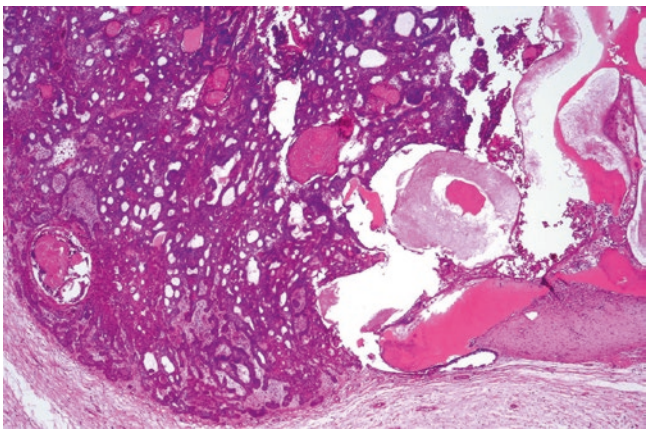

Fig. 4.32 Lesion showing features of ameloblastoma but also containing aggregates of ghost cells and dentin, enamel, and dental papilla tissue. Whether this lesion should be labeled as dentinogenic ghost cell tumor with associated immature odontoma or odontoameloblastoma is arbitrary

ryngioma that also is defined by ameloblastomatous epithelium with ghost cells [122]. This is interesting from a histogenetic viewpoint but does not pose any diagnostic problems in view of the different locations of these lesions, the one lying intraorally, the other intracranially.

Treatment and prognosis Treatment consists of enucleation for intraosseous cases or excision for the peripheral ones. Recurrences may occur [123].

4.3.3.3 Ameloblastic Fibroma

Definition Ameloblastic fibroma is a neoplasm lacking a hard tissue component, only displaying soft tissues similar to those found in the immature tooth germ. Recently, it has been proposed that there are two variants of this lesion, lesions in patients aged above 22 years probably being true neoplasms, while those in younger patients may be either true neoplasms or odontomas in early stages of development [124].

Epidemiology It is one of the less common odontogenic tumors [125]; the mean age of occurrence is 14.8 years but cases may be seen from as young as only 7 weeks up to as old as 62 years [111, 126].

Clinical aspects Ameloblastic fibromas present as a painless swelling of the posterior mandible or are discovered due to disturbances in tooth eruption. Radiographically, the tumor appears as a well-demarcated expansile radiolucency, often in connection with a malpositioned tooth.

Microscopy The epithelial part of ameloblastic fibroma consists of branching and anastomosing epithelial strands that form knots of varying size. These knots have a peripheral rim of columnar cells that embraces a loosely arranged spindle-shaped epithelium. The epithelial strands lie in a immature, myxoid cell-rich, mesenchyme. The amount of epithelium may vary among different cases and regionally within an individual case. There is no formation of dental hard tissues and mitotic figures, either in epithelium or mesenchyme, are extremely rare; when easily found, they should rise concern about the benign nature of the case.

Ameloblastic fibroma may contain granular cells: whether these lesions should be called *granular cell ameloblastic fibroma* or *granular cell odontogenic fibroma* is still controversial (see also Sect. 4.3.2.2) [99].

Another issue to be discussed in this context is the recent description of a lesion for which the term *primordial odontogenic tumor* is proposed. This lesion also combines immature mesenchyme and columnar epithelium but in an arrangement that differs from ameloblastic fibroma, the epithelium forming an outer covering layer [127]. Whether this morphology indeed qualifies for a novel entity awaits reports of more cases.

Differential diagnosis Ameloblastoma and ameloblastic fibroma share similar epithelial components but, at variance with ameloblastic fibroma, in which immature, embryonic, cell-rich myxoid stroma is found, the stromal component of ameloblastoma consists of mature fibrous connective tissue. Areas similar to ameloblastic fibroma may also be observed in the hyperplastic dental follicle [14, 90, 92]. In such cases, the radiographic appearance makes the distinction: a radiolucent rim surrounding an unerupted tooth in the case of a dental follicle and an expansive radiolucent jaw lesion in the case of an ameloblastic fibroma.

Treatment and prognosis Treatment consists of enucleation and curettage, but in some cases recurrence may occur [124]. Sometimes, ameloblastic fibroma may progress to malignancy: such lesions are characterized by increased cellularity, nuclear pleomorphism, and mitotic activity of the mesenchymal component and are known as ameloblastic fibrosarcoma (see Sect. 4.3.4.7).

4.3.3.4 Ameloblastic Fibro-Odontoma

Definition Ameloblastic fibro-odontomas are lesions that combine a soft tissue component, similar to ameloblastic fibroma, with the presence of dentin and enamel. In rare cases, only dentin is present and these tumors are called *ameloblastic fibro-dentinoma* [53].

Epidemiology Ameloblastic fibro-odontomas are rare [125] and occur primarily within the first two decades,

Clinical aspects Such tumors preferentially affect the posterior jaw areas, the mandible being more commonly involved than the maxilla [111, 112]. Most cases of ameloblastic fibro-odontoma present as painless swelling or are discovered due to disturbances in tooth eruption. Radiographically, the tumor presents as a well-demarcated expansive radiolucency with a radiopaque center.

Microscopy The tumor consists of a tissue component being identical to ameloblastic fibroma to which dental hard tissues – dentin and enamel – are added. Dentin may be formed either as eosinophilic mineralized material containing tubuli, just as in normal teeth, but it may also appear as a homogeneous eosinophilic mass with sparse-included mesenchymal cells or epithelial remnants. It always lies in close association with adjacent epithelium and forms the scaffold for the deposition of the more purple enamel matrix that is laid down at the epithelial–dentin interface by columnar epithelial cells that have reached their terminal differentiation as ameloblasts. The dental hard tissues are arranged haphazardly without any reminiscence to the orderly structure characterizing normal teeth (Fig. 4.33).

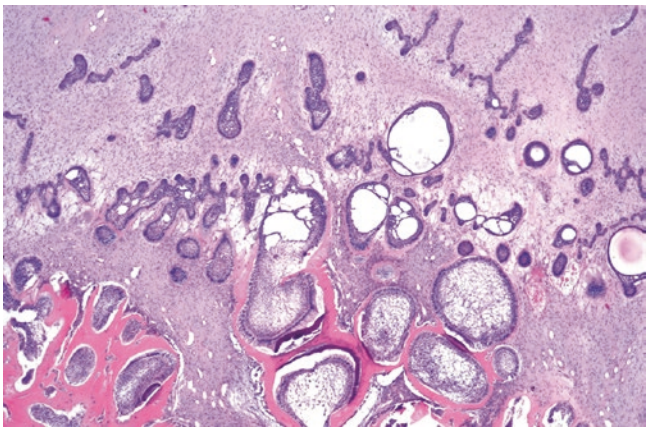

Fig. 4.33 Ameloblastic fibro-odontoma combines the soft tissue elements of an ameloblastic fibroma with the deposition of dental hard tissues, such as enamel and dentin. Cavities in the homogeneous eosinophilic dentin contain high-columnar ameloblasts lying down enamel matrix (*deep purple*)

Differential diagnosis Hyperplastic dental follicles may show focal areas with the appearance of ameloblastic fibro-odontoma, but they appear as a radiolucent rim surrounding an unerupted tooth. Additional differential diagnostic considerations are the same as those already mentioned (see Sect. 4.3.3.3): ameloblastic fibro-odontomas can be distinguished from ameloblastoma by the presence of cellular myxoid tissue, dentin, and enamel.

Treatment and prognosis Treatment consists of enucleation and curettage. Recurrence is rarely seen [128].

4.3.3.5 Odontoma: Complex Type

Definition Complex odontoma, one of the more common odontogenic lesions [125], is a hamartomatous lesion composed of a haphazard conglomerate of dental hard tissues.

Epidemiology In view of its asymptomatic clinical course, complex odontomas may remain occult for long period of times and the wide age distribution of this lesion (2–74 years) mostly reflects the time when the lesion was found rather than the age at which it formed [112].

Clinical aspects Complex odontomas may reveal their existence by disturbances in tooth eruption, missing teeth, or jaw expansion, preferentially in the posterior mandible (Fig. 4.34). Quite often, they are incidental findings on radiographs taken for other purposes. In such cases, an amorphous calcified mass is seen that may be connected with the crown of an unerupted tooth.

Microscopy Complex odontomas consist of a usually well-delineated mass of dental hard tissues in a haphazard arrangement. The bulk of the lesion consists of dentin recognizable by the presence of tubuli. Enamel plays a minor role, usually confined to small rims in cavities in the dentin mass. The stroma consists of mature fibrous connective tissue.

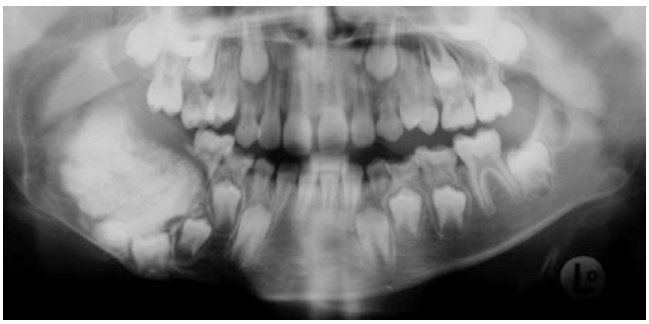

Fig. 4.34 Panoramic radiograph showing radiodense mass in connection with a tooth germ, a picture typical for complex odontoma

Differential diagnosis Odontomas may contain areas identical to the calcifying cystic odontogenic tumor including ghost cells [129–131]. Odontoma-like structures may also occur in the hyperplastic dental follicle.

Treatment and prognosis Treatment consists of conservative removal and recurrences are not seen.

4.3.3.6 Odontoma: Compound Type

Definition Compound odontoma is a malformation consisting of tiny teeth that may vary in number from only a few to numerous. These teeth do not resemble any of the normal teeth but usually are cone shaped.

Epidemiology Compound odontoma is one of the more frequently encountered odontogenic lesions [125]. Data on age of occurrence show the same wide range as with complex odontoma: 0.5–73 years of age which is due to the fact that compound odontomas may also remain unnoticed for a long time.

Clinical aspects In contrast with almost all other odontogenic lesions that have the posterior mandible as preferred site, compound odontomas have a definite predilection for the anterior maxilla [111, 112]. They may cause swelling or disturbed tooth eruption. Radiographically, a radiolucency containing multiple toothlike radiopaque structures is seen.

Microscopy Histologically, they show the normal arrangement of a centrally placed fibrovascular pulp tissue surrounded by dentin and with an outer surface covered by enamel in the crown area and cementum in the root part.

Differential diagnosis Compound odontomas may contain areas similar to a calcifying cystic odontogenic tumor including ghost cells (see Sect. 4.3.3.2) [129–131].

Treatment and prognosis Treatment consists of enucleation and there is no recurrence.

4.3.3.7 Odontoameloblastoma

Definition Odontoameloblastoma is a very rare neoplasm that combines the features of ameloblastoma and odontoma, including the presence of enamel and dentin [53]. Its status as an entity is debatable; ameloblastoma developing in an odontoma, ameloblastic fibro-odontoma with a prominent epithelial component, and dentinogenic ghost cell tumor with associated odontoma could be more appropriate diagnostic labels for these rare tumors.

Clinical aspects Radiographically, the soap-bubble appearance of ameloblastoma is combined with radiopaque masses due to the odontoma component [132].

Treatment and prognosis The ameloblastoma component (see Sect. 4.3.1.1) determines clinical presentation and behavior and the treatment should be planned accordingly.

4.3.4 Odontogenic Tumors: Malignant

Both odontogenic epithelium and odontogenic mesenchyme may show malignant neoplastic degeneration, causing either odontogenic carcinomas or odontogenic sarcomas [53, 133]. All entities to be mentioned show the clinical presentation and course as well as the radiographic appearance of an intraosseous malignant tumor.

4.3.4.1 Malignant Ameloblastoma

Definition Malignant (metastasizing) ameloblastoma is an ameloblastoma that metastasizes in spite of an innocuous histologic appearance (as reported in Sect. 4.3.1.1).

Microscopy The primary tumor does not show any specific features different from conventional ameloblastomas that do not metastasize. Therefore, this diagnosis can only be made retrospectively, after the occurrence of metastatic deposits that most commonly occur in the lung, but occasionally are seen elsewhere (Fig. 4.35) [134–136]. It is thus the clinical behavior and not the histology that justifies a diagnosis of malignant ameloblastoma [53, 137]. This definition profoundly differs from that mentioned earlier, when malignant ameloblastoma was reported as a neoplasm showing the pattern of conventional ameloblastoma combined with cytological features of malignancy, a definition based on morphological features and not on clinical behavior [5]. To avoid possible misinterpretations [133, 136], the term malignant ameloblastoma should be restricted to metastasizing ameloblastomas, while an ameloblastoma showing aggressive morphological features (i.e., cytologic atypia and prominent mitotic activity) should be called ameloblastic carcinoma (see Sect. 4.3.4.2) [138]. For the same reasons, the indiscriminate use of designations such as atypical ameloblastoma to denote lesions with fatal outcome due to either metastasis, histologic atypia, or relentless local spread [139] is strongly discouraged.

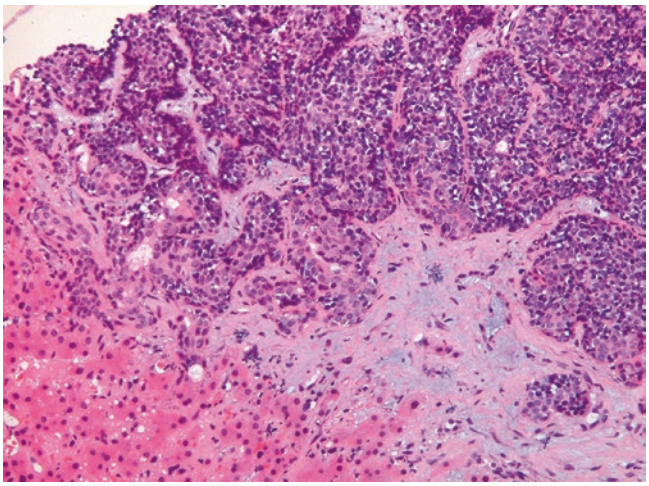

Fig. 4.35 Ameloblastoma metastatic to liver, thus in retrospect qualifying as malignant ameloblastoma

4.3.4.2 Ameloblastic Carcinoma

Definition Ameloblastic carcinoma shows histologic features of both ameloblastoma and squamous cell carcinoma [53, 140]; the tumor may arise de novo or from a preexisting benign odontogenic tumor or cyst [133].

Epidemiology Ameloblastic carcinoma shows a wide age range, with a mean age of 49.2 years, and affects men more frequently than females [141].

Clinical aspects Most cases occur in the mandible as asymptomatic swellings, and, radiographically, perforation of the cortical bone plate and extension into the adjacent soft tissues are not uncommon and may lead to the suspicion of a malignant lesion.

Microscopy Ameloblastic carcinoma is characterized by the architectural pattern of ameloblastoma but composed of cells that, though showing columnar morphology and peripheral palisading, also exhibit pronounced cytological atypia and mitotic activity, thus allowing the separation of ameloblastic carcinoma from ameloblastoma (Figs. 4.36 and 4.37).

Treatment and prognosis Complete surgical resection is advised. Metastatic deposits may appear after a variable timespan (few months to several years), most frequently in

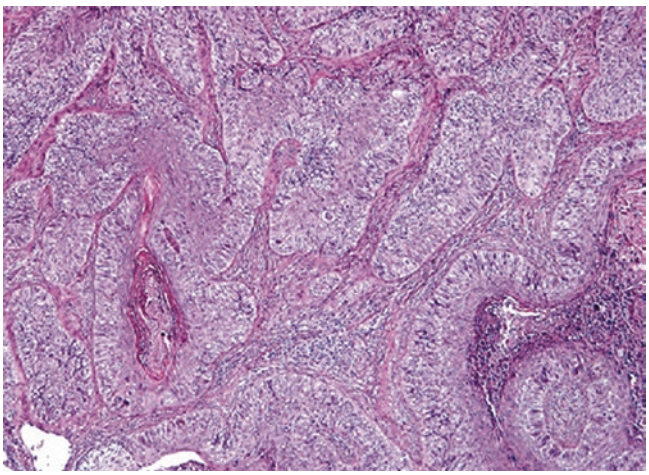

Fig. 4.36 Ameloblastic carcinoma contains large and hypercellular aggregates of tumor cells, vaguely resembling those of a follicular ameloblastoma

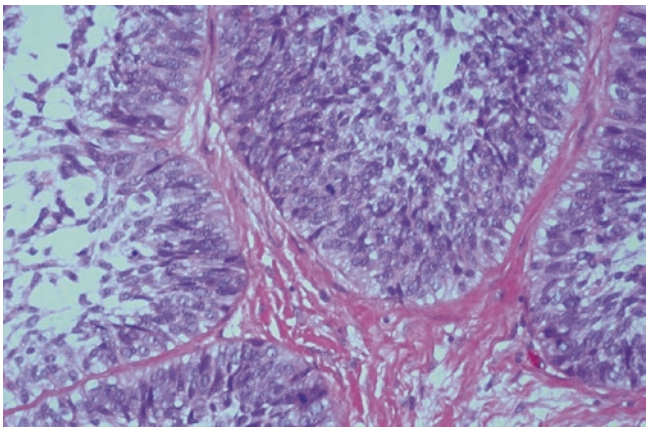

Fig. 4.37 At higher magnification, ameloblastic carcinoma combines the presence of epithelial nests with peripheral palisading and cytonuclear atypia

the lungs and lymph nodes [141–143]. The 5- and 10-year survival rates are reported to be 72.9 % and 56.8 %, respectively, whereas in case of metastasis a 5-year survival rate of only 21.4 % was obtained [141].

4.3.4.3 Primary Intraosseous Carcinoma

Definition Primary intraosseous carcinoma is a squamous cell carcinoma arising within the jaws, having no initial connection with the oral mucosa, and presumably developing from residues of the odontogenic epithelium [53].

Epidemiology This tumor is more common in adult males (mean age: 52 years) and mainly occurs in the posterior mandible [144].

Clinical aspects Swelling of the jaw and pain most often are the presenting signs, and the tumor appears as a radiolucent lesion, whereas aggressive behavior, such as infiltrative borders or bone perforation, usually is restricted to cases also showing rapid growth.

Microscopy The tumor is composed by atypical squamous cells, ranging from well to poorly differentiated [136, 140], which do not display the morphological characteristics of the ameloblastic epithelium (e.g., columnar shape, reverse polarity, stellate reticulum). Attention should be paid to the exclusion of a possible metastatic origin from the skin, the antral/nasal mucosa, or distant organs.

This tumor may also arise from still recognizable precursor lesions, such as the epithelial lining of an odontogenic cyst [145, 146], or from the enamel epithelium [147].

Treatment and prognosis Complete resection and postoperative radiotherapy seem to provide the best results. In view of frequent metastases to regional lymph nodes and lungs, the prognosis is poor with almost 50 % of the patients failing locoregionally within the first 2 years of follow-up [144].

4.3.4.4 Clear Cell Odontogenic Carcinoma

Definition A primary intraosseous carcinoma not showing prominent squamous differentiation and containing a distinct neoplastic clear cell population.

Clear cell odontogenic carcinoma was initially reported as clear cell odontogenic tumor [148], the latter designation having been disregarded as locally aggressive behavior, and distant metastases may characterize the clinical course of this lesion [149].

Epidemiology The tumor is mostly seen in the fifth to eighth decades with a striking predilection for the female gender.

Clinical aspects Progressive jaw expansion with teeth loosening is the most frequent presentation, the anterior mandible being the preferred localization [150–152]. Radiologically, it appears as an ill-defined radiolucency.

Microscopy The tumor is most frequently composed of cells with clear cytoplasm, hyperchromatic nuclei, and well-defined borders, arranged in strands or large nests [150]. These cells coexist with variable proportions of smaller polygonal cells with eosinophilic cytoplasm and dark nuclei (Figs. 4.38 and 4.39). In rarer instances, this tumor may be exclusively composed of a clear cell population, and, occasionally, focal squamous cell differentiation has also been reported [151]. The possible arrangement of the cells at the periphery of the nests in a palisading pattern raised the possibility that this tumor entity may somehow be related to ameloblastoma with clear cells.

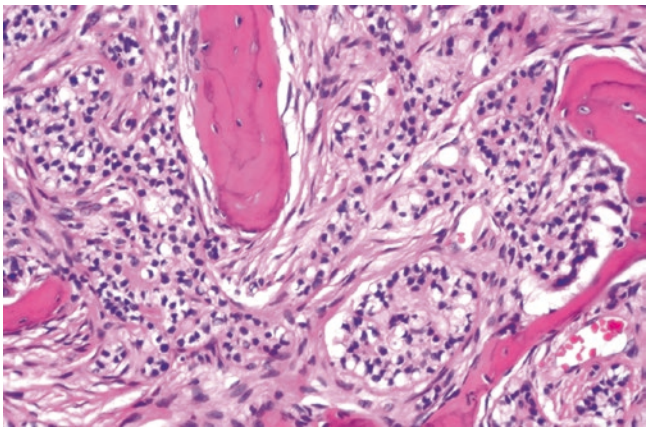

Fig. 4.38 Clear cell odontogenic carcinoma is characterized by clear cells forming epithelial nests

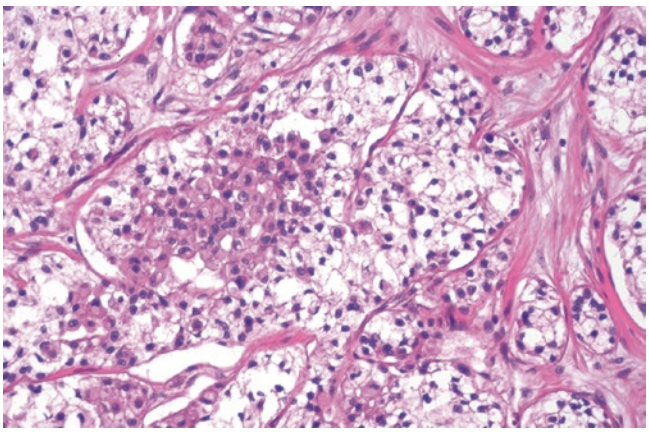

Fig. 4.39 At higher magnification, clear cell odontogenic carcinoma contains clear cells as well as eosinophilic cells

Special stains and immunohistochemistry The clear cells stain variably positive for glycogen with PAS and are diastase sensitive but negative with mucin stains, and they show distinct immunoreactivity for epithelial markers such as keratin AE1/AE3, cytokeratins 8–18, cytokeratin 19, and epithelial membrane antigen [152].

Differential diagnosis This includes metastatic renal cell carcinoma, the clear cell variant of mucoepidermoid carcinoma, and ameloblastoma with clear cells. Metastatic renal cell carcinoma mainly can be ruled out on clinical grounds, but the typical CD10 immunoreactivity of this tumor may also contribute in distinguishing between the differential diagnostic possibilities. The clear cell variant of mucoepidermoid carcinoma can be properly identified with stains for mucin production (e.g., mucicarmin stain) but differentiation from ameloblastoma with clear cells may be problematic, and it has been proposed that these lesions may be histogenetically related, as previously mentioned [153]. However, lack of calretinin immunoreactivity may help to rule out ameloblastoma. Also, clear cell carcinoma of minor salivary gland origin should be considered in the differential diagnosis (see Chap. 5), but whether this is a distinct lesion is questionable as recent data suggest that clear cell odontogenic carcinoma and salivary clear cell carcinoma may be linked biologically [154].

Treatment and prognosis Wide surgical resection is advised as recurrent disease occurs in more than 50 % of cases with prolonged follow-up, metastases are found in lymph nodes as well as in the lungs, and the skeleton and tumor-related death also has been reported [150, 152].

4.3.4.5 Malignant Epithelial Odontogenic Ghost Cell Tumor (Ghost Cell Odontogenic Carcinoma)

Definition Malignant epithelial odontogenic ghost cell tumor, also called *ghost cell odontogenic carcinoma* according to the latest WHO classification [53], is a tumor that combines the elements of a benign calcifying cystic odontogenic tumor with a malignant epithelial component.

Etiology and pathogenesis This tumor apparently arises (one-third of reported cases) from malignant transformation of a preexisting benign calcifying cystic odontogenic tumor [118].

Epidemiology It may occur at any age but most cases have been reported in adults (peak in the fourth decade); males are affected more often (2:1) than females.

Clinical aspects Only a few cases of this tumor have been reported so far, thus precluding any definitive conclusions regarding its clinicopathologic features. Nevertheless, most affected patients complained of progressive jaw expansion, the maxilla being more frequently affected than the mandible. Rapid growth and extension into the adjacent tissue have also been reported. Radiologically, it appears as a radiolucent lesion with indistinct borders sometimes displaying multilocularity and tooth root displacement or resorption.

Microscopy This tumor is composed of strands of odontogenic epithelium, sometimes showing focal ameloblastoma-like features (e.g., peripheral palisading) admixed with variable proportions of mummified cells with indistinct cell borders and "ghost" nuclei. Also, eosinophilic calcified (dentinoid) amorphous material can be present in variable amounts. Mitotic figures are frequent (Fig. 4.40).

Treatment and prognosis Usually, the tumor invades adjacent tissues, and malignancy is demonstrated by locally aggressive growth, while distant metastases have been occasionally recorded [155]. Consequently, wide surgical excision is recommended with prolonged follow-up.

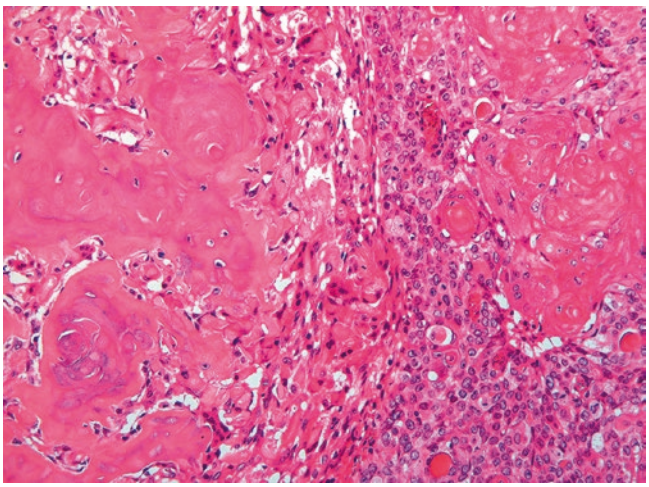

Fig. 4.40 Ghost cell odontogenic carcinoma characterized by the presence of atypical ameloblastomatous epithelium together with dysplastic dentin and ghost cell aggregates

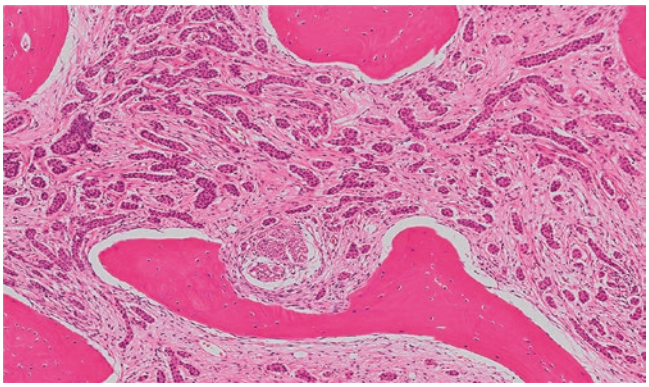

Fig. 4.41 Epithelial strands of bland epithelium lying in a fibrous background characterize sclerosing odontogenic carcinoma

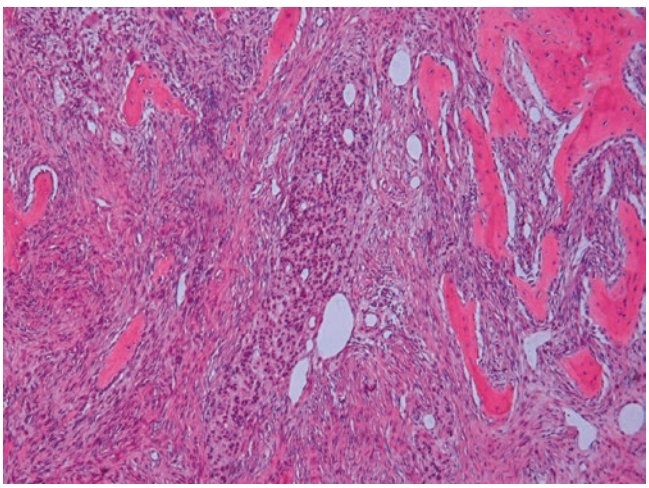

Fig. 4.42 Sclerosing odontogenic carcinoma with fibrous dysplasia-like bony component. The epithelial areas lie in a fibrous background that also contains trabeculae of woven bone without any osteoblastic rimming

4.3.4.6 Sclerosing Odontogenic Carcinoma

Definition Sclerosing odontogenic carcinoma, first described in 2008 [156], is an exceedingly rare tumor, characterized by a dense sclerotic collagenous stroma that may obscure the neoplastic epithelial component.

Epidemiology Sclerosing odontogenic carcinoma presents in adults with a slight prevalence for the male gender.

Clinical aspects In the few cases reported thus far, the mandible was more frequently affected than the maxilla, and the tumor appeared as an ill-defined expansile mass with possible perforation of the cortical plate and extension into the adjacent soft tissues. Radiologically, it appears radiolucent and is associated with tooth displacement and root resorption [157].

Microscopy Sclerosing odontogenic carcinoma is composed of small aggregates or thin cords of polygonal epithelial cells with eosinophilic cytoplasm occasionally showing some cytoplasmic clearing and embedded in a sclerotic collagenous stroma. The tumor cells show nuclear hyperchromatism and some pleomorphism but no conspicuous mitotic activity; perineural invasion and infiltration of the skeletal muscles has also been reported (Fig. 4.41).

Recently, sclerosing odontogenic carcinoma in association with fibro-osseous lesions (osseous dysplasia, fibrous dysplasia, and ossifying fibroma) of the jaws has been reported [158], but evidences are too few to exclude that this was just an incidental finding (Fig. 4.42).

Immunohistochemistry The tumor cells are consistently immunoreactive for epithelial markers (cytokeratins AE1/AE3, 5/6, and 19) and for e-cadherin. Sometimes, due to extreme stromal sclerosis, immunohistochemistry will reveal a much larger epithelial component than anticipated when only examining the hematoxylin and eosin-stained sections.

Differential diagnosis Conventional clear cell odontogenic carcinoma, calcifying epithelial odontogenic tumor, and primary intraosseous carcinoma should be considered in the differential diagnosis. The former usually shows larger clusters of clear tumor cells admixed with variable proportions of cells with eosinophilic cytoplasm while the neoplastic cells of calcifying epithelial odontogenic tumor usually show distinct cell borders and are embedded in a fibrous stroma containing amyloid. Also, a variable number of intralesional calcifications usually are detectable in calcifying epithelial odontogenic tumor. Primary intraosseous carcinoma usually shows prominent squamous cell differentiation that is undetectable in sclerosing odontogenic carcinoma.

Although sclerosing odontogenic carcinoma displays some morphological diversity from clear cell odontogenic carcinoma (e.g., smaller aggregates of tumor cells, prominent

sclerosing stroma), a complete separation of these neoplasms may not be justified [138, 159], but, in consideration of the more attenuated clinical behavior of the former, it may be advisable to keep them separate until more evidence will accumulate.

Treatment and prognosis Its malignant behavior is mostly proven by lack of peripheral demarcation, and infiltration into adjacent tissue and perineural spaces that may be responsible for frequent recurrences. Nevertheless, distant metastases have not been recorded so far. Consequently, segmental resection is currently considered curative.

4.3.4.7 Odontogenic Sarcomas

Definition A malignant odontogenic neoplasm, containing a malignant mesenchymal component, admixed with an epithelial component that may show either benign or malignant features.

Epidemiology These very rare tumors may occur at any age with a preference for young adulthood (third decade) and a slight preference for the male gender.

Clinical aspects Jawbone swelling is the most frequent presenting sign, and they appear as radiolucent lesions, occasionally containing multiple radiopaque masses.

Microscopy The WHO discerns between ameloblastic fibrosarcoma, ameloblastic fibrodentinosarcoma, and ameloblastic fibro-odontosarcoma [53]. A*meloblastic fibrosarcoma* consists of malignant mesenchymal (fibrosarcoma-like) cells, showing nuclear pleomorphism and mitotic activity, lying in a fibromyxoid stroma admixed with epithelial islands similar to those present in ameloblastoma or ameloblastic fibroma [160]. When dentin is also present, the lesion is designated *ameloblastic fibrodentinosarcoma*, and with the addition of enamel, it becomes an *ameloblastic fibro-odontosarcoma*. However, this subclassification has no prognostic relevance [161]. These tumors may arise de novo or from a preexisting ameloblastic fibroma or ameloblastic fibro-odontoma [162].

Those extremely rare lesions that combine carcinomatous and sarcomatous elements, recognizable as odontogenic in view of the presence of an epithelial component resembling ameloblastic carcinoma, should be considered to represent cases of *odontogenic carcinosarcoma* or *odontogenic carcinoma with sarcomatous proliferation* (Figs. 4.43 and 4.44).

Treatment and prognosis Odontogenic sarcomas are considered low-grade neoplasms, with a high propensity to local dissemination but only limited metastatic potential. The treatment of choice is surgical resection.

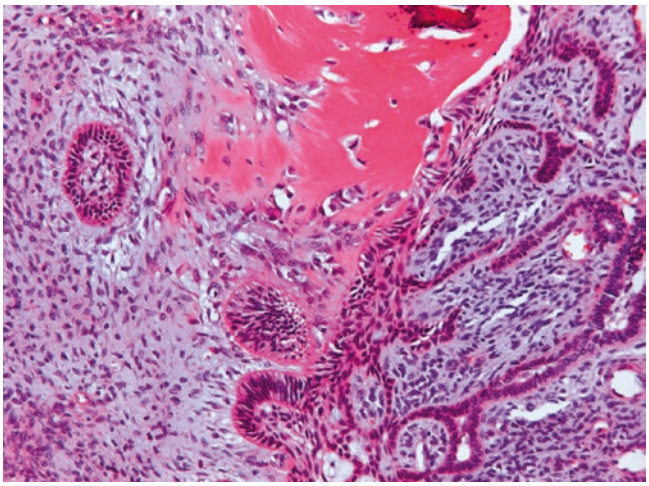

Fig. 4.43 Odontogenic carcinosarcoma. At the interface of atypical mesenchymal tissue and atypical ameloblastomatous epithelium, dysplastic dentin is formed. See Fig. 4.44 for detail

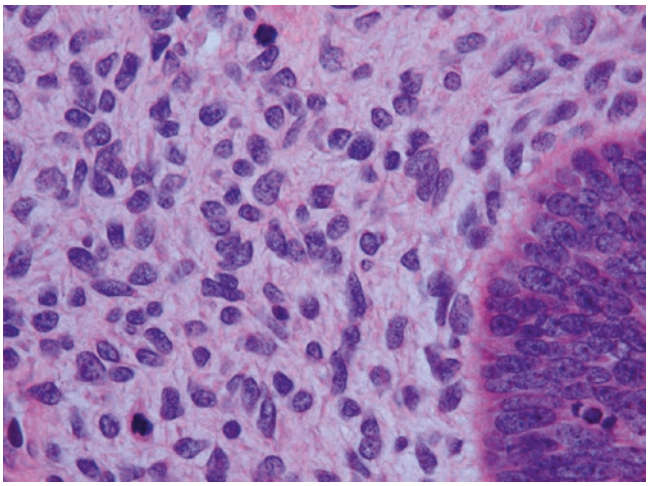

Fig. 4.44 Detail from Fig. 4.43. Picture from odontogenic carcinosarcoma showing mitotic activity in both mesenchymal and epithelial components

4.4 Bone Lesions: Non-odontogenic

4.4.1 Introduction

This section includes discussion of bone lesions that have the maxillofacial bones as site of predilection or are even confined to this location whereas bone lesions that also may occur in other parts of the skeleton are included for differential diagnostic considerations. Fibro-osseous lesions and giant cell lesions belong to the first group. Within the second group, osteoblastoma, osteosarcoma, and lesions producing cartilage, as well as other skeletal lesions are presented.

4.4.2 Benign Lesions: Reactive and Inflammatory

4.4.2.1 Osteoma and Tori

Definition Osteomas are exophytic bony lesions usually located at the outer surface of one of the maxillofacial bones.

Epidemiology In the maxillofacial skeleton, osteomas most commonly occur in the frontal and ethmoid sinus; less often, the maxillary antrum and the sphenoid sinus are involved [163]. They may also occur in the jaw bones either sporadic or as manifestation of Gardner syndrome [164].

Clinical aspects Paranasal osteomas as group are common lesions [165]. Clinically, they cause sinusitis and headache or other signs of sinonasal disease. Similar bony outgrowths at the palate or mandible are called *tori*. Radiologically, they have a radiodense appearance.

Microscopy Lesions are composed of dense lamellar bone with sparse marrow cavities filled with fatty or fibrous tissue. Sometimes, central areas may show more loose and vascular connective tissue containing slender trabeculae of woven bone lined with osteoblasts.

Central jaw osteomas pose a diagnostic problem as they need to be differentiated from other similar lesions of the jaws, such as central ossifying fibroma, localized chronic sclerosing osteomyelitis, osseous dysplasia, osteoblastoma, cementoblastoma, and complex odontoma [166].

4.4.2.2 Osteomyelitis

Definition Inflammatory disorder of the bone leading to bone necrosis and sclerosis. When seen after irradiation, e.g., for head and neck cancer, it is called osteoradionecrosis.

Epidemiology Osteomyelitis mainly involves the mandible. Although it may be seen at any age, children are rarely affected.

Etiology Osteomyelitis may occur through extension of infection of the dental pulp or as a complication after tooth extraction. In case of osteoradionecrosis, the poor vascularization of the jaw bone due to irradiation causes increased vulnerability to infection. Also, bisphosphonates, a class of drugs that prevent the loss of bone mass and therefore are used to treat osteoporosis and related disorders such as Paget's disease, bone metastasis, multiple myeloma, and other conditions that feature bone fragility, have been associated with the development of necrosis and subsequent infection of the jaw bone [167].

Clinical aspects There are five types: *acute suppurative osteomyelitis*, *chronic suppurative osteomyelitis*, *chronic focal sclerosing osteomyelitis*, *chronic diffuse sclerosing osteomyelitis*, *and proliferative periostitis*. Radiographs show an ill-defined mixed radiodense and radiolucent lesion. In case of acute osteomyelitis, pain and fever occur, and intraoral examination may reveal sinuses or dead bone sequestered through mucosal defects. In chronic osteomyelitis, slight discomfort may be the sole symptom.

Microscopy Acute suppurative osteomyelitis shows bone marrow cavities infiltrated with neutrophils. The bony trabeculae are necrotic and may be lined with multinucleated osteoclasts. Usually, this form of osteomyelitis evolves into chronic suppurative osteomyelitis which also may arise de novo. Besides bone sequesters surrounded by numerous neutrophilic granulocytes, also granulation tissue is present. Sinuses are formed partly lined by squamous epithelium from the oral mucosa. In less severe cases, fibrosis and development of a chronic inflammatory infiltrate may also be seen. When the inflammation is mild, the jaw bone responds by bone formation. This form of osteomyelitis is known as chronic sclerosing which may be focal as well as diffuse. Dense sclerotic bone masses are seen together with a bone marrow exhibiting edema and small foci of lymphocytes and plasma cells. When the inflammation mainly involves the periosteum, the disease is called proliferative periostitis or called periostitis ossificans. Histologically, one sees bony trabeculae that lie in a linear parallel pattern. The intervening stroma is composed of fibrous connective tissue sparsely infiltrated with lymphocytes and plasma cells (Fig. 4.45).

Differential diagnosis Both focal and diffuse chronic sclerosing osteomyelitis must be distinguished from other bone lesions, especially the fibro-osseous ones (see Sect. 4.4.3). Edematous marrow with sprinkled lymphocytes and dense sclerotic bone allow its distinction from the fibro-osseous lesions with their cellular fibroblastic stroma. Paget's disease

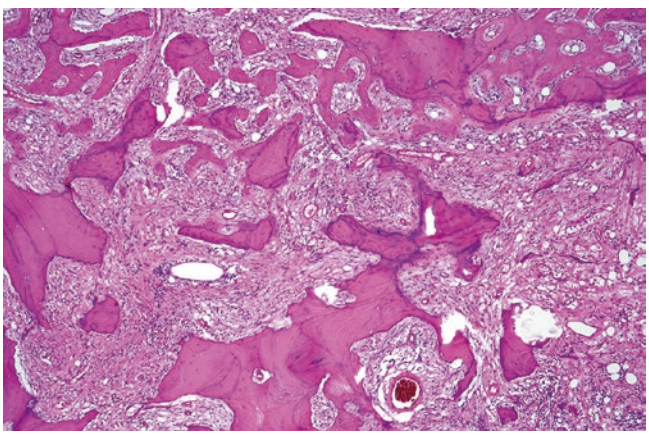

Fig. 4.45 Dense sclerotic lamellar bone and fibrous bone marrow containing lymphocytes characterize chronic sclerosing osteomyelitis

enters the differential diagnosis as it may mimic chronic sclerosing osteomyelitis radiologically but its histology differs profoundly; bone marrow containing dilated thin-walled blood vessels, numerous osteoclasts, and prominent reversal lines in the bone causing the proverbial mosaic pattern are typical for Paget's disease and absent in chronic sclerotic osteomyelitis. Finally, in case of osteoradionecrosis occurring after radiotherapy for squamous cell carcinoma, sinuses lined by squamous epithelium should not be mistaken for recurrent tumor.

Treatment and prognosis Treatment consists of antibiotics and surgery. Unless the sequestra are removed, the disease will not heal. Especially in case of chronic sclerotic osteomyelitis, the disease may respond poorly to treatment and run a protracted course.

4.4.3 Fibro-Osseous Lesions

The current classification of maxillofacial fibro-osseous lesions includes fibrous dysplasia, ossifying fibroma, and osseous dysplasia [168, 169]. Table 4.3 gives an overview of the various entities in this group. In previous classifications, efforts to discern between bone and cementum caused designations as cemento-ossifying fibroma, cementifying fibroma, or cemental dysplasia. However, none of the proposed differences between cementum and bone has withstood the test of time, and therefore terms such as cementifying or cemental have been dropped as being unwanted and confusing [170].

4.4.3.1 Fibrous Dysplasia

Definition Fibrous dysplasia is composed of cellular fibrous tissue containing trabeculae of woven bone. It occurs in three clinical subtypes: monostotic which affects one single bone, polyostotic which affects multiple bones, and Albright syndrome in which multiple bone lesions are accompanied by skin hyperpigmentation and endocrine disturbances [168].

Epidemiology Craniofacial fibrous dysplasia usually is of the monostotic type [171]. Mostly, the disease occurs during the first three decades although, occasionally, cases are seen at an older age. The maxilla is more often involved than the mandible. In the maxilla, fibrous dysplasia may extend by continuity across suture lines to involve adjacent bones [168].

Etiology Activating missense mutations of the gene encoding the α subunit of the stimulatory G protein are a consistent finding in the various forms of fibrous dysplasia [172, 173].

Clinical aspects Fibrous dysplasia clinically presents itself as a painless swelling of the involved bone. Radiographically, the classical appearance is described as orange-skin or ground-glass radiopacity without defined borders [168].

Microscopy Fibrous dysplasia shows replacement of the normal bone by moderately cellular fibrous tissue containing irregularly shaped trabeculae consisting of woven bone without rimming osteoblasts that fuse with adjacent bone (Fig. 4.46). Jaw lesions may also occasionally show lamellar bone (Fig. 4.47). Sometimes, tiny calcified spherules may be present [171].

Differential diagnosis Fibrous dysplasia has to be distinguished from other lesions characterized by the combination of fibrous tissue and bone: ossifying fibroma, osseous dysplasia, low-grade osteosarcoma, and sclerosing osteomyelitis. However, none of these is composed of woven bone trabeculae fusing with adjacent uninvolved bone. Ossifying fibroma and osseous dysplasia both show much variety in appearances of mineralized material and stromal cellularity, low-grade osteosarcoma invades through the cortical bone into soft tissues, and sclerosing osteomyelitis shows coarse trabeculae of lamellar bone, whereas the intervening stroma is not cellular but edematous with sprinkled lymphocytes [169]. Sometimes, focal hemorrhage with clustering of osteoclastic giant cells may lead to confusion with central giant cell granuloma, espe-

Table 4.3 Fibro-osseous lesions [168, 169]

Fibrous dysplasia	
Ossifying fibroma	Conventional
	Juvenile trabecular
	Juvenile psammomatoid
Osseous dysplasia	Periapical osseous dysplasia
	Focal osseous dysplasia
	Florid osseous dysplasia
	Familial gigantiform cementoma

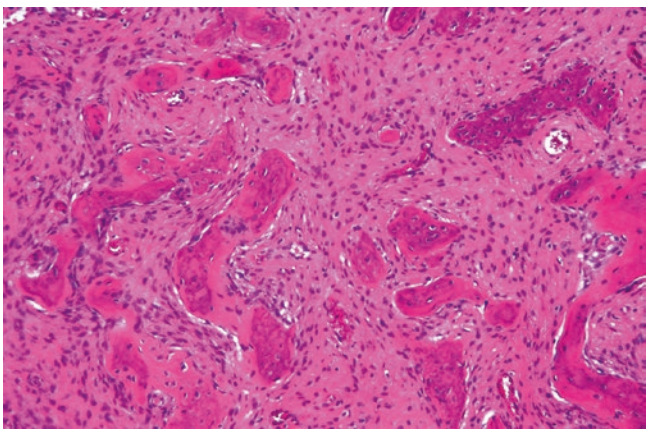

Fig. 4.46 Fibrous dysplasia of the classical type; fibrocellular tissue containing curvilinear trabeculae of woven bone without osteoblastic rimming

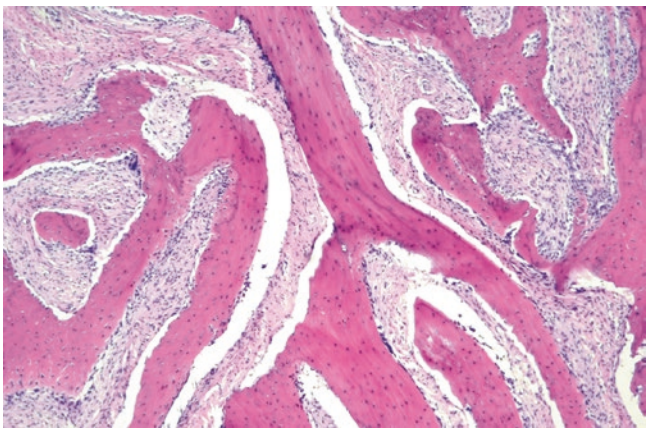

Fig. 4.47 In the jaws, fibrous dysplasia may consist of both woven and lamellar bone as shown in this photomicrograph taken with the use of partly polarized light to enhance the visibility of the lamellar collagenous scaffold of the bone

cially if there is excessive metaplastic bone formation in the latter. However, in all equivocal cases, detection of the diagnostic mutation will be decisive provided that undecalcified or EDTA decalcified material is available [174].

Radiological mimics of fibrous dysplasia are meningioma with hyperostosis as may occur in the walls of the paranasal sinuses [175] and intraosseous hemangioma that occasionally may be accompanied with excessive reactive bone formation [176].

Other entities occurring in the head and neck area that may mimic fibrous dysplasia radiologically are the so-called Bullough lesion and odontomaxillary dysplasia. Bullough lesion is a lesion of the temporal bone that presents as a retroauricular soft tissue mass with calcific densities, confined to the soft tissues on the outer table of the skull without intraosseous involvement. The lesion is characterized histologically by rounded and ovoid zones of ossification within a bland fibrous stroma and so is different from fibrous dysplasia both by its location and histology (Fig. 4.48) [177].

Odontomaxillary dysplasia is a developmental condition that presents early in life and is characterized by asymptomatic unilateral enlargement of the maxilla. Radiographically, the bone of the affected region exhibits a localized, ill-defined increased bone density owing to coarse, irregular trabeculae with a variable vertical orientation (Fig. 4.49). Teeth in the same jaw area may show an abnormal shape which is not a feature of fibrous dysplasia. Moreover, the histopathology of the affected bone also differs from fibrous dysplasia with thickened, irregularly shaped trabeculae of immature woven bone with bone marrow spaces changed into loose paucicellular fibrous tissue [178].

Treatment and prognosis Usually, fibrous dysplasia is a self-limiting disease. Therefore, treatment is only required in case of problems due to local increase in size of the affected bone. Sometimes, an osteosarcoma may arise in fibrous dysplasia [179].

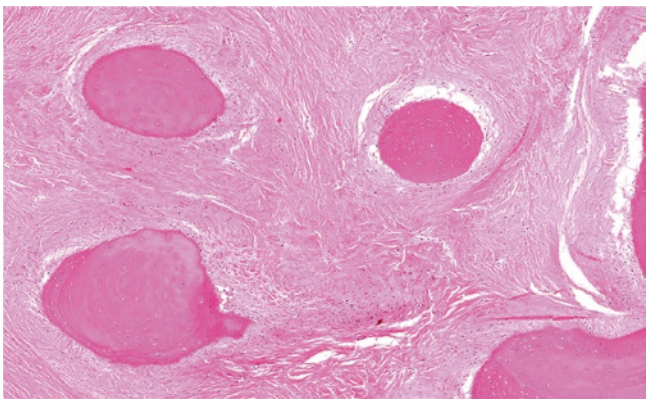

Fig. 4.48 Rather cell-poor fibrous tissue containing smoothly outlined bone fragments with a rim of cellular periosteum and radiating collagenous fibers characterize the so-called Bullough lesion

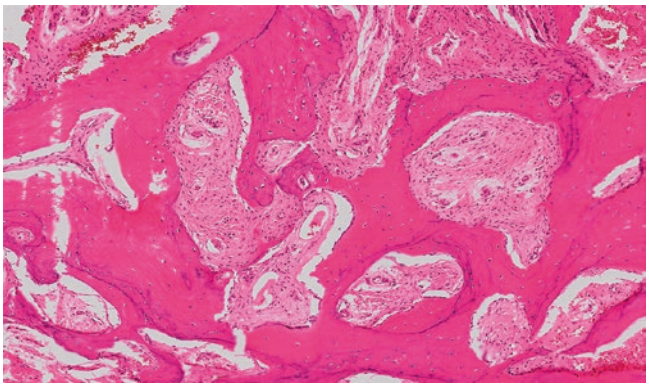

Fig. 4.49 Both lamellar and woven bone in a haphazard arrangement define odontomaxillary dysplasia

4.4.3.2 Ossifying Fibroma

Definition Ossifying fibroma, formerly also called *cemento-ossifying fibroma*, is a well-demarcated lesion composed of fibrocellular tissue and mineralized material of varying appearances.

Epidemiology Ossifying fibroma occurs most often in the second through fourth decades. The lesion shows a predilection for females and is mostly seen in the posterior mandible [170]. Multifocality may occur, and if a patient shows more than only one ossifying fibroma or when there are other family members with this lesion, one should consider the possibility of the hyperparathyroidism–jaw tumor syndrome (HPT-JT), an autosomal dominantly inherited disorder that combines jaw lesions with the appearance of ossifying fibroma with neoplastic and/or cystic lesions in the parathyroid glands and the kidneys [180].

Two subtypes of ossifying fibromas for which further details are given when discussing the microscopy (see below) show a slightly different epidemiology; they occur at a younger age, and one of them, the juvenile psammomatoid ossifying fibroma, most frequently involves the bony walls of the paranasal sinuses [181].

Clinical aspects Clinically, ossifying fibroma causes expansion of the involved bone leading to a palpable swelling. Radiographically one sees a demarcated lesion that may have radiodense as well as radiolucent areas depending on the various contributions of soft and hard tissue components to an individual lesion [168].

Microscopy Ossifying fibroma is composed of fibrous tissue that may vary in cellularity from areas with closely packed cells displaying mitotic figures to almost acellular sclerosing parts within one and the same lesion. The mineralized component may consist of plexiform bone, lamellar bone, and acellular mineralized material, all sometimes occurring together in one single lesion (Figs. 4.50 and 4.51).

Juvenile psammomatoid and *juvenile trabecular ossifying fibroma* are subtypes with a histomorphology different from the more conventional type of ossifying fibroma [182]. Juvenile trabecular ossifying fibroma consists of cell-rich fibrous tissue with bands of cellular osteoid together with slender trabeculae of plexiform bone lined by a dense rim of enlarged osteoblasts (Fig. 4.52). Sometimes these trabeculae may anastomose to form a lattice. Mitoses are present, especially in the cell-rich areas. Also multinucleated giant cells, pseudocystic stromal degeneration, and hemorrhages may be present. These latter components are not haphazardly distributed throughout the lesion but cluster together and form curvilinear strands that even may be noticed at gross evaluation (Figs. 4.53 and 4.54) [183].

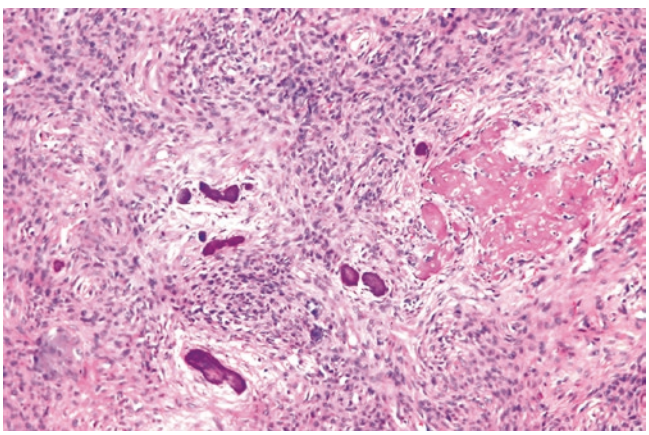

Fig. 4.50 Ossifying fibroma contains both cell-rich and cell-poor areas as well as well-structured bone and amorphous calcified material

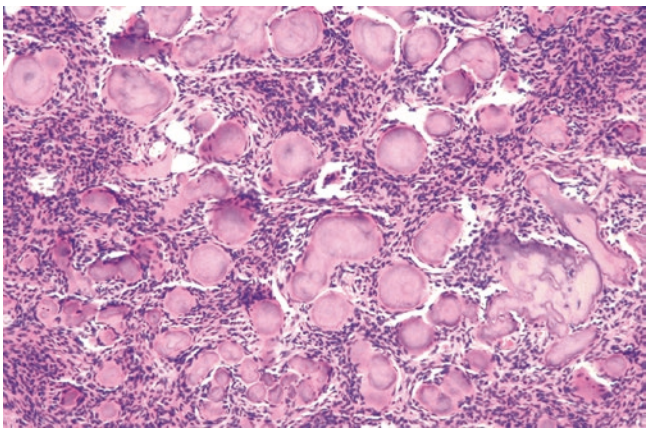

Fig. 4.51 Ossifying fibroma may also contain more smoothly contoured bony elements, formerly thought to represent cementum

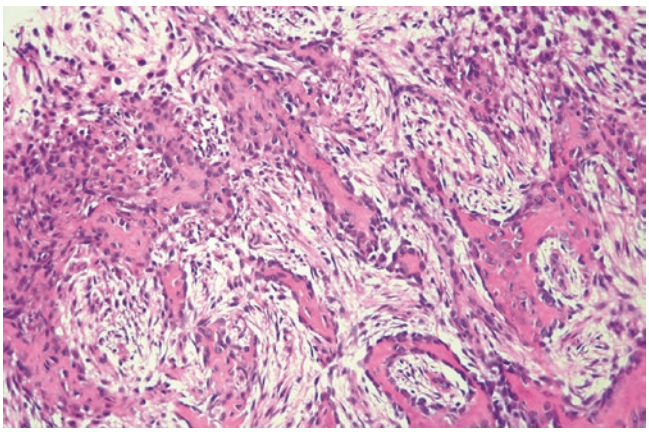

Fig. 4.52 The plump osteoblasts that line the bony trabeculae in juvenile trabecular ossifying fibroma are a prominent feature in this lesion

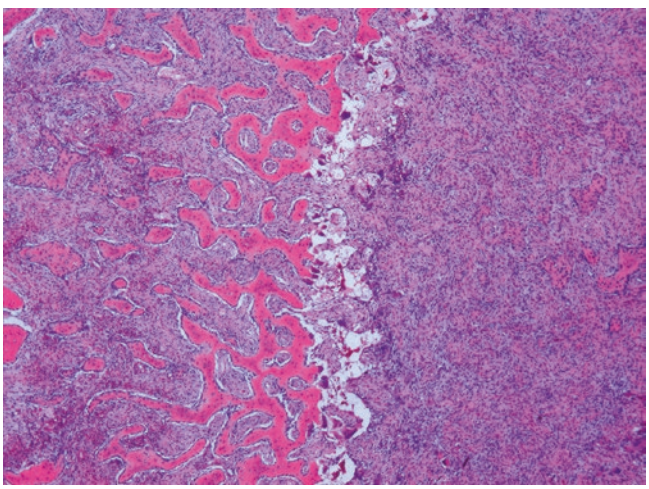

Fig. 4.53 Juvenile trabecular ossifying fibroma contains curvilinear strands composed of hemorrhage, osteoclastic giant cells, and pseudocystic stromal degeneration. These strands are also visible to the naked eye, see Fig. 4.54

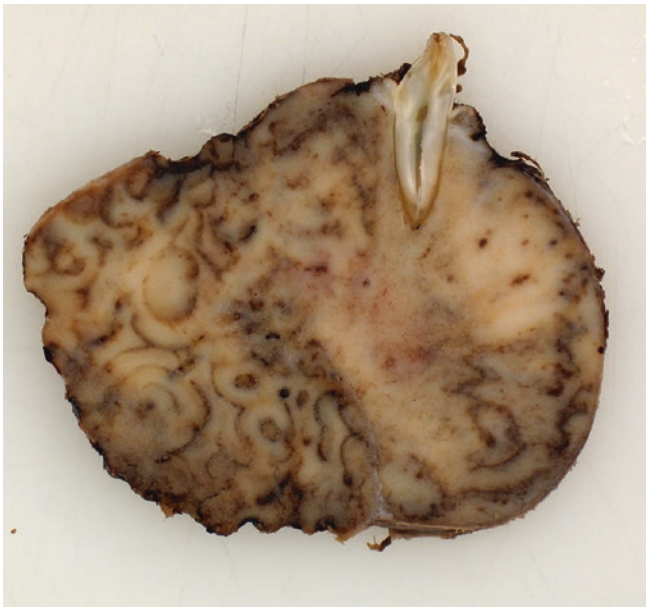

Fig. 4.54 Cut surface of juvenile trabecular ossifying fibroma showing the diagnostic curvilinear strands of which the microscopical counterpart is shown in Fig. 4.53

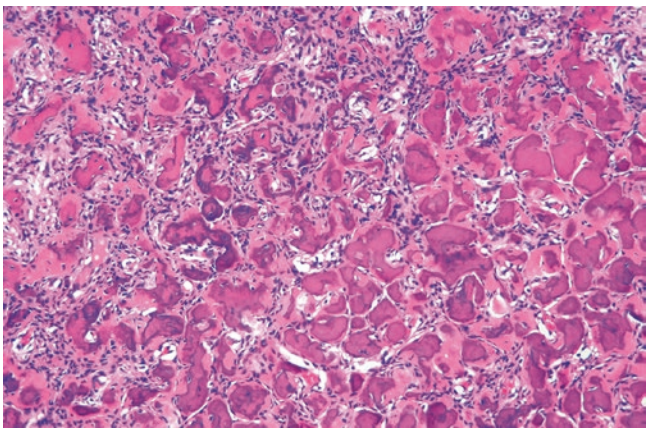

Fig. 4.55 Psammomatoid ossicles in a cellular stroma characterize juvenile psammomatoid ossifying fibroma. A comparison with Fig. 4.51 shows that these particles are not as smoothly outlined as the ones occurring in conventional ossifying fibromas

Juvenile psammomatoid ossifying fibroma is characterized by a fibroblastic stroma containing small ossicles resembling psammoma bodies, hence its name. The stroma varies from loose and fibroblastic to intensely cellular. The spherical or curved ossicles are acellular or include sparsely distributed cells (Fig. 4.55). They should not be confused with the cementum-like deposits that are present in conventional ossifying fibroma. These particles have a smooth contour whereas the ossicles in juvenile psammomatoid ossifying fibroma have a peripheral radiating fringe of collagen fibers. Ossicles may coalesce to form trabeculae. Sometimes, juvenile psammomatoid ossifying fibroma contains basophilic

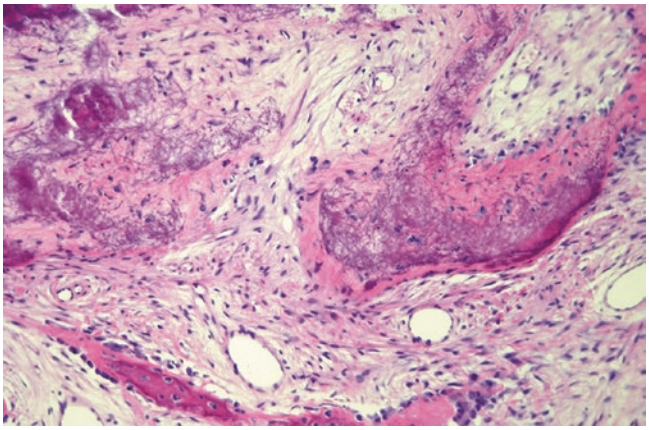

Fig. 4.56 Threadlike calcifications in an eosinophilic matrix are also often present in juvenile psammomatoid ossifying fibroma

concentrically lamellated particles as well as irregular threadlike or thornlike calcified strands in a hyalinized background (Fig. 4.56). Other features such as trabeculae of woven bone as well as lamellar bone, pseudocystic stromal degeneration, and hemorrhages resulting in areas similar to an aneurysmal bone cyst, multinucleate giant cells, and mitotic figures can also be observed. Sometimes, this type of ossifying fibroma may turn into a large bone cavity, diagnostic lesional tissue being confined to small areas in its wall.

Genetics Chromosomal abnormalities have been observed in ossifying fibromas [184–186]. Data are still too scarce to determine their pathogenetic significance.

Differential diagnosis Ossifying fibroma may be confused with fibrous dysplasia. The most important distinguishing feature is the presence of demarcation and/or encapsulation in ossifying fibroma as opposed to the merging with its surroundings as shown by fibrous dysplasia. In addition, the variation in cellularity as well as in appearances of mineralized material distinguishes ossifying fibroma from fibrous dysplasia. Moreover, as already mentioned, mutation analysis will be decisive in difficult cases as ossifying fibroma lacks this genetic marker [187].

To distinguish ossifying fibroma from osseous dysplasia (see next section), data on clinical presentation and radiographic appearance are indispensable. Osseous dysplasia usually is asymptomatic, being an incidental finding on radiographs whereas ossifying fibroma shows the features of an expanding benign neoplasm both radiologically and clinically.

Juvenile psammomatoid ossifying fibroma has to be differentiated from meningioma with psammoma bodies; immunohistochemistry positive for EMA rules out juvenile psammomatoid ossifying fibroma. Moreover, the psammomatoid ossicles in juvenile psammomatoid ossifying

fibroma are clearly different from the acellular spherical real psammoma bodies [188].

Juvenile trabecular ossifying fibroma may be confused with osteosarcoma due to its cellularity and mitotic activity. However, atypical cellular features are not seen, and recognizing the recently described curvilinear garlands of osteoclastic giant cells, stromal pseudocysts, and extravasated erythrocytes will also be helpful in ruling out osteosarcoma [183]. Moreover, the lesion is demarcated from its surroundings whereas osteosarcoma does not respect cortical borders [169]. In cases with abundant giant cells, it may turn out difficult to make the distinction between juvenile trabecular ossifying fibroma and central giant cell granuloma, especially in case of metaplastic ossification in the latter. This item will be discussed below (see Sect. 4.4.4).

Treatment and prognosis Excision of ossifying fibromas usually yields permanent cure.

4.4.3.3 Osseous Dysplasia

Definition Osseous dysplasia is a pathologic process of unknown etiology located in the tooth-bearing jaw areas in the vicinity of the tooth apices and thought to arise from proliferation of periodontal ligament fibroblasts that may deposit bone as well as cementum. Alternative designations include cemental dysplasia or cemento-osseous dysplasia.

Clinical aspects The condition occurs in various clinical forms that are distinguished by clinical and radiological features. *Periapical osseous dysplasia* occurs in the anterior mandible and involves only a few adjacent teeth. A similar limited lesion occurring in a posterior jaw quadrant is known as *focal osseous dysplasia* [189]. *Florid osseous dysplasia* is nonexpansile, involves two or more jaw quadrants, and occurs in middle-aged black females [168]. *Familial gigantiform cementoma* is expansile, involves multiple quadrants, and occurs at young age. This type of osseous dysplasia shows an autosomal dominant inheritance with variable expression, but sporadic cases without a history of familial involvement have also been reported [190, 191]. Simple bone cysts may be seen with florid as well as focal osseous dysplasia [50]. The variation in ratio of soft tissue to hard tissue is reflected in the radiographic appearance, lesions being either predominantly radiolucent, predominantly radiodense, or mixed.

Microscopy All subtypes have the same histomorphology: cellular fibrous tissue, trabeculae of woven as well as lamellar bone, and spherules of cementum-like material (Fig. 4.57). The proportions of fibrous tissue and mineralized material may vary, and it has been shown that these lesions are initially fibroblastic but over the course of several years may show increasing degrees of calcification. Osseous dysplasia lacks encapsulation or demarcation but tends to merge with the adjacent cortical or medullary bone [168].

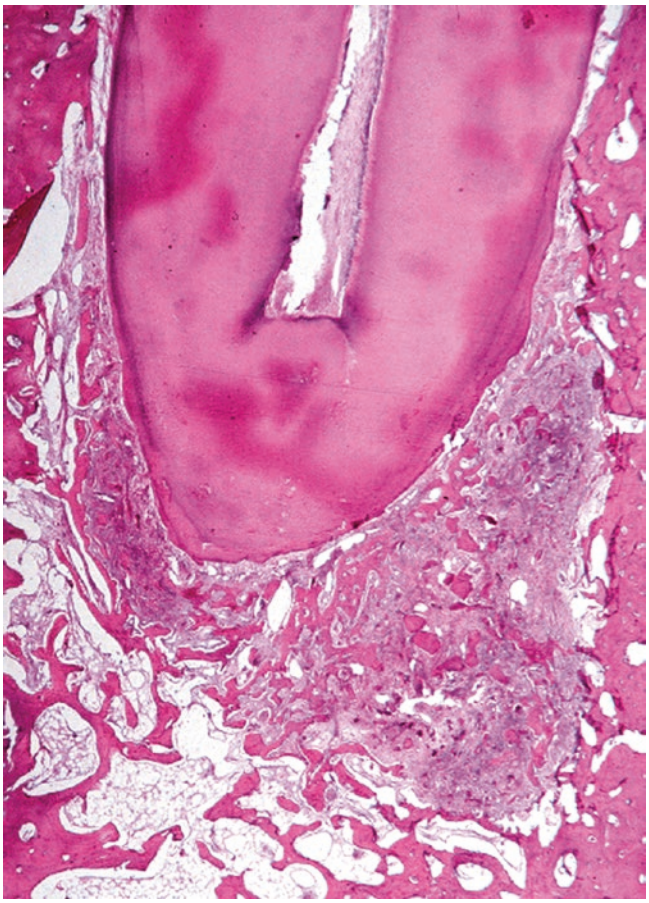

Fig. 4.57 Osseous dysplasia typically lies at the root tip. It consists of fibrous tissue containing mineralized material of varying appearances

Differential diagnosis Osseous dysplasia has to be distinguished from ossifying fibroma. Osseous dysplasia is a mixed radiolucent–radiodense lesion with ill-defined borders in the tooth-bearing part of the jaws, either localized or occupying large jaw areas depending on the type. In contrast, ossifying fibroma usually is a localized lesion that expands the jaw [192].

Osseous dysplasia has also to be differentiated from sclerosing osteomyelitis [193]. Sclerotic lamellar bone trabeculae and well-vascularized fibrous tissue with lymphocytes and plasma cells define sclerosing osteomyelitis, whereas cementum-like areas and fibrocellular soft tissue are lacking [194]. Another mimicker of osseous dysplasia is Paget's disease. When occurring in the jaw, this disease may be associated with extensive hypercementosis, thus radiologically resembling florid osseous dysplasia [195]. Histology however is different as has already been mentioned when discussing the differential diagnosis of Paget's disease versus chronic sclerosing osteomyelitis (see Sect. 4.4.2.2).

Moreover, in osseous dysplasia, the radiodense deposits are not attached to the roots of the teeth in contrast with Paget's disease.

The distinction between fibrous dysplasia and osseous dysplasia can be made on radiographs, osseous dysplasia involving only the jaw area that surrounds the root apices, whereas fibrous dysplasia usually shows a more diffuse involvement with bone expansion. Moreover, the distinction can be made histologically through the wide morphological spectrum of mineralized material observed in osseous dysplasia, whereas in fibrous dysplasia, this is confined to woven bone only, with the exception of lesions in the posterior maxilla, but this is a jaw area in which osseous dysplasia rarely occurs.

Treatment The various forms of osseous dysplasia do not require treatment unless necessitated by complications such as infection of sclerotic bone masses as may occur in florid osseous dysplasia or facial deformity as may be seen in familial gigantiform cementoma.

4.4.4 Giant Cell Lesions

Central giant cell granuloma, cherubism, and aneurysmal bone are defined by the presence of osteoclastic giant cells and are taken together because of their mainly identical histological features. The first two entities are confined to the jaws; the third may occur at other sites as well.

4.4.4.1 Central Giant Cell Granuloma

Definition A jaw lesion characterized by the presence of hemorrhages and osteoclastic giant cells. In older literature, the term giant cell reparative granuloma is occasionally employed due to a presumed traumatic etiology.

Epidemiology Central giant cell granuloma mostly is seen before the age of 30. The lesion is restricted to the jaws, the mandible being more often involved than the maxilla.

Clinical aspects Central giant cell granuloma manifests itself as a localized jaw swelling. Radiographically, it is a radiolucent lesion that may be either uni- or multilocular. Multiple giant cell lesions may occur in association with Noonan syndrome as well as with neurofibromatosis [196, 197].

Microscopy Central giant cell granuloma shows osteoclast-like giant cells lying in a fibroblastic background tissue. The fibroblastic tissue may vary in cellularity from very dense to cell-poor. Mitotic figures may be encountered but are usually not numerous and not atypical. The giant cells mostly cluster in areas of hemorrhage but they also may lie more dispersed among the lesion (Fig. 4.58). Bone formation, if present, usually is confined to the periphery of the lesion or occurs in less cellular fibrous septa that may divide the lesion in separate foci, thus considered not to be part of the lesion but to represent metaplastic stromal changes.

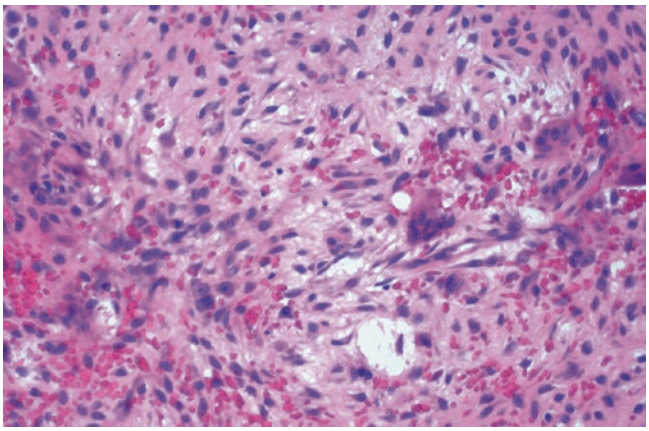

Fig. 4.58 Central giant cell granuloma shows osteoclast-like giant cells in a loose fibrocellular stroma. Cherubism has an identical appearance

Differential diagnosis Lesions with a histologic appearance identical to central giant cell granuloma may occur in the gingiva and are called giant cell epulis (see Chap. 3). Sometimes, lesions combine the appearance of giant cell granuloma with odontogenic fibroma [101]. If there is recurrence, hyperparathyroidism should be ruled out as the brown tumors associated with this latter disease are identical to giant cell granuloma. Also, one has to distinguish between giant cell granuloma and true giant cell tumor. There has been much discussion whether giant cell granulomas with a more aggressive behavior than usually observed could represent a gnathic manifestation of this latter lesion [198, 199]. Occasionally, central giant cell granulomas are reported in the temporomandibular joint area in which case chondroblastoma (see Sect. 4.4.5.3) should be considered also.

In case of metaplastic bone formation, confusion may arise between central giant cell granuloma and juvenile trabecular ossifying fibroma as both lesions also share hemorrhagic areas and osteoclastic giant cells. However, in the latter lesion, hemorrhages and osteoclastic giant cells are not randomly distributed but arranged in garlands (see Sect. 4.4.3.2), and bone deposition occurs in cell-rich stromal areas resulting in extremely cellular osteoid, a pattern clearly different from the metaplastic bone deposition in the more fibrous parts of the giant cell granuloma.

Treatment and prognosis As the lesion is not encapsulated, removal is sometimes followed by recurrence.

4.4.4.2 Cherubism

Definition Lesion similar to giant cell granuloma but involving two or more jaw quadrants bilaterally in a symmetrical way.

Epidemiology The disease occurs in young children, often with a history of other afflicted family members.

Etiology and pathogenesis The genetic defect responsible for cherubism has been localized to chromosome 4p16.3 [200]. Occasionally, neurofibromatosis may lead to a phenotype similar to cherubism [201].

Clinical aspects The expansion of the affected jaw areas causes the angelic face leading to the lesion's designation: cherubism. With the onset of puberty, the lesions loose their activity and may mature to fibrous tissue and bone. Radiologically, involved jaw areas show expanding multilocular radiolucencies with displacement of tooth germs.

Microscopy As already mentioned, the histology of cherubism is similar to giant cell granuloma. The clinical presentation makes the difference between both lesions and leaves little room for an alternative diagnosis. Sometimes, there may also be a component consisting of immature odontogenic tissue due to developing tooth germs lying within the lesional tissue. This is a fortuitous finding without any clinical relevance.

Treatment and prognosis Treatment is restricted to cosmetic bone reduction.

4.4.4.3 Aneurysmal Bone Cyst

Definition The aneurysmal bone cyst is an expanding radiolucent lesion that is characterized by blood-filled spaces lined by fibrous septa containing osteoclast-like multinucleated giant cells and delicate spicules of osteoid. Most cases arise primarily, but approximately 20–30 % are secondary to an identifiable, preexisting lesion. These preexisting lesions most frequently associated with secondary aneurysmal bone cyst (in order of frequency) include giant cell tumor of bone, chondroblastoma, chondromyxoid fibroma, and fibrous dysplasia.

Epidemiology In a series of 238 cases of aneurysmal bone cyst, only 5 were in the maxillofacial skeleton [202], and as case series from the head and neck show that it may take a lot of years to collect a significant number of cases, it can be concluded that the lesion is rare at this site [203]. The lesion does not show gender predilection and most often occurs from the second till the fourth decade of life.

Clinical aspects Aneurysmal bone cyst usually presents as a bony swelling, at radiology showing a multilocular radiolucent appearance. Pain may also be a prominent presenting sign [202].

Microscopy Histologically, primary aneurysmal bone cyst shows cellular fibrous septa that surround blood-filled spaces and contain uniform fibroblasts with scattered osteoclast-like giant cells. Giant cells seen in aneurysmal bone cyst are mainly related to the vascular spaces or hemorrhagic foci in the septa. Osteoid spicules that usually run parallel to the surface of the aneurysmal spaces are seen in the fibrous septa. Occasionally, chondroid foci may be present. In case of a secondary aneurysmal bone cyst, finding the associated neoplasm may require more extensive sampling.

Differential diagnosis In the jaw bones, aneurysmal bone cyst should be distinguished from central giant cell granuloma that lacks the large, blood-filled spaces and the septa with osteoid spicules. The differential diagnosis with telangiectatic osteosarcoma that may arise with extragnathic lesions has no practical relevance for the head and neck area.

Genetics Primary aneurysmal bone cysts are genetically characterized by recurrent chromosomal abnormalities of which t(16;17)(q22;p13) is the most frequent [204].

Treatment and prognosis Aneurysmal bone cysts are usually treated by curettage and bone grafting with varying recurrence rates of 20–70 % in different series and with different treatment modalities [205].

4.4.5 Bone Tumors: Benign

4.4.5.1 Osteoblastoma

Definition Osteoblastoma is a rare, bone-forming neoplasm characterized by interconnecting trabeculae of woven bone and rimmed by prominent osteoblasts. Histologically, the lesion resembles osteoid osteoma and a size of 1.5 cm has been mentioned as border, larger lesions being osteoblastoma and smaller ones osteoid osteoma [206]. However, within the jaw, criteria to discern between osteoid osteoma and osteoblastoma are poorly respected in reported cases, and, hence, in this text both will be taken together under the heading osteoblastoma as advocated by Jones et al. [207].

Epidemiology Osteoblastoma has been stated to account for approximately 1 % of primary bone tumors. Approximately 10–15 % arise within the bones of the craniofacial skeleton, in most cases the mandible. The lesion shows an age range from 3 to 77 years of age with a mean age of 23.5 years. Females are slightly more afflicted than men [207, 208].

Clinical aspects Patients present with an expanding painful mass; radiographically, osteoblastomas show a mixed radiodense and radiolucent appearance, depending on the extent of intralesional mineralization.

Microscopy Osteoblastomas are composed of trabeculae of neoplastic woven bone that are rimmed prominently by osteoblasts with scattered osteoclasts. The intertrabecular space contains a loose vascular connective tissue with foci of extravasated red blood cells. Mitoses may occur but are not numerous.

Epithelioid multinodular osteoblastoma is a subtype of osteoblastoma characterized by multiple nodules of epithelioid cells, most with a lacy, blue-bone matrix. Frequently, there are also sheets of cells with almost no matrix at all (Figs. 4.59 and 4.60). This histological variant has the jaw bones as the most common site [209]. Similar cases have also been described under the designation aggressive osteoblastoma [210, 211].

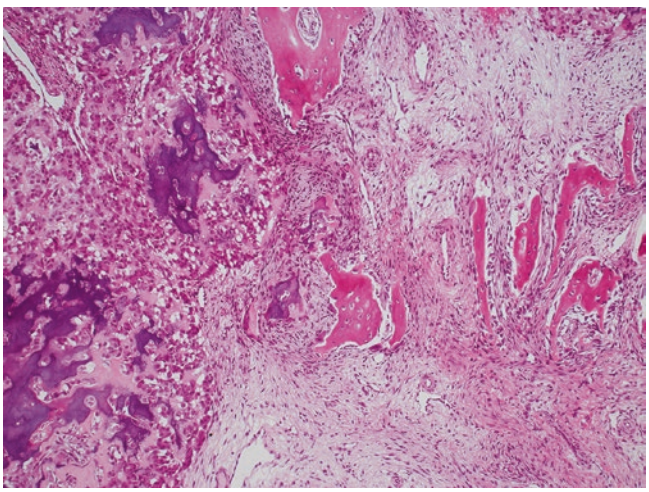

Fig. 4.59 Multinodular epithelioid osteoblastoma. Irregularly mineralized bone is surrounded by large areas of epithelioid osteoblasts. Lesion extends into adjacent jaw bone that shows remodeling (Picture kindly provided by Dr. Mary Toner, Dublin Dental University Hospital, Ireland)

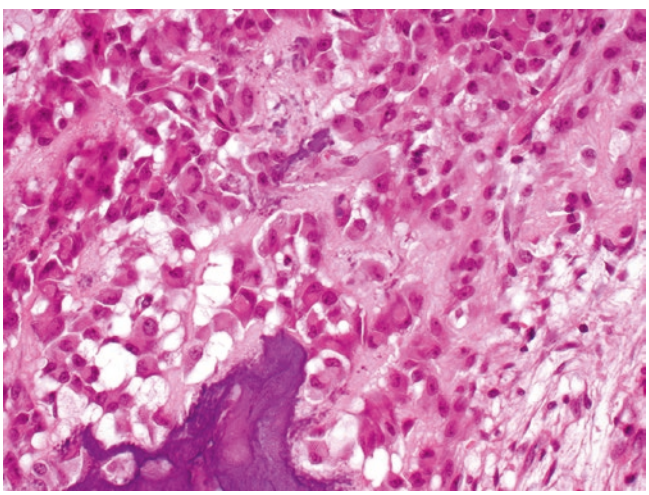

Fig. 4.60 Detail of multinodular epithelioid osteoblastoma showing the characteristic appearance of the osteoblasts with ample cytoplasm thus acquiring an epithelioid morphology. This may be mistaken for calcifying epithelial odontogenic tumor (Picture kindly provided by Dr. Mary Toner, Dublin Dental University Hospital, Ireland)

Differential diagnosis The differential diagnosis of osteoblastoma includes osteosarcoma and fibro-osseous lesions. Osteosarcomas demonstrate invasive growth by which adjacent jaw bone is engulfed. This type of bone involvement has to be differentiated from the nodular growth pattern of the epithelioid (aggressive) osteoblastoma type in which there may be tumor nodules separated from each other by broad fibrous septa with reactive bone, a morphology that should not be mistaken for invasion but as representing reactive jaw bone remodeling.

The fibro-osseous lesions demonstrate a fibroblastic stroma that lacks the vascularity and hemorrhage typical of osteoblastoma, and although osteoblastic rimming may occur in some ossifying fibromas, especially in the juvenile trabecular subtype, the stromal features as mentioned above allow distinction between both entities. Moreover, osteoblastoma lacks the garlands composed of osteoclastic giant cells, pseudocystic stroma degeneration, and hemorrhage that are typical for this ossifying fibroma variant and described in detail under that specific heading (see Sect. 4.4.3.2).

Occasionally, the epithelioid (aggressive) osteoblastoma may mimic a calcifying epithelial odontogenic tumor, due to the presence of matrix deposits surrounded by large aggregates of epithelioid osteoblasts [210]. In doubtful cases, immunohistochemistry will solve the diagnostic problem by demonstrating the expression of epithelial markers in the former lesion.

Finally, lesions with the morphology of an osteoblastoma but showing continuity with the root surface of an adjacent tooth should be diagnosed as cementoblastoma (see Sect. 4.3.2.3).

Treatment and prognosis The treatment of osteoblastoma is surgical excision. En bloc resection is usually curative; curettage may occasionally result in local recurrence [208, 212]. Malignant transformation of osteoblastoma to osteosarcoma is rare [213, 214]. Prognosis of the epithelioid (aggressive) osteoblastoma does not appear to be different from the more common variant of this tumor [214].

4.4.5.2 Chondromyxoid Fibroma

Definition Chondromyxoid fibroma is a benign tumor characterized by lobules of spindle-shaped or stellate cells with abundant myxoid or chondroid intercellular substance.

Epidemiology Chondromyxoid fibroma accounts for only 1.8 % of all benign neoplasms of bone. The lesion has a predilection for patients in the second and third decades of life. The long bones are the sites most frequently affected, particularly the tibia. Out of 278 cases of chondromyxoid fibroma, 15 tumors involved the skull and facial bones [215]. The mandible is more often involved than the maxilla and there is no gender predilection [216]. Still less usual head and neck sites are the nasal septum and the temporal bone [217, 218].

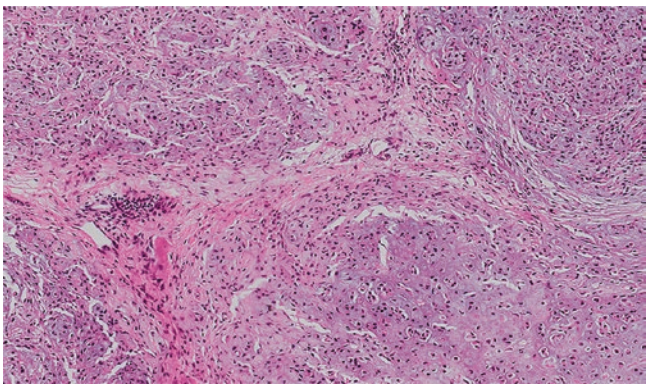

Fig. 4.61 Cell-rich lobules of chondroid tissue are separated by fibrous septa. This is typical for chondromyxoid fibroma

Clinical aspects Jaw swelling is the most common presenting sign, pain only rarely reported. On radiographs, the most typical presentation is that of a purely radiolucent lesion with well-defined sclerotic margins [216].

Microscopy The histologic features include the presence of stellate or spindle-shaped cells in a myxoid background and arranged in lobules (Fig. 4.61). The interlobular tissue is cellular and also composed of oval or spindle-shaped cells. Multinucleated giant cells may be present at the border of the lobules in approximately 50 % of tumors. Calcification and/or hyaline cartilage is seen in a minority of cases. In some cases, the lobularity is not very conspicuous. Focally, some nuclear atypia may be noted but the overall nuclear morphology is rather uniform.

Differential diagnosis The main histologic differential diagnosis is with chondrosarcoma that may also have a lobulated growth pattern, myxoid matrix, and spindling of the nuclei. However, chondromyxoid fibromas typically contain cells with small, oval- to spindle-shaped nuclei with only focally some atypia whereas the nuclei in chondrosarcomas are usually larger, hyperchromatic, and with a more widely distributed nuclear atypia. In chondromyxoid fibromas of the skull base, chordoma may be considered as an alternative diagnosis. Chordomas contain epithelial cells arranged in nests and cords and with vacuolated cytoplasm. Moreover, the chordoma cells are positive with cytokeratin markers. Further details on chordoma are mentioned below (see Sect. 4.4.6.4).

Treatment and prognosis The therapeutic approach is controversial. Jaw resection has been recommended for extensive lesions whereas smaller lesions may be treated by curettage only [216].

4.4.5.3 Chondroblastoma

Definition Chondroblastoma is a benign, cartilage-producing neoplasm.

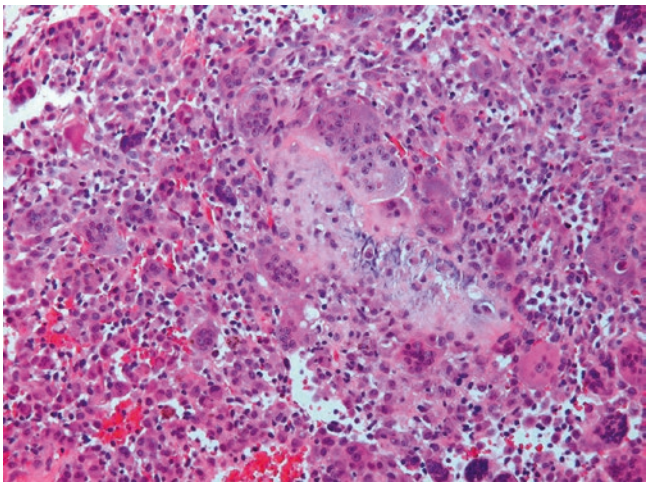

Fig. 4.62 Chondroblastoma is composed of chondroid matrix with threadlike calcifications, lying in a dense cellular background that contains multinucleated giant cells

Epidemiology Chondroblastoma accounts for 1–2 % of all bone tumors and typically occurs at the ends of the long bones. Less than 10 % of all chondroblastomas occur in the maxillofacial skeleton [215]. Specific data on head and neck cases are rare. In one series it was shown that there is a male predominance and that the majority of cases occur in the lateral part of the temporal bone. Moreover, there is a wide age range [219].

Clinical aspects Patients with chondroblastomas generally present with localized pain. When the tumor is located in the temporal bone, hearing loss may occur. Radiologic findings were not suggestive of a specific diagnosis, other than probably benign. Chondroblastomas involving the temporal bone are usually lucent, expansile lesions with sharp margins. These features are not suggestive of a specific diagnosis [215, 219].

Microscopy Chondroblastomas are composed of mononuclear cells and chondroid matrix (Fig. 4.62). The mononuclear cells have an oval to elongated nucleus that may display a characteristic longitudinal groove. Sometimes these cells contain brown granular pigment (Fig. 4.63). Mitotic figures are usually present but not numerous. Multinucleated giant cells are a common feature. Chondroid matrix varies in amount. Approximately one-third of chondroblastomas exhibit calcification, usually occurring in a chicken wire-like pattern. Secondary aneurysmal bone cyst formation may occur in one-third of cases [215].

Differential diagnosis In the head and neck area, the main differential diagnosis is central giant cell granuloma. Giant cell granuloma however does not contain the large cells with brown pigment and neither is deposition of chondroid matrix or chicken wire calcification present in this lesion. Moreover,

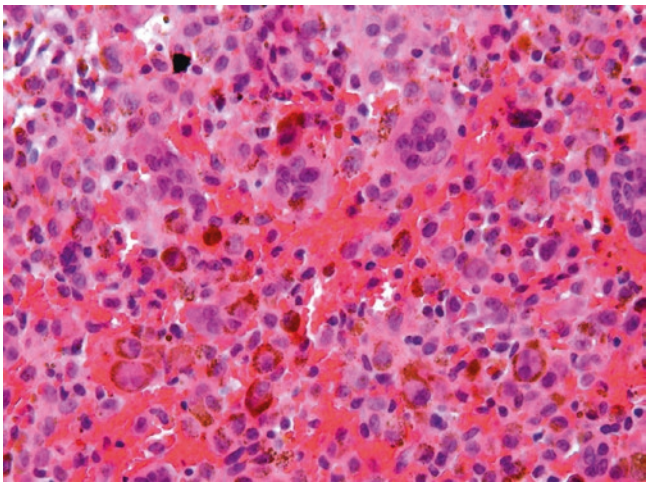

Fig. 4.63 Epithelioid cells containing coarse granular brown pigment are a typical component of chondroblastoma

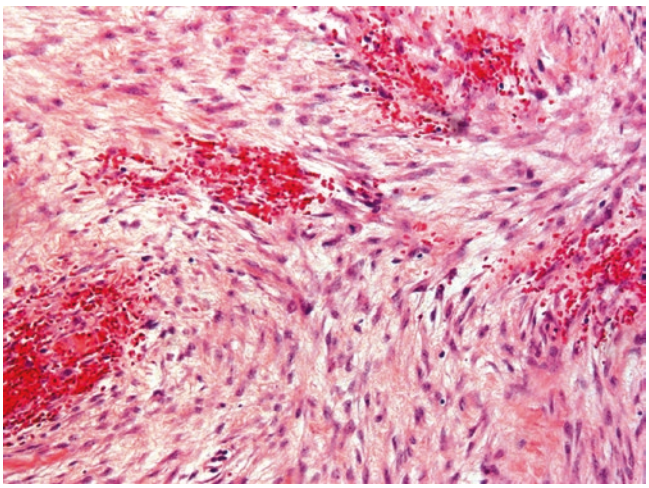

Fig. 4.64 Desmoplastic fibroma showing slightly enlarged elongated spindle cells in a loose edematous background that may contain tiny hemorrhages. Also some mitotic activity may occur

occurrence of a giant cell granuloma in the temporomandibular joint region is highly unusual.

Treatment and prognosis Curettage is the preferred treatment. Depending on the location and extent of the lesion, a wider margin may be necessary in achieving permanent cure. Reported recurrence rates vary from 6 to 15 % [215].

4.4.5.4 Desmoplastic Fibroma

Definition Desmoplastic fibroma is a benign intraosseous neoplasm that is recognized as the intraosseous counterpart of soft tissue fibromatosis in both gnathic and extragnathic sites. It has a propensity for locally aggressive behavior and local recurrence [220].

Epidemiology Desmoplastic fibroma is a rare bone tumor that may involve the jaws, predominantly the mandible in about one-fifth of cases. There is no gender predilection and on average, patients are 15.1 years old at the time of the final diagnosis [221].

Clinical aspects Asymptomatic swelling of the jaw is the most often encountered sign at initial presentation. Radiographs show a radiolucent and expansile lesion, an appearance shared with a lot of other intraosseous lesions and hence not specific.

Microscopy Histology shows a proliferation of uniform spindle cells in a collagenous background with varying degrees of myxoid changes. The cells are arranged in a parallel fashion mixed with whorling bundles (Fig. 4.64).

Immunohistochemistry The tumor cells usually show focal reactivity for smooth muscle actin while being nonreactive for S100 protein and muscle-specific actin [220].

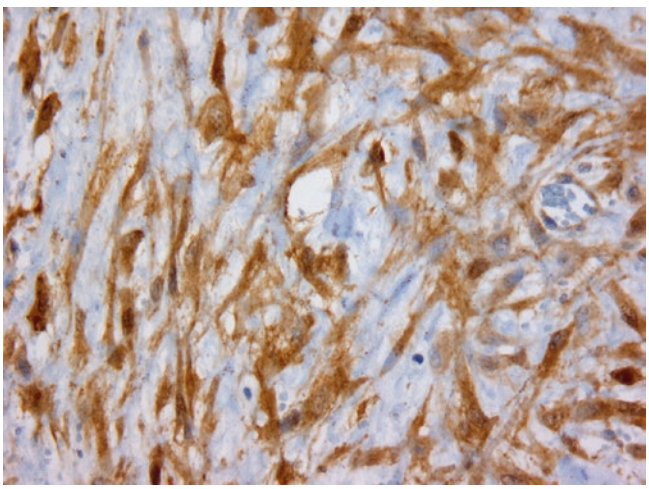

Fig. 4.65 High-power view of desmoplastic fibroma showing expression of beta-catenin, mainly cytoplasmatic but occasionally also nuclear

Nuclear expression of beta-catenin is also occasionally observed (Fig. 4.65) [222].

Genetics Data on genetic aberrations in desmoplastic fibroma are limited. Trisomy 8 and trisomy 20 as well as a rearrangement involving chromosomes 11 and 19 have been reported [223]. Whether aberrations in the beta-catenin pathway as are present in soft tissue desmoid-type fibromatosis are also observed in desmoplastic fibroma is yet unsettled, one report denying this [222], but in another study they were demonstrated [224]. The issue of genetic alterations in desmoid-type fibromatosis is more extensively discussed in Chap. 12.

Differential diagnosis Low-grade fibrosarcoma and odontogenic fibroma are the most important lesions to differentiate from desmoplastic fibroma. In fibrosarcoma, the cells

often are arranged in the so-called "herring bone" pattern, and moreover the nuclei are not as monotonous as in desmoplastic fibroma. In odontogenic fibroma, the cells are not arranged in bundles but more randomly distributed in a background that may vary from densely fibrous to immature myxoid and may contain odontogenic epithelium as well as calcifications (see Sect. 4.3.2.2). Moreover, neither fibrosarcoma nor odontogenic fibroma show expression of smooth muscle actin.

Solitary congenital fibromatosis (infantile myofibromatosis) of bone is most commonly seen in the craniofacial bones of patients 2 years old or younger. It may be distinguished from desmoplastic fibroma by its more nodular growth pattern and different immunophenotype (see Chap. 12 for more extensive description of this entity).

Finally, it has to be emphasized that desmoplastic fibroma arising within bone cannot be separated from a desmoid-type fibromatosis that secondarily involves bone. In these cases, clinical and radiographic features are critical for an appropriate categorization.

Treatment and prognosis Cases treated with either resection or excision showed less postsurgical recurrence, when compared to ones treated by curettage [220].

4.4.5.5 Non-ossifying Fibroma

Definition The non-ossifying fibroma is a benign lesion most commonly seen in the metaphyses of the long bones in children. Synonyms in use for this lesion are histiocytic fibrous defect, metaphyseal fibrous defect, fibrous cortical defect, fibrous xanthoma, and histiocytic xanthogranuloma [225].

Terms most often used are non-ossifying fibroma and fibrous cortical defect, the former used for larger lesions, the latter for the smaller ones.

Multiple lesions of non-ossifying fibroma may occur within the context of Jaffe–Campanacci syndrome where they are seen in association with café au lait spots, mental retardation, hypogonadism, and congenital ocular or cardiac anomalies [226].

Epidemiology In the head and neck region, the lesion is rare with only 20 cases reported until now; all of them occurred in the mandible, both in the ascending ramus and body. The majority of patients were female and mean age in this series was 19 years and 7 months which is higher than for the extragnathic cases.

Clinical aspects In the jaws, non-ossifying fibroma may present as an expanding lesion but may be asymptomatic as well. The radiographic appearance usually is that of a well-demarcated multilocular radiolucency with sclerotic borders.

Microscopy Histologically, non-ossifying fibroma consists of spindle-shaped fibroblasts lying in a storiform pattern and admixed with variable numbers of multinucleated giant cells and foam cells and depositions of hemosiderin pigment. Occasionally, foci of osseous metaplasia can be seen.

Differential diagnosis Main differential diagnoses are benign fibrous histiocytoma and central giant cell granuloma. Benign fibrous histiocytoma mimics non-ossifying fibroma histologically but occurs with greater frequency in older patients and is often symptomatic. Central giant cell granuloma shows much higher numbers of osteoclastic giant cells than are usual for non-ossifying fibroma [227].

Treatment and prognosis The lesions respond favorably to curettage and may even show spontaneous resolution [225].

4.4.5.6 Langerhans Cell Histiocytosis

Definition Langerhans cell histiocytosis (LCH), also called histiocytosis X, is characterized by a clonal proliferation of pathologic cells with the characteristics of Langerhans cells (LCs), in single or multiple organs. Its clinically more widespread manifestation is known as Letterer–Siwe disease [228]. Further discussion will concentrate on the intraosseous LCH, more widely known as eosinophilic granuloma.

Epidemiology A review of 1,120 cases of LCH yielded 114 cases with oral involvement. The great majority of cases occurred in males, with 40 % of the lesions having developed before the patient had reached the age of 10 years. The mandible was involved in 73 % of the cases, and the posterior jaw region was the predominant site. Extraoral involvement occurred in 70 %, with the most common sites being the skull and lower extremity [229].

Etiology and pathogenesis The question, whether LCH is a reactive rather than a neoplastic disorder, has not yet been solved. Because of familial clustering and twin studies, it has been proposed that genetic alterations affecting cell proliferation, cell cycle regulation, and/or apoptosis could be responsible for the development of LCH. Moreover, genetic alterations are supportive evidence of a neoplastic nature. The alternative hypothesis suggests that LCH results from increased survival rather than uncontrolled proliferation of LCH cells and that the expansion of regulatory T-cells may be involved in the failure of the host immune system to eliminate LCH cells [228].

Clinical aspects Clinically, gingival inflammation, tooth mobility, delayed healing of extraction wounds, jawbone swelling, and pain have been observed as presenting signs. Radiologically, LCH can show periodontal destruction with alveolar bone loss, involving a small group of teeth and often

exposing the roots of the teeth. In other cases, the lesion causes radiolucent areas in the mandible body, angle, or ascending ramus [230].

Microscopy The classical histopathologic feature of LCH is the presence of cells with the LC phenotype, with varying proportions of macrophages, T-lymphocytes, eosinophils, and multinucleated giant cells. The LC phenotype can be confirmed by the presence of Birbeck granules on electron microscopy or by demonstration of CD1a on immunohistochemistry. Occasionally, the diagnostic cells may be overlooked initially due to a predominance of eosinophils.

Differential diagnosis In case of extensive concomitant inflammation, lesions may be mistaken for osteomyelitis. However, the common abundance of eosinophils in a biopsy from a bone destructing lesion should raise the suspicion of LCH, and demonstration of the LCs by immunohistochemistry will be decisive.

Treatment and prognosis The treatment of LCH depends on the extent and severity of disease at diagnosis. Patients with localized disease generally have a high chance of spontaneous remission and a favorable outcome. In most unifocal bone lesions, a simple curettage or even a biopsy can provide diagnostic tissue and will often result in healing, and more extensive surgical resection is usually not recommended. Local therapies include steroids and irradiation. Intralesional instillation of steroids is an effective and safe treatment modality when therapy is required for a limited number of bony lesions [228]. For oral lesions, the preferred treatment was surgical curettage, and the prognosis was generally very favorable, as evidenced by the overall recurrence rate of 16% [229].

4.4.5.7 Melanotic Neuroectodermal Tumor of Infancy

Definition Melanotic neuroectodermal tumor of infancy was in the past also known as retinal anlage tumor or melanotic progonoma due to a presumed derivation from cells destined to form the retina.

Epidemiology Most of the lesions occur before the age of 1 year. The majority of them occur in the anterior maxilla [231, 232].

Etiology and pathogenesis Cells derived from the neural crest play a major role in the formation of the jaws and teeth. These cells are also thought to be the source from which the melanotic neuroectodermal tumor of infancy develops [1, 2, 233].

Clinical aspects Clinically, melanotic neuroectodermal tumor of infancy manifests itself as a rapidly growing blue tissue mass, usually at the anterior alveolar maxillary ridge. Radiologically, bone resorption may be seen although this is difficult to evaluate in the delicate bony structures of the infantile maxilla. Tooth germs are displaced and may lie within the tumor mass.

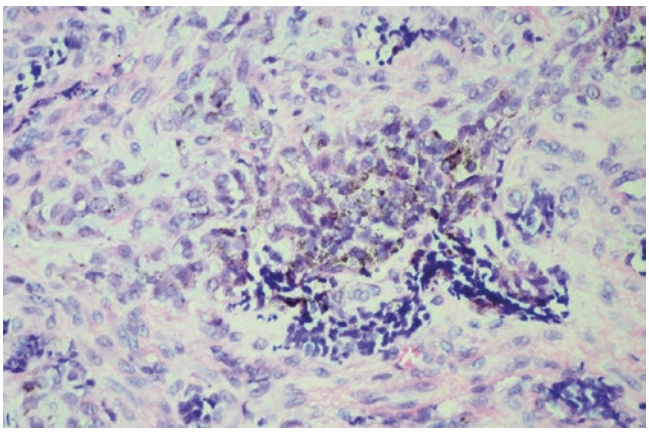

Fig. 4.66 Melanotic neuroectodermal tumor of infancy consists of small dark cells and larger cells with vesicular nuclei. Melanin is usually associated with the latter cell population

Microscopy The tumor shows dense fibrous stroma with nests composed of two different cell types: centrally placed small dark cells without any discernable cytoplasm and peripherally located larger cells with vesicular nuclei and ample cytoplasm with melanin pigment (Fig. 4.66) [231, 234, 235]. Maturation of the small cells to ganglion cells has been reported [236]. Although the cells may be atypical, mitotic figures are rare [234]. Sometimes, a transition of the large cells to osteoblasts forming tiny bony trabeculae can be observed [237]. The lesion is not encapsulated.

Immunohistochemistry Immunohistochemically, the large cells are positive for a wide variety of cytokeratins, neuron-specific enolase, S100, HMB45, and chromogranin. The small cells show positivity for CD56, neuron-specific enolase, synaptophysin, and chromogranin [234]. This pattern can be summarized as evidence for neural, melanocytic, and epithelial differentiation. In addition, the large cells have been shown to be positive for vimentin [237].

Differential diagnosis The highly specific age distribution and histomorphology leaves no room for differential diagnostic considerations. However, quite often, immature odontogenic tissues form part of the material excised or biopsied, due to the early age of occurrence and the close association of the tumor with tooth germs. This should not be mistaken as evidence for some type of odontogenic tumor.

Treatment and prognosis Conservative excision usually forms adequate treatment. Recurrences have been described. Metastases are exceptionally rare [235]. There are no histological features predicting a more aggressive behavior [234].

4.4.5.8 Myoepithelial Tumors

Definition Myoepithelial tumors of bone are rare lesions being morphologically and immunophenotypically similar to their counterparts in salivary glands and soft tissue [238].

Epidemiology Myoepithelial tumors of bone can occur at any age.

Clinical aspects Radiographically, the reported tumors show a radiolucency with a well-circumscribed sclerotic margin. Some lesions erode the cortex and push into adjacent soft tissue. CT scanning shows homogeneous dense tumors with well-defined margins. On MRI, neoplasms have a hypointense signal on T1-weighted images and a heterogeneous signal intensity on T2-weighted sequences. They enhance uniformly with gadolinium [239].

Macroscopy Lesions are described as mucoid or cartilaginous-like in appearance. Their cut surface are white, red gray, or pink and focally hemorrhagic [239].

Microscopy The tumors show the same histologic spectrum as their salivary gland counterparts [239]. Strands of epithelial cells with a cytoplasm that varies from eosinophilic to clear and lying in a fibromyxoid background with some chondroid differentiation may dominate the picture (Fig. 4.67). When there is no nuclear atypia, tumors are best classified as benign; in case of moderate to severe nuclear atypia with vesicular or coarse chromatin and prominent nucleoli, cases may behave in a malignant fashion [240].

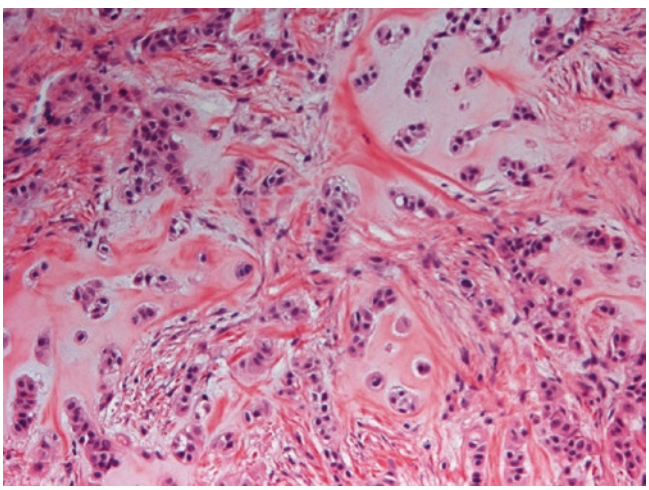

Fig. 4.67 Intraosseous myoepithelioma. Eosinophilic cells and matrix production mimic calcifying epithelial odontogenic tumor

Immunohistochemistry Confirmation of myoepithelial differentiation requires expression of epithelial markers (keratin and/or EMA) as well as either S100 or GFAP [241].

Genetics *EWSR1* rearrangement is common with up to 70% [238, 242]. Described fusion partners are *POU1F1*, *PBXI*, and *ZNF444* [238].

Differential diagnosis In case of a predominant epithelial component, the lesion may be mistaken for an epithelial or a mixed odontogenic tumor as this is the most likely possibility when one encounters a lesion with an apparently epithelial component within the jaw bone. This especially concerns tumors such as calcifying epithelial odontogenic tumor or odontogenic fibroma with abundant odontogenic epithelium. Furthermore, in case of nuclear atypia, metastatic carcinoma in the jaw bone enters the differential diagnosis. However, demonstrating a myoepithelial immunophenotype with the appropriate markers will lead to the correct diagnosis. Recognition of the possibility of an intraosseous myoepithelioma requires a high level of suspicion due to its rarity.

Treatment and prognosis Although experience with primary myoepithelial tumors of bone is rather limited, it seems that histopathologically bland tumors behave in a benign manner, and for them, conservative surgery appears to be sufficient treatment. In case of histological signs of malignancy, recurrences and metastases may occur [243].

4.4.6 Bone Tumors: Malignant

4.4.6.1 Osteosarcoma

Definition Osteosarcoma is composed of neoplastic cells that deposit a collagenous matrix, which may transform into bone through deposition of calcium salts.

Epidemiology Most cases occur in the long bones; approximately 10% of all cases occur in the maxillofacial skeleton, most frequently the jaw bones [244]. Jaw osteosarcomas may occur at any age, but they tend to occur approximately a decade later than osteosarcomas of the long bones, with the majority of patients being older than 30 years of age [208]. Males are affected more frequently than females. Mandible and maxilla are equally involved; in rare instances, the tumor may occur in other bones of the craniofacial area.

Clinical aspects Patients most frequently present with swelling and pain. Radiographic studies show a poorly defined mixed radiodense and radiolucent lesion, usually with soft tissue extension. Tumors may show a symmetrical widening of the periodontal ligament space which may be an extremely helpful diagnostic sign [245].

Microscopy Almost 50 % of the osteosarcomas that occur in the jaw bones are of the chondroblastic subtype [208]. These tumors show chondroid lobules that are bordered by a hypercellular rim of atypical cells that lie down osteoid. Sometimes osteoid production may be sparse and in those cases it may be difficult to distinguish between an osteosarcoma and a chondrosarcoma. Osteosarcomas of the osteoblastic, fibroblastic, or myxoid subtype also occur in the jaw bones.

Differential diagnosis Occasionally, osteosarcoma may be difficult to distinguish from a fibro-osseous lesion (Fig. 4.68). This especially is the case with well-differentiated osteosarcomas [246]. In those instances examination of the relationship between lesional bone and its surroundings is extremely important. Fibro-osseous lesions do not spread into soft tissues but remain confined within a cortical bone layer or a strip of fibrous periosteum. Moreover, entrapment of preexistent cancellous jaw bone by tumor bone is never observed in fibro-osseous lesions (Fig.4.69). Furthermore, low-grade osteosarcoma frequently shows amplification of *MDM2* and *CDK4*, and demonstrating these alterations either at the gene level with fluorescent in situ hybridization or at the protein level with immunohistochemistry will be helpful in distinguishing this lesion from other lesions characterized by deposition of bone in a fibrocellular background [247].

Occasionally, in maxillary cases an epithelioid appearance of osteoblasts may cause confusion with a salivary gland tumor (Fig. 4.70). Moreover, chondroblastic osteosarcoma should not be confused with chondrosarcoma, a point more extensively discussed in the next paragraph. Finally, nodular fasciitis involving the mandibular periosteum may mimic periosteal osteosarcoma in case of associated metaplastic ossification. This condition, also called periosteal fasciitis, is more extensively discussed in Chap. 12 under the heading nodular fasciitis.

Treatment and prognosis Osteosarcomas of the jaw are more likely to recur locally after treatment whereas distant metastases are observed less often than with the more common osteosarcomas arising in the long bones. The optimal treatment is complete resection. In a recent series, the metastatic rate was less than 20 %, and complete resection of the tumors resulted in a long-term survival of over 80 % after 10 years, whereas neoadjuvant or adjuvant treatment failed to show any additional favorable effect [248]. Therefore, the role of multimodality treatment should be critically scrutinized.

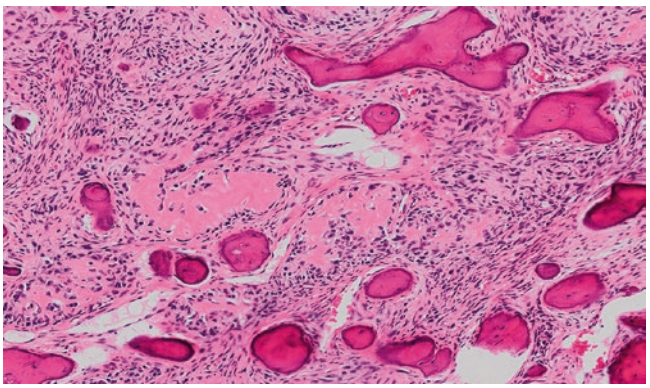

Fig. 4.68 Cell-poor bony fragments lying in a fibrous tissue suggest ossifying fibroma. However, the areas with atypical osteoid lined by enlarged cells reveal the true nature of this lesion; osteosarcoma with areas mimicking ossifying fibroma

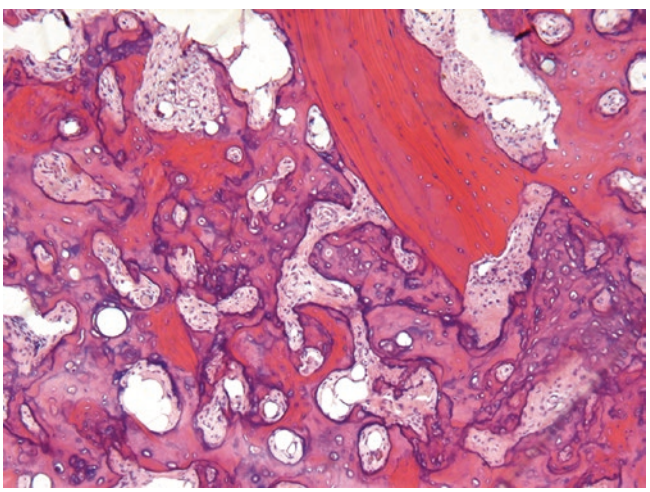

Fig. 4.69 Entrapment of lamellar bone by poorly mineralized osteoid are diagnostic for osteosarcoma invading adjacent jaw bone

4.4.6.2 Chondrosarcoma

Definition Chondrosarcoma is a malignant neoplasm characterized by the formation of cartilaginous matrix by neoplastic cells.

Epidemiology Head and neck chondrosarcoma is rare, accounting for less than 12 % of all cases of chondrosarcoma. The majority of affected patients are in the fourth decade of life, with a slight predilection for male patients.

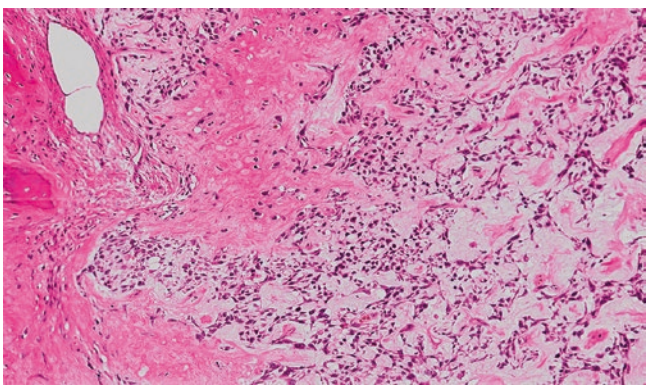

Fig. 4.70 Sometimes, the cells in osteosarcoma may lie in strands in a myxoid background. This pattern may mimic a salivary gland malignancy

The sinonasal cavities including the maxillary antrum are the locations most often involved [215, 249].

Clinical aspects Pain, swelling, and nasal obstruction are common complaints of patients with chondrosarcoma of the jaw and facial bones. Presenting symptoms vary depending on the site of origin of the tumor. Radiographs reveal a space-occupying lesion with bone destruction but without specific diagnostic features. Sometimes, chondrosarcoma manifests itself as a mainly exophytic mass with only limited invasion of the underlying bone [250].

Microscopy The histologic features of chondrosarcoma in the jaws and facial bones are the same as those in other bones. The tumor shows a lobulated growth pattern, hypercellularity, and cytologic atypia. Occasionally the chondrocytes are more spindle shaped, forming a lattice in a myxoid background. Chondrosarcomas are divided in three different grades: grade I tumors being moderately cellular and containing hyperchromatic plump nuclei of uniform size with occasional binucleated cells; grade II tumors being more cellular and with a greater degree of nuclear atypia, hyperchromasia, and nuclear size; and grade III tumors still more cellular and pleomorphic with easily detectable mitoses [251]. This grading system has been shown to be of prognostic significance for chondrosarcomas overall, but its usefulness could not be confirmed in a series of head and neck cases [215].

Clear cell chondrosarcoma and mesenchymal chondrosarcoma are specific histologic subtypes. The first is rarely seen in the head and neck area and will not be further mentioned here [252]; for the other, see the next paragraph.

Differential diagnosis Chondrosarcoma should not be confused with chondroblastic osteosarcoma which is not a remote pitfall since chondroid differentiation in osteosarcoma of the jaw is quite common. The distinction between both sarcoma types should be made by looking for the presence of osteoid formed by neoplastic cells, a feature not to be confused with bone shells bordering cartilaginous nodules or with endochondral ossification of matrix laid down by neoplastic chondrocytes. Both are secondary phenomena in chondrosarcomas and no arguments for a diagnosis of osteosarcoma.

In the area of the temporomandibular joint, presence of cartilage nodules may be due to *synovial chondromatosis*. Lack of histological atypia and careful clinical and radiologic documentation will lead to the appropriate diagnosis [253].

Occasionally, a nodular increase in cartilage in the nasal septum due to reactive changes may be misinterpreted as low-grade chondrosarcoma. In this case, conservative removal of any excess of tissue and keeping the patient under follow-up may be the most prudent management.

A peripheral chondrosarcoma should not be confused with *osteochondroma*, a cartilage-capped osseous projection protruding from the surface of the affected bone. These lesions have been reported in the skull base, maxillary sinus, zygomatic arch, and mandible. In the mandible, they occur most frequently at the condyle or coronoid processes [254]. Presence of a hyaline cartilage cap with chondrocytes that recapitulate the arrangement of a growth plate are sufficient distinct to allow differentiation of this lesion from a chondrosarcoma. For cases occurring in the skull base, chordoma enters the differential diagnosis. However, chondrosarcomas lack the physaliferous cells typically seen in chordoma. Moreover, the chordomas are positive with keratin markers, whereas chondrosarcomas are negative (see Sect. 4.4.6.4).

In extragnathic chondrosarcomas, detecting mutations in either *IDH1* or *IDH2* genes has been mentioned as a helpful diagnostic tool [247] but whether this also applies to chondrosarcoma of the maxillofacial bones is presently unknown.

Treatment and prognosis The most common cause of death is related to direct extension of the tumor. Distant metastasis is rare; if it occurs, deposits are most likely to be seen first in the lungs. Treatment is by surgical removal with wide margins. Radiation and chemotherapy are reported to have of little to no value. Overall survival in a series from the Mayo Clinic has been reported as 80.7 %, 65.3 %, and 56 % after 5, 10, and 15 years, respectively [215].

4.4.6.3 Mesenchymal Chondrosarcoma

Definition Mesenchymal chondrosarcoma is a malignant small round cell neoplasm with focal cartilaginous differentiation.

Epidemiology Mesenchymal chondrosarcoma represents approximately 1 % of all chondrosarcomas. It affects a very wide age range, but the peak frequency is in the second decade of life. Approximately 25 % of mesenchymal chondrosarcomas occur in the maxillofacial skeleton, in particular in the mandible and maxilla [215, 255].

Clinical aspects The most common presenting symptom of head and neck mesenchymal chondrosarcoma is a swelling or mass that may or may not be painful. Additional symptoms are those of a space-occupying mass and depend on location, e.g., diplopia for sinonasal cases and loosening of the teeth for cases in maxilla or mandible. Radiological examination shows features of a bone destructing tissue mass with spread in adjacent soft tissues or, if at that site, in sinonasal cavities.

Microscopy Mesenchymal chondrosarcoma consists of two distinct components: well-differentiated hyaline cartilage and an undifferentiated, small round cell malignancy

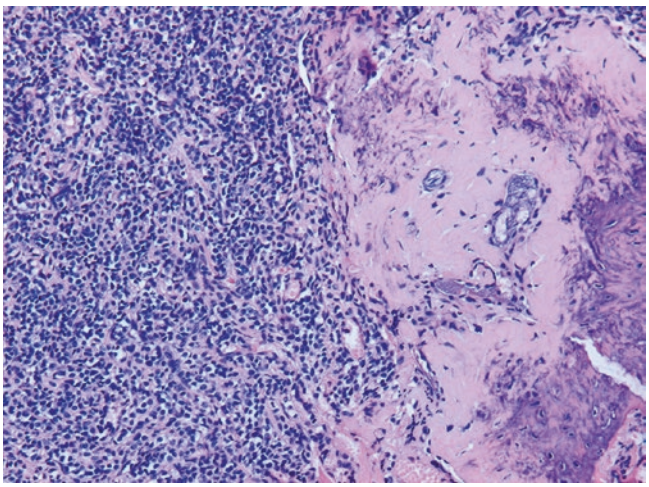

Fig. 4.71 Mesenchymal chondrosarcoma shows a small round blue cell component in close association with partly mineralized chondroid matrix

(Fig. 4.71). The proportions of each of them may vary among individual cases. The chondroid areas usually show features of low-grade chondrosarcoma although, sometimes, the matrix is more fibrous than hyaline and with a peculiar light blue hue when stained with hematoxylin and eosin. In this matrix, areas of calcification or ossification may occur. The high-grade small cell component is composed of cells with hyperchromatic nuclei, inconspicuous nucleoli, and scant cytoplasm. These cells lie in fields and sheets and sometimes surround branching thin-walled vessels.

Immunohistochemistry The cartilaginous areas are positive for S100 protein as is common for chondroid tissue. The small cells are usually positive with CD99 and may also show focal immunoreactivity with myogenic markers desmin, MyoD1, and smooth muscle actin. There is no expression of myogenin, cytokeratins, and HMB-45 [215].

Genetics A *HEY1–NCOA2* fusion has been identified in mesenchymal chondrosarcomas and the consistent detection of this finding identifies it as a marker of potential diagnostic value [256].

Differential diagnosis When the cartilaginous component is absent in a biopsy sample, the tumor may be confused with a spectrum of small blue round cell neoplasms. They include synovial sarcoma, Ewing's sarcoma, olfactory neuroblastoma, rhabdomyosarcoma, undifferentiated carcinoma, and malignant lymphoma. Appropriate use of immunohistochemistry and molecular markers will be needed to rule out or to confirm these alternative diagnoses. Moreover, deeper cuts or more extensive gross sampling may reveal the presence of cartilage not present in the initially examined material.

Treatment and prognosis Management is primarily surgical. Although adjuvant radiation appears to convey some benefit by reducing tumor bulk when these lesions have extended beyond bony confines, there is no evidence to suggest that this is associated with improved outcome. Chemotherapy does not appear to be effective in the limited experience documented thus far. Patients with complete local control following resection should be followed closely for development of distant metastasis, which signifies a worse clinical outcome [255]. The 5-year and 10-year survival rates have been reported to range from 35 to 60 % and 20 to 40 %, respectively [215].

4.4.6.4 Chordoma

Definition Chordoma is a bone tumor that is aggressive, locally invasive, and has a poor prognosis. These tumors are thought to arise from transformed remnants of the notochord, and they have a predilection for the axial skeleton, with the most common sites being the sacrum, skull base, and spine.

Epidemiology Mostly chordomas occur at either the cranial or caudal end of the vertebral column [257]. Available data indicate an almost equal distribution in the skull base (32 %), mobile spine (32.8 %), and sacrum (29.2 %) [258]. Chordomas show a slight male predominance; they may occur at any age [257].

Clinical aspects Chordomas manifest themselves by destroying adjacent structures resulting in cranial nerve dysfunction. Rarely, they cause a swelling in the neck due to lateral growth. Radiographically they appear as destructive bone lesions with a surrounding soft tissue mass [258].

Microscopy Three different types of chordoma are discerned: conventional, chondroid, and dedifferentiated. Conventional chordoma consists of lobules separated from each other by fibrous bands. These lobules contain ovoid cells with small, dark nuclei and homogeneous eosinophilic cytoplasm. Other cells show large vesicular nuclei and abundant cytoplasm with vacuoles. Sometimes, these cells contain only one single vacuole causing a signet-ring appearance or vacuoles surrounding the nucleus: these latter cells represent the so-called physaliferous cells thought to be pathognomonic for chordoma. In general, the cell density is maximal at the periphery of the lobules; more centrally, the cells lose their epithelial cohesion and may lie isolated in abundant mucoid matrix (Fig. 4.72). Although there may be atypia, mitotic figures are infrequent. The lesion invades adjacent structures.

Chondroid chordoma denotes a variant of chordoma that contains cartilaginous areas indistinguishable from chondrosarcoma [257]. Probably however, chondrocytic differentiation in chordomas represents a focal maturation

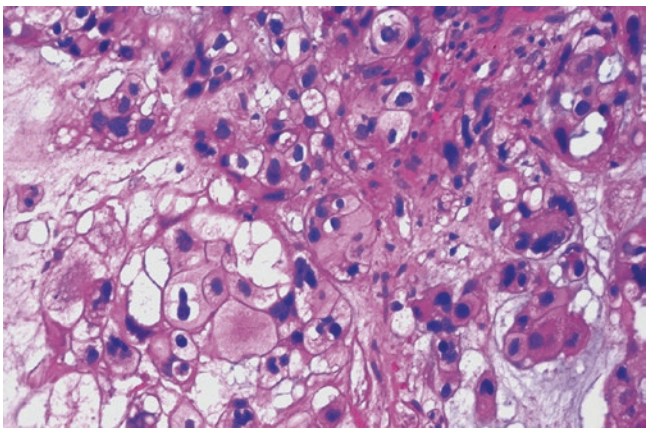

Fig. 4.72 At high magnification, the vacuolated nature of the chordoma cells are clearly visible as is their epithelial cohesion

process [259]. Neither has the distinction between conventional and chondroid chordoma any clinical significance. Chondroid chordomas behave in a manner that is clinically similar to chordomas, with the same prognosis [260]. Dedifferentiated chordomas are those lesions that contain areas of chordoma as well as an additional malignant mesenchymal component that may be either a fibrosarcoma, an osteosarcoma or, mostly, a poorly differentiated sarcoma [261].

Immunohistochemistry Chordoma is characterized by positivity for S100 as well as vimentin and a broad variety of epithelial markers [262]. Moreover, the brachyury transcription factor, known to be involved in notochordal development, has been shown to be a highly specific chordoma marker [263–265]. This brachyury expression in more than 90 % of chordomas makes it a unique, specific marker that together with other sensitive markers like cytokeratin, epithelial membrane antigen, and S100 protein substantiates a diagnosis of chordoma.

Differential diagnosis Chordoma has to be distinguished from chondrosarcoma. Positivity for epithelial markers is a consistent feature in chordomas and absent in chondrosarcoma [262]. Other look-alikes such as extraskeletal myxoid chondrosarcoma, myxoid liposarcoma, and myxopapillary ependymoma also lack positivity for epithelial markers [266]. Chordoid meningiomas may also mimic chordoma but there are no physaliferous cells, and there is also no positivity for cytokeratins in this meningioma subtype [267].

The differential diagnosis of chordoma should also include pleomorphic adenoma, especially when the lesion presents itself as a submucosal sinonasal or nasopharyngeal mass. Both lesions may show epithelial clusters as well as single cells with vacuolated cytoplasm lying in a mucoid matrix. Moreover, positivity for S100, vimentin, and epithelial markers is displayed by both. Positivity for myoepithelial markers however is restricted to pleomorphic adenoma [268].

Treatment and prognosis The site of skull base chordoma precludes radical surgical treatment. Although chordomas are not typically metastatic on presentation, distant metastasis may occur. 5 % of chordomas show metastasis to the lungs, bone, skin, and brain at the time of initial presentation, and as high as 65 % are metastatic in very advanced disease [258]. However, patient survival seems to be less affected by distant metastasis than by local progression of the disease. Mostly, therapy consists of debulking and irradiation. In a recent review across multiple institutions that encompassed 560 patients treated for cranial chordoma, the survival rate among these patients was 63 % and 16 % for 5-year and 10-year survivals, respectively. There was no difference between 5-year survival in patients with chordoma with histological chondroid features and those with chordoma possessing typical histology. When patients who only received surgery were compared to those patients who were treated with surgical intervention in combination with adjuvant radiation treatment, no difference in survival rate was found. The authors conclude that adjuvant radiation therapy and histological type were not associated with an improvement of survival rates [269].

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Major and Minor Salivary Glands

5

Silvana Di Palma, Roderick H.W. Simpson, Alena Skalova, Isabel Fonseca, Ilmo Leivo, and Stephan Ihrler

Contents

S. Di Palma, MD, FRCPath (✉)
Department of Histopathology, Royal Surrey County Hospital, University of Surrey, Egerton Road, Guildford GU2 7XX, Surrey, UK
e-mail: sdipalma@nhs.net

R.H.W. Simpson, MD
Department of Anatomical Pathology, Foothills Medical Centre, University of Calgary, 1403, 29th Street NW, Calgary T2N 2T9, AB, Canada
e-mail: roderick.simpson@cls.ab.ca; roderick.simpson@doctors.org.uk

A. Skalova, MD, PhD
Department of Pathology, Faculty of Medicine in Plzen, Charles University Prague, Plzen, Czech Republic
e-mail: skalova@fnplzen.cz; skalova@medima.cz

I. Fonseca, MD, PhD
Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

Serviço de Anatomia Patológica, Instituto Português de Oncologia Francisco Gentil - Lisboa, Lisbon, Portugal
e-mail: ifonseca@medicina.ulisboa.pt; ifonseca@ipolisboa.min-saude.pt

I. Leivo, MD, PhD
Department of Pathology and Forensic Medicine, Institute of Biomedicine, University of Turku, Turku, Finland
e-mail: ilmo.leivo@utu.fi

S. Ihrler
Laboratory for Dermatopathology and Oral Pathology, Munich, Germany
e-mail: ihrler@dermpath-muenchen.de

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5.1 Introduction

5.1.1 Normal Salivary Glands

The salivary glands include paired major glands (parotid, submandibular and sublingual) and thousands of minor glands throughout the upper aerodigestive tract.

Structurally the salivary glands are made up of three components. Firstly, the peripheral acinus with luminal, saliva-producing acinar cells and abluminal myoepithelial cells. Secondly, the short intercalated duct with luminal cuboidal intercalated duct cells and abluminal myoepithelial cells. Thirdly, the long striated and excretory ducts, both with luminal oxyphilic cells and inconspicuous abluminal basal cells. These basal cells, stained by cytokeratin subtypes 14 and 5/6, as well as p63, comprise an important pluripotent reserve cell pool for cellular regeneration, for different types of reactive metaplasia and possibly also for the complex tumorigenesis of salivary glands [1–3].

Myoepithelial cells have contractile properties that assist in the secretion of saliva. Similar cells are found in the breast and in the tracheobronchial and sweat glands. They are plentiful in the acini and intercalated ducts, but are largely absent from striated ducts. They are thin and spindle shaped and situated between the basement membrane and epithelial cells, and ultrastructurally they possess long cytoplasmic processes. They display features of both smooth muscle and epithelium. Accordingly, immunohistochemistry shows strong staining with alpha smooth muscle actin (αSMA), calponin, smooth muscle myosin heavy chain (SMMHC) [4], h-caldesmon [5], S-100 protein [6], as well as with some cytokeratins (e.g. subtype 14) and p63 [2, 3]. Scattered nests of sebaceous cells can be seen in normal parotid and minor salivary glands [3, 7].

The acinar component in the parotid glands consists predominantly of serous cells, whilst in the submandibular and minor glands, it comprises a mixture of serous and mucous cells and in the sublingual glands purely mucous cells. Serial sectioning has shown an average of 20 lymph nodes within each parotid, and they may be affected by inflammatory processes and neoplasms, both primary and metastatic [8]. It may be difficult, especially in small biopsies, to distinguish lesions of intraparotid lymph nodes from lymphatic tissue in inflammatory lesions (i.e. lymphoepithelial sialadenitis) or from reactive tumor-associated lymphatic tissue [9, 10].

5.1.2 Developmental Disorders

Agenesis, aplasia, hypoplasia and atresia of glands or of the main ducts are all extremely rare. In contrast, parenchymal inclusions in intraparotid lymph nodes are very common [1], and epithelial tumors may develop from them [11]. Extranodal heterotopia is rare and can be subdivided into high (involvement of the ear, pituitary, mandible, etc.) or low forms (lower neck, thyroid). Accessory parotid glands comprising salivary tissue separate from the main gland, adjacent to Stensen's duct, are found in 20 % of people.

5.2 Obstructive Disorders

5.2.1 Mucus Escape Reaction (Extravasation Mucocoele/Ranula)

Definition Pooling of saliva in a cavity not lined by epithelium.

Epidemiology Most patients are under 30 years of age, and the minor salivary glands (especially of the lower lip) are most often affected.

Etiology and pathogenesis The relative incidence in lower lip is 65 %, buccal mucosa 10 %, palate 4 %, parotid 0.6 % and submandibular and lingual glands 1.2 %. The pathogenesis is in most cases traumatic severance of a duct (e.g. from biting), leading to mucus pooling.

Clinical aspects It presents in the lip as a raised, often blue, dome-shaped swelling of the mucosa usually 2–10 mm in diameter. Cases developing from the sublingual gland in the floor of mouth where it is known as ranula (Latin for 'small frog') are usually significantly larger.

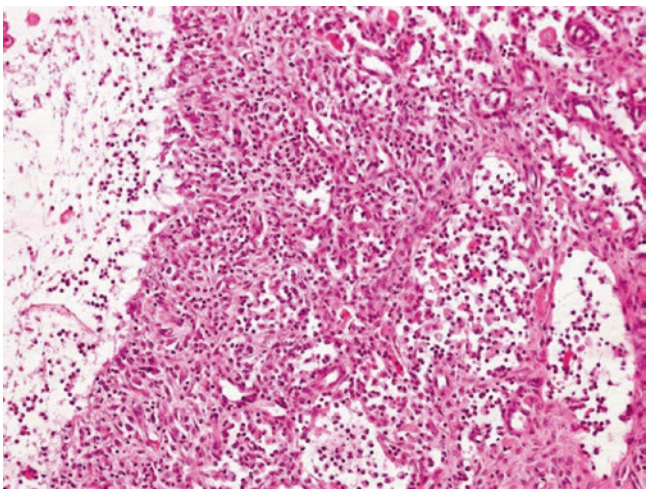

Fig. 5.1 Extravasation mucocoele (mucous escape reaction): mucin-filled cavity lined with granulation tissue and macrophages

Macroscopy Pseudocyst with a white wall containing mucus-like material.

Microscopy Shows a well-defined mucin-filled cavity lined not by epithelium but by granulation tissue and macrophages (Fig. 5.1).

Differential diagnosis The most important differential diagnosis in cases with numerous clear cell macrophages is with low-grade mucoepidermoid carcinoma. The latter includes squamous, intermediate (some of which have clear cytoplasm) and mucinous goblet cells. The differential diagnosis may be difficult in a small incisional biopsy, but immunohistochemistry separates CD68-positive macrophages from cytokeratin-positive tumor cells.

Treatment and prognosis Treatment consists of complete surgical excision. Recurrences can occur for incompletely excised lesions.

5.2.2 Chronic Sclerosing Sialadenitis of the Submandibular Gland (Küttner Tumor)

Definition A tumor-like condition affecting either one or both submandibular glands.

Epidemiology It can occur at any age (mean 45 years) and affects both sexes.

Etiology and pathogenesis It is a consequence of ductal obstruction resulting from several different causes. In up to 80 % of cases, large calculi in the excretory ducts are generally considered to be responsible, with a lower percentage due to small intraparenchymal microliths [12, 13]. In addition, IgG4-related sclerosing disease is a factor in rare cases (see below).

Clinical aspect Patients present with recurrent or persistent unilateral submandibular pain and or swelling frequently associated with eating.

Macroscopy The submandibular gland is replaced by white and firm tissue resembling a tumor.

Microscopy The histopathological picture of chronic sclerosing sialadenitis varies depending on the stage of the process; it evolves from just scattered lymphoplasmacytic aggregates to severe changes of acinar atrophy and heavy chronic inflammation with germinal centre formation, leading eventually to an end stage of destruction of the lobular architecture and scarring. The inflammation is centred on the acini rather than ducts, although minor intraductal aggregates of neutrophils are often present. Lymphoepithelial lesions are absent or very rare [9, 10, 12, 13].

Differential diagnosis The main histological differential diagnosis in surgically resected submandibular glands is hyper-IgG4 disease (see Sect. 5.4.1) and malignant lymphoma (see Sect. 5.10.3), whilst Sjögren-type lymphoepithelial sialadenitis (see Sect. 5.10.1) is only rarely diagnosed in this gland in the absence of parotid involvement.

Treatment and prognosis The prognosis is good after complete surgical removal.

5.2.3 Chronic Sialectatic Parotitis

Definition A tumor-like inflammatory condition affecting parotid glands.

Epidemiology An uncommon disease occurring mostly in (male) children and only rarely in adults.

Etiology and pathogenesis Although the pathogenesis is unknown, an allergic reaction has been postulated.

Clinical aspect Patients present with chronic, recurrent and mostly bilateral and painful swelling of the parotid glands.

Macroscopy Biopsies are rarely indicated or performed for chronic sialectatic parotitis.

Microscopy Histology shows chronic lymphocytic infiltration around large, interlobular ducts with prominent germinal centres and ductal cysts, devoid of Sjögren-type lymphoepithelial lesions.

Differential diagnosis Clinical correlation is necessary for a correct diagnosis. There is no association with Sjögren's syndrome or HIV infection [10].

Treatment and prognosis Surgical treatment is reserved only for extreme cases, as the prognosis is that of a recurrent and chronic disease. In the juvenile variant, the disease often subsides at the end of puberty.

5.3 Infections of Salivary Glands

Definition Infective disease of salivary glands caused by bacteria, fungi and viruses.

5.3.1 Bacteria and Fungi

Epidemiology Both are rather infrequent causes of infections in Europe or North America, but tuberculosis is not uncommon in Africa.

Etiology and pathogenesis The changes are secondary to inflammation caused by the infective agent, such as tuberculosis, cat scratch disease, syphilis, tularaemia, brucellosis and toxoplasmosis.

Clinical aspect The clinical presentation may be that of a salivary gland mass.

Macroscopy Surgical excision is rarely performed.

Microscopy Inflammation with or without granulomata may involve the gland itself or the intraparotid lymph nodes. Special stains for microorganisms are recommended, but may not always identify the causative organisms, particularly in tuberculosis. It may be said (particularly in high incidence areas) that any granulomatous inflammation is tuberculosis until clinically proven otherwise.

Differential diagnosis The differential diagnosis includes non-infectious granulomatous disorders, in particular sarcoidosis (see below).

Treatment and prognosis The patient is managed with medical therapy, which usually resolves the infection.

5.3.2 Viruses

Definition Infective lesion caused by viruses.

Epidemiology Several viral diseases may cause glandular infiltration by chronic inflammatory cells but are rarely biopsied. The most common are mumps and cytomegalovirus (CMV) infection. Mumps (epidemic parotitis) presents as an acute illness occurring in children and young adults. Following the introduction of a vaccine from attenuated live mumps virus, the incidence of the disease has decreased significantly.

CMV may be localised to the salivary gland or may involve the glands as part of a systemic disease in either the new-born or in immunocompromised adults particularly those with HIV (human immunodeficiency virus)/AIDS (acquired immune deficiency syndrome). The diagnosis of CMV is made by finding the characteristically enlarged acinar cells with intranuclear inclusions [14, 15].

Microscopy The salivary glands show a non-specific chronic inflammatory cell infiltrate.

Differential diagnosis The histopathological differential diagnosis is with other infections and chronic inflammatory infiltrates, and the diagnosis is best confirmed by virology tests.

Treatment and prognosis Disease in the salivary glands tends to resolve without morbidity.

5.3.2.1 HIV-Related Disease

The most characteristic lesion seen in HIV/AIDS is cystic lymphoid hyperplasia (see below – Sect. 5.7.4). Other lesions seen in the salivary glands in AIDS include infections due to immune compromised state, lymphoma and intraparotid lymphadenopathy [16].

5.4 Miscellaneous Inflammatory Disorders

5.4.1 Hyper-IgG4 Disease

Definition A fibrous inflammatory disease of exocrine glands with excess IgG4-positive plasma cells.

Epidemiology Patients are usually adults, and there is no sex predilection.

Etiology and pathogenesis The true incidence is not known. In the head and neck, hyper-IgG4 disease seems to comprise a small proportion of cases of chronic sclerosing submandibular sialadenitis.

Clinical aspect Patients present with a tumor-like mass alone or associated with systemic IgG4-related sclerosing disorders such as autoimmune pancreatitis, autoimmune cholangitis, lesions in retroperitoneum/mediastinum and

orbital swellings. The so-called pseudotumour of the orbit is part of the manifestations of hyper-IgG4 disease.

Macroscopy The salivary gland is replaced by white and firm tissue which mimics a malignant tumor.

Microscopy The histology shows preservation of salivary gland lobules, but there is significant inter- and intralobular fibrosis with acinar atrophy. There are no lymphoepithelial lesions, but there is a lymphoplasmacytic infiltrate, which contains numerous IgG4-positive plasma cells. In a series of 13 cases [17], there was a mean of 229/high-power field (HPF) (range 75–608) IgG4-positive plasma cells and an overall IgG4/IgG ratio of 0.86. This significantly higher number of IgG4-positive plasma cells contrasts with the lower number of IgG4-positive plasma cells seen in chronic sialadenitis, not otherwise specified [18].

Differential diagnosis The main histological differential diagnosis is classical chronic sclerosing submandibular sialadenitis associated with lithiasis and Sjögren-type lymphoepithelial sialadenitis (both mainly devoid of IgG4-positive plasma cell) [19, 20].

Treatment and prognosis Diagnosis in multisystemic disease is important as immunosuppressive therapy (systemic corticosteroid) is very effective [18–20].

5.4.2 Sarcoidosis

This multisystem granulomatous disorder of unknown etiology is an important and relatively frequent cause of granulomatous inflammation in intraparotid lymph nodes or the salivary glands themselves. The histological picture is typically that of multiple well-demarcated non-necrotising granulomata composed of epithelioid histiocytes with or without scattered multi-nucleate giant cells; asteroid and Schaumann bodies may be present on occasions.

5.4.3 Kimura's Disease

This is seen predominantly in Oriental patients and frequently affects the salivary glands. Microscopy shows acinar atrophy and fibrosis, often affecting surrounding ducts, and a heavy lymphoid infiltrate with formation of irregularly shaped follicles, together with numerous eosinophils often forming abscesses, typically within germinal centres. There is also a proliferation of high endothelial venules with slit-like lumina lined by non-vacuolated cuboidal or atrophic endothelial cells containing pale oval nuclei. Recurrences sometimes occur after excision.

5.5 Miscellaneous Non-inflammatory Disorders

There are a variety of non-infectious inflammatory conditions such as xanthogranulomatous sialadenitis, Rosai-Dorfman disease [21] and amyloidosis [22] that will not be discussed here.

5.5.1 Necrotising Sialometaplasia (Salivary Gland Infarction)

Definition Reactive vascular and/or inflammatory condition of salivary glands.

Epidemiology This is a rare condition affecting mostly the intraoral minor salivary glands – palate in particular – of adults, mainly male, patients.

Etiology and pathogenesis The classical necrotising sialometaplasia is a reaction pattern of salivary glands following salivary gland infarction. Although the underlying pathogenesis is generally considered to be ischaemia, in addition to trauma such as from an ill-fitting denture, often no predisposing factor is found.

Clinical aspect Patients present with an ulcer in the palate which is often biopsied with a clinical suspicion of malignancy. The symptoms may also appear after oral surgery for unrelated causes. Also, there has been a case where the trauma was caused by a stalk of a peach, the patient had eaten few weeks before.

Macroscopy The specimens are usually received as biopsy fragments.

Microscopy Microscopy in the early stages shows partial necrosis of salivary lobules with later a moderate chronic inflammatory infiltrate and immature or mature squamous metaplasia, partly or totally replacing the lobules (Fig. 5.2) [23, 24].

Differential diagnosis Due to reactive cellular atypia and increased proliferative and mitotic activity, there is a superficial resemblance to mucoepidermoid and squamous cell carcinoma. The preserved lobular architecture of the lesion together with the clinical situation (mostly postoperative) is of paramount importance for the correct diagnosis [2, 24].

Treatment and prognosis Once the diagnosis has been confirmed, the behaviour is that of a benign self-healing disease.

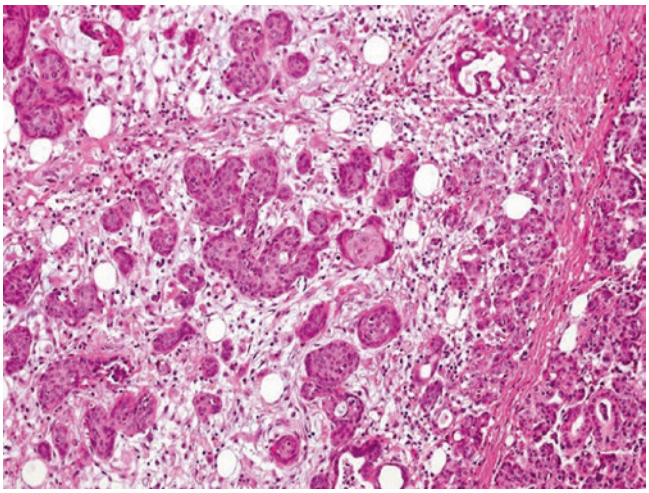

Fig. 5.2 Necrotising sialometaplasia. Most of the ducts and acini are replaced by mature non-keratinizing squamous epithelium. The lobular architecture of the gland is preserved

5.5.2 Sialadenosis

Definition Sialadenosis is a non-inflammatory disease of the salivary glands characterised by recurrent and painless bilateral swelling mainly of the parotid glands.

Epidemiology It is most frequent in young women, but its exact incidence is not known as the parotid enlargement may be interpreted as accumulation of facial fat clinically.

Etiology and pathogenesis There is an association with eating disorders (e.g. anorexia, bulimia), malnutrition, chronic alcoholism and liver cirrhosis and with some drugs such as antihypertensive agents and antidepressant [25]; also, sialadenosis has been related to some endocrine disorders (diabetes mellitus, ovarian and thyroid insufficiencies). Recent data suggest that the underlying process may be a disorder of the autonomic nervous system affecting salivary gland innervation, causing a secondary functional atrophy of myoepithelial cells [25].

Clinical aspect Patient complains of cosmetically disfiguring swelling of parotid glands which may be mildly painful.

Microscopy Histologically, there is generalised enlargement of the serous acinar cells (two or three times the normal size) with slight compression of striated ducts but totally devoid of inflammation.

Differential diagnosis As morphometric measurements usually are not available or performed, the subjective impression of enlarged acini should always be correlated with the clinical findings.

Treatment and prognosis If the predisposing factors (eating disorder, drugs, alcohol, etc.) can be corrected, sialadenosis tends to regress.

5.5.3 Adenomatoid Hyperplasia of Salivary Glands

Adenomatoid hyperplasia of salivary glands can be subdivided into two categories as follows:

5.5.3.1 Acinar Adenomatoid Hyperplasia

Definition Acinar adenomatoid hyperplasia (AAH) is a hyperplastic disorder of salivary glands.

Epidemiology It is a rare condition mostly affecting intraoral minor salivary glands, the palate in particular. It can affect all ages, although most patients are between 30 and 60 years old. There is a slight male predominance.

Etiology and pathogenesis The etiology is unknown; possible relevant factors include local trauma due to denture or tobacco smoking.

Clinical aspect This lesion is usually asymptomatic, often being noted on routine oral or dental examination. Most cases occur in the palate, but other minor salivary glands can be involved [26].

Macroscopy Examination reveals a nodular submucosal swelling up to 30 mm in diameter; usually biopsy fragments are provided.

Microscopy Histology shows hyperplastic mucous or seromucous acini with preserved lobular arrangement usually devoid of significant inflammation or fibrosis.

Differential diagnosis The histological diagnosis is a matter of exclusion of other hyperplastic and neoplastic conditions of minor salivary glands.

Treatment and prognosis The treatment tends to be surgical and the prognosis is good.

5.5.3.2 Intercalated Duct Hyperplasia (Also Known as Ductal Adenomatoid Hyperplasia)

Definition Hyperplastic disorder of intercalated ducts.

Epidemiology Relatively rare, idiopathic condition.

Etiology and pathogenesis The etiology is unknown, but there is an association in literature between intercalated duct

hyperplasia/adenoma and benign and malignant tumors of the salivary glands [27–29]. The recognition in literature of intercalated duct hyperplasia has shed light about the histogenesis of some tumors such as epithelial-myoepithelial carcinoma as a tumor of ductal origin and also perhaps explained why in hybrid carcinomas of the salivary glands, equally very rare, the most frequent combination is that of EMC and adenoid cystic carcinoma [28].

Clinical aspect The clinical presentation is that of the associated tumor where intercalated duct hyperplasia represents an incidental finding at histological examination.

Macro Appearance is also that of the associated tumor for which the specimen is surgically resected.

Microscopy It is either a well-circumscribed single nodule or multiple unencapsulated foci of proliferating intercalated ducts. The ducts are small in size and are lined by an inner layer of cuboidal cells and an outer layer of myoepithelial cells [27].

Differential diagnosis When not associated with another tumor of salivary gland, the differential diagnosis is with a small basal cell adenoma.

Treatment and prognosis The treatment is surgical, and the prognosis is that of the accompanying tumor. Intercalated ductal hyperplasia in itself has a good prognosis.

5.5.4 Irradiation Changes

Definition Cytohistological changes of salivary glands following radiotherapy.

Epidemiology Irradiation changes are relatively common in patients with head and neck cancers since radiotherapy is used as part of their treatment.

Etiology and pathogenesis Acini (particularly serous) are very sensitive to radiation.

Clinical aspect The common clinical sign is tenderness and swelling of the irradiated glands, which is followed by xerostomia as a common complication.

Macroscopy The glands have increased consistency, but the lobular architecture is preserved.

Microscopy The early changes are those of swelling, vacuolation and necrosis of ductal and acinar cells [30]. An initial acute inflammatory response is later followed by chronic, non-specific inflammation with acinar atrophy, dilatation of excretory ducts and squamous and mucous metaplasia. The metaplastic cells may display cellular atypia and cytoplasmic vacuolisation. In more advanced stages, there is a major loss of acinar parenchyma, fibrosis, lipomatosis, chronic inflammation and changes in arteries.

Differential diagnosis Is mostly with squamous cell and mucoepidermoid carcinoma. In irradiation changes, the cytological atypia is confined within ducts and acini, whilst mucoepidermoid and squamous cell carcinomas replace the normal tissue.

Treatment and prognosis Dependent on the extent of salivary gland involvement, loss of saliva production may be progressive and irreversible. Recent experimental studies have shown that radiation-damaged salivary glands can be restored and reacquire their morphology and function.

5.5.5 Tissue Changes Following Fine Needle Aspiration

Definition Histological changes of normal and pathological tissue of salivary glands following fine needle aspiration (FNA).

Epidemiology Not very common phenomenon despite the common use of FNA in the preoperative assessment of salivary gland lesions [31].

Etiology and pathogenesis Possible causes include trauma by pressure or by FNA [32] with vascular damage and aggravated sensitivity of oncocytic cells to hypoxia.

Clinical aspect These are non-specific and are those of a salivary gland mass for which FNA is being undertaken. In some cases tumor-like changes can develop such as so-called xanthogranulomatous sialadenitis with an exuberant inflammation after total or subtotal tumor necrosis following FNA.

Macroscopy The surgical specimen may contain the needle tract and areas of necrosis and haemorrhage.

Microscopy These comprise focal necrosis and inflammation up to subtotal or total infarction with or without reactive pseudo-malignant changes [31–33]. This is most frequent in Warthin's tumor [34]. A reticulin stain may identify the ghost architecture, and immunohistochemistry with an antimitochondrial antibody may highlight residual oncocytic differentiation of the epithelial cells [35]. Significant infarction has also been noted in acinic cell carcinoma, but the histological diagnosis was not compromised [36].

Differential diagnosis The necrotic and pseudo-malignant changes can be confused with carcinoma of squamous or mucoepidermoid type.

Treatment and prognosis Surgical excision is usually curative. Despite some worrisome histological findings, there is general agreement that FNA of salivary gland lesion is a safe procedure, and it does not usually alter the histological diagnosis [36].

5.6 Oncocytic Lesions

Definition Lesions composed of oncocytic cells which possess densely eosinophilic and granular cytoplasm due to excess mitochondria. Two cell types are recognised: light and dark. The former contain abundant eosinophilic cytoplasm with central, round nuclei and visible nucleoli; the latter possess pyknotic nuclei, with relatively scanty densely eosinophilic cytoplasm. Recognition of these two cell types is essential in the differential diagnosis of primary and metastatic tumors with oncocytic characteristics (see Sect. 5.8.5).

Epidemiology Oncocytic change is frequent in salivary glands, particularly in older people. The cells can be confirmed by an antimitochondrial antibody [35, 37].

Etiology and pathogenesis Research in mitochondrial DNA mutations suggests that a molecular genetic abnormality interferes with mitochondrial DNA which leads to the increased number of enlarged mitochondria in the cytoplasm of oncocytic cells (mitochondriopathy) [38].

In salivary glands, three main types of oncocytic lesions are recognised.

5.6.1 Focal and Diffuse Oncocytosis

Definition Small microscopic foci of oncocytic metaplasia, usually of ducts, but occasionally also acini.

Epidemiology Focal oncocytosis occurs with increasing frequency with advancing age (Fig. 5.3) [2]. In contrast, diffuse oncocytosis of the parotid is extremely rare.

Etiology and pathogenesis Genetic changes affecting mitochondrial DNA may have a role (see above) [38].

Clinical aspects There are no specific clinical signs unless oncocytosis is associated with a salivary gland tumor causing a clinical mass. Specimens for histological examination usually are removed for other reasons (primary and metastatic tumors, neck dissection, etc.).

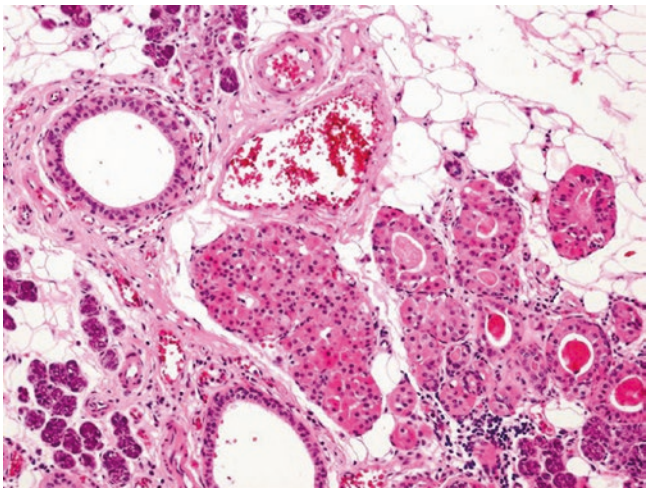

Fig. 5.3 Focal oncocytosis of the parotid gland. Some ducts and acinar cells show cytoplasmic oncocytic features

Macroscopy The affected salivary gland may show involutional fatty changes only.

Microscopy Histological examination shows ducts and acini replaced by *light* and *dark* oncocytic cells involving one or more lobules (focal oncocytosis) or involving virtually the whole gland (diffuse oncocytosis – very rare and often bilateral).

Differential diagnosis In small biopsy specimens, it may be difficult to distinguish from Warthin's tumor and oncocytoma or other tumors showing oncocytic metaplasia, e.g. pleomorphic adenoma, myoepithelioma and mucoepidermoid carcinoma (see Sects. 5.8.1, 5.8.2, 5.8.4, 5.8.5 and 5.9.3).

Treatment and prognosis The prognosis is favourable with or without surgical resection.

5.6.2 Multifocal Nodular Oncocytic Hyperplasia

Definition Multifocal nodular oncocytic hyperplasia (MNOH) is a partial replacement of salivary gland ducts and acini by multiple foci of oncocytic cells.

Epidemiology It is a rare condition observed from time to time in routine surgical pathology. Adults are affected and the lesion can be bilateral.

Etiology and pathogenesis Etiology is unknown.

Clinical aspects Parotid swelling – sometimes bilateral – is the common clinical presentation.

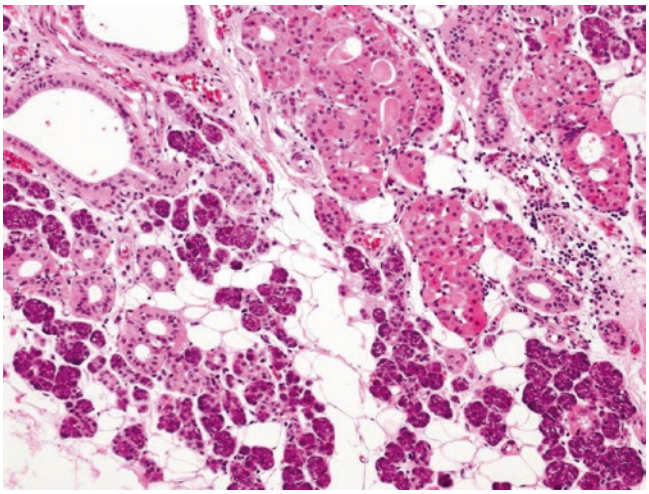

Fig. 5.4 Multiple nodular oncocytic hyperplasia. Nodules composed of oncocytic cells that can show clear change

Macroscopy The parotid shows multiple nodules of varying size, but the diagnosis is essentially microscopic.

Microscopy The oncocytic nodules appear to engulf normal acini giving a false impression of invasion. The nodules are circumscribed, but not usually encapsulated, but elicit no reaction in the surrounding normal parenchyma. The oncocytic cells often have clear and only faintly granular cytoplasm (Fig. 5.4). If a capsule or part of it is noted, a diagnosis of oncocytoma should be favoured. The two conditions can coexist, but there is little agreement on how to distinguish oncocytoma in a background of MNOH.

Differential diagnosis The main differential diagnosis is solid-type acinic cell carcinoma. The immunohistochemical demonstration of a small component of basal cells (CK5/6, p63 positive) is in favour of an oncocytic tumor and excludes acinic cell carcinoma. A clear cell appearance of MNOH can be mistaken for other clear cell neoplasms of salivary glands [37, 39, 40].

Treatment and prognosis The treatment is surgical. Frequent recurrences are due to the diffuse, multifocal character of this lesion.

5.6.3 Ductal Oncocytosis

Definition Hyperplastic/metaplastic oncocytic disorder affecting ducts of minor salivary glands.

Epidemiology Ductal oncocytosis is relatively uncommon. It affects minor salivary glands, larynx in particular, followed by the floor of the mouth.

Clinical aspects Small and painless nodule of the floor of the mouth.

Macroscopy Small biopsy fragments or surgical specimen of the salivary gland.

Microscopy The ductal epithelium is replaced by oncocytic cells. Sometimes the architecture is papillary resembling Warthin's tumor [41].

Differential diagnosis The main differential is with Warthin's tumor which occurs almost exclusively in the parotid.

Treatment and prognosis Resection of the lesion is curative.

5.7 Cysts

Definition Cystic lesions arising mainly from the ductal system of salivary glands.

Epidemiology Some are relatively frequent; the commonest are mucocoeles (80 %) including ranula, ductal cysts (11 %) and cystic lymphoepithelial lesions (7 %) [42]. Those associated with genetic/inherited disorders are particularly rare (see Table 5.1).

Etiology and pathogenesis Cysts can be classified according to the epidemiology and pathogenesis.

Clinical aspect The most common clinical presentation is that of a painless mass in the salivary gland.

Macroscopy The cyst can be single or composed of multiple locules of varying size.

Microscopy, differential diagnosis, treatment and prognosis They will be discussed for each subtype (see Sects. 5.7.1, 5.7.2, 5.7.3, 5.7.4, 5.7.5 and 5.7.6).

Table 5.1 Cystic and pseudocystic lesions of salivary glands

1.	Dysgenetic, e.g. polycystic dysgenetic disease
2.	Acquired cysts lined with epithelium, e.g. simple ductal cysts
3.	Cystic lymphoepithelial lesions (solitary lymphoepithelial cyst (see Sect. 5.7.3), lymphoepithelial sialadenitis (see Sect. 5.10.1), cystic lymphoepithelial lesion of AIDS (see Sect. 5.7.4)
4.	Pseudocysts without an epithelial lining, e.g. extravasation mucocoele including ranula (see Sect. 5.2.1)
5.	Cystic change in neoplasms, e.g. in Warthin's tumor, in variants of mucoepidermoid and acinic cell carcinomas, rarely in pleomorphic adenoma
6.	Miscellaneous other cysts

5.7.1 Salivary Polycystic Dysgenetic Disease

Definition This very rare condition resembles cystic anomalies of other organs, such as the kidney, liver and pancreas, although no association has been described [43].

Epidemiology Rare disorder. Some cases are familial [44], and almost all cases have occurred in females. Most patients manifest in childhood, a few in adulthood. It affects the parotid glands almost exclusively, usually bilaterally.

Etiology and pathogenesis The report of familial cases suggests a possible genetic transmission. The postulated ducts of origin in salivary polycystic dysgenetic disease are the intercalated ducts.

Clinical aspects The children complain of recurrent parotid gland swellings.

Macroscopy The parotid shows multiple small cysts.

Microscopy The glands maintain the lobular architecture, and some lobules are affected more severely than others. The cysts vary in size up to a few mm, and they are irregular in shape. The lining epithelium is flat, cuboidal to low columnar resembling the epithelium of intercalated ducts. The lumina contain secretion with spherical microliths. Remnants of salivary acini are seen between the cysts, and thick fibrous interlobular septa are often prominent.

Differential diagnosis The differential diagnosis includes sclerosing polycystic adenosis (see Sect. 5.7.5), cystadenomas and benign and malignant tumors with a cystic component. The young age of patients and the bilateral nature of the lesions are helpful diagnostic markers.

Treatment and prognosis The treatment tends to be surgical for cosmetic reasons only. The prognosis is that of a benign lesion.

Cystic fibrosis can also involve the duct system of salivary glands with preference for submandibular gland, sublingual gland and minor salivary glands. The histological changes observed are due to the abnormal mucous plugging of excretory ducts with characteristic deposition of dense eosinophilic material in the ducts. Recent data have identified the main genetic defects of cystic fibrosis [45] with new treatment strategies [46].

5.7.2 Salivary Duct Cyst

Definition Form of retention cyst mostly seen in parotid gland.

Epidemiology Rare acquired cyst that can occur at any age, although usually in patients over 30 years old.

Etiology and pathogenesis This acquired cyst is due to dilatation of a salivary duct following obstruction due to different reasons [47].

Clinical aspect A painless swelling in one parotid (85 % of cases).

Macroscopy Well circumscribed and unilocular, usually 10–30 mm in size; they contain viscous brown fluid.

Microscopy The wall of the cyst comprises dense fibrous tissue, 1–3 mm thick, and there is often mild to moderate chronic inflammation, but no dense lymphoid infiltrate. The epithelial lining comprises one or a few layers of cuboidal or columnar cells with occasional goblet cells and rarely squamous epithelium [42].

Differential diagnosis Mostly with lymphoepithelial cyst which has a more dense lymphoid infiltrate.

Treatment and prognosis The cyst is cured with surgical excision.

5.7.3 Benign Lymphoepithelial Cyst

Definition An epithelial lined cyst surrounded by extensive lymphoid infiltrate.

Epidemiology This is a rather infrequent cyst of parotid gland with a slight male predominance and mean age of onset at 46 years (range 18–79) [48, 49].

Etiology and pathogenesis Benign lymphoepithelial cysts are thought to arise from embryological salivary gland inclusions in intraparotid lymph nodes [10, 50].

Clinical aspects Painless, usually solitary but occasionally bilateral mass in parotid gland.

Macroscopy The average diameter of the cyst is 25 mm but may reach 70 mm.

Microscopy Histology shows the lining epithelium to be cuboidal, columnar, lymphoepithelial, squamous or a combination thereof. Small number of goblet cells may be present (Fig. 5.5). This lining is surrounded by abundant lymphoid tissue composed of small lymphocytes, plasma cells and germinal centres. Lymphoepithelial lesions are not a feature [10].

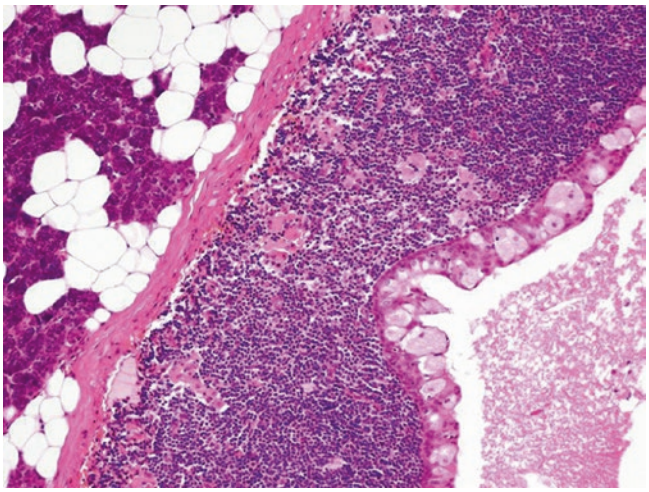

Fig. 5.5 Simple, benign lymphoepithelial cyst. The cavity is lined with columnar and cuboidal cells with scattered goblet cells. The surrounding tissue contains small lymphocytes and macrophages. Beyond this is a capsule and subcapsular space resembling that of a lymph node

Differential diagnosis The main differential diagnosis is with Sjögren's syndrome lymphoepithelial sialadenitis with cystic component (see Sect. 5.10.1) and cystic lymphoepithelial lesion of AIDS (see Sect. 5.7.4). Clinicopathological correlation may be necessary for definitive diagnosis [10].

Treatment and prognosis Benign lymphoepithelial cysts are not known to recur after surgical excision.

5.7.4 Cystic Lymphoepithelial Lesion of AIDS

Definition A diffuse and/or cystic, usually bilateral, enlargement of parotid glands.

Epidemiology Cystic lymphoepithelial lesion has been seen in about 5 % of HIV-positive patients; however, this disease has become relatively less common in Western countries since the availability of effective antiretroviral therapy.

Etiology and pathogenesis Infection by HIV virus, but the exact pathogenesis is unknown.

Clinical aspects Cystic lymphoepithelial lesion of AIDS can be the first clinical manifestation of HIV disease, and thus histological identification of it means a diagnosis of AIDS.

Macroscopy Cystic and enlarged parotid gland.

Microscopy Microscopic examination shows a dense lymphoid infiltrate including exaggerated follicular hyperplasia. There is an elaborate dendritic reticulum cell network within which there is evidence of active HIV replication, although the exact histogenesis of this lesion is not understood. Plasma cells (polytypic) are often numerous. The glandular parenchyma is atrophic, and multiple cystic spaces are seen. The cysts are dilated striated ducts, and the lining sometimes shows squamous metaplasia. The cysts are infiltrated by lymphoid cells including a variable number of marginal zone B cells, and there are multiple typical lymphoepithelial lesions [15].

Differential diagnosis There is considerable histomorphological overlap with lymphoepithelial sialadenitis (see Sect. 5.10.1), but the cysts are usually larger [9, 15, 50].

Treatment and prognosis The lymphoid infiltrate is polyclonal, and although it usually does not progress to lymphoma, nevertheless patients with HIV disease are in general at risk of developing aggressive B-cell lymphomas, most commonly Burkitt's and diffuse large B-cell lymphoma [51].

5.7.5 Sclerosing Polycystic Adenosis

Definition Sclerosing polycystic adenosis (SPA) is a recently described rare salivary gland lesion, originally thought to be a process somewhat analogous to epithelial proliferative lesions of the breast such as fibrocystic disease and sclerosing adenosis [52]. Subsequent demonstration of clonality suggests that it is probably a neoplasm [53].

Epidemiology SPA is a rare lesion of salivary glands. It occurs within a broad age range with a mean of 40 years (range 9–84 years of age). SPA is slightly more common in females with a male-to-female ratio approximately 2:3. Location of SPA is mostly in the parotid gland, less commonly in the submandibular gland, and minor salivary glands of oral mucosa.

Etiology and pathogenesis The nature of this lesion was initially believed to be reactive or inflammatory [52]. However, using X chromosome-linked human androgen receptor (HUMARA analysis), later investigators have shown that SPA is a clonal process [53]. It not infrequently harbours intraductal dysplastic epithelial proliferations including cases where the degree of atypia and structural changes reaches that of ductal carcinoma in situ (DCIS) [54].

Clinical aspects Patients typically present with slow-growing mass; some of them have pain or sensation. Onset of symptoms ranges from 10 days to 2 years. One patient, in addition, had a history of chronic recurrent parotitis.

Macroscopy Grossly, most tumors are firm or rubbery, well circumscribed and surrounded by normal salivary gland tissue. The tumors range in size from 3 to 70 mm in greatest dimensions. The cut surface is pale and glistening with multiple small cystic spaces ranging from 1 to 3 mm in diameter.

Microscopy Histological examination shows a well circumscribed partly encapsulated mass with preservation of the lobular architecture and a variable amount of inflammatory infiltrate in a sclerotic stroma (Fig. 5.6). Multiple dilated ducts are often lined by a flattened bilayered epithelium. The ductal cells comprise a spectrum of vacuolated, foamy, apocrine and mucous appearances (Fig. 5.7), and focal squamous metaplasia may be also present. The hallmark of the lesions is the presence of large acinar cells with numerous coarse eosinophilic periodic acid-Schiff (PAS)-positive cytoplasmic granules (Fig. 5.8). Some ducts contain solid and cribriform epithelial proliferations with vacuolated foamy cells having a sebaceous-like appearance. In all cases, there is focal intraluminal epithelial proliferation giving rise to solid, microcystic and cribriform structures. In most cases, nuclear polymorphism is noted, ranging in severity from mild up to severe and then amounting to low-grade DCIS (Fig. 5.9). In places, tiny cell aggregates and small ducts embedded in sclerotic stroma reminiscent of stromal invasion can also be seen [55]. The ductal and

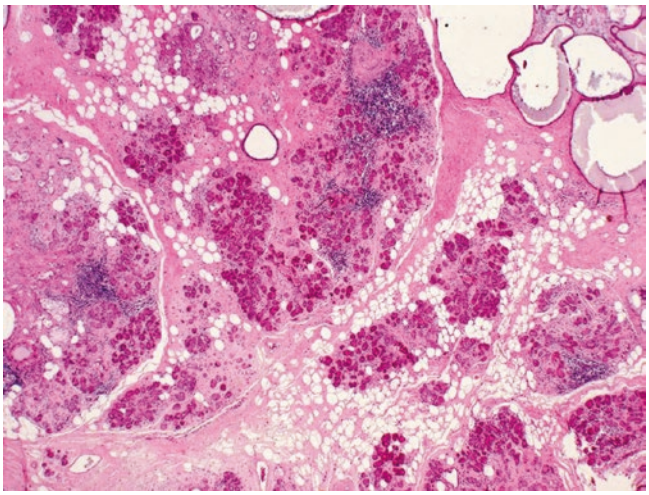

Fig. 5.6 Sclerosing polycystic adenosis. Histological examination shows a well-circumscribed partly encapsulated mass with preservation of the lobular architecture and a variable amount of inflammatory infiltrate in a sclerotic stroma

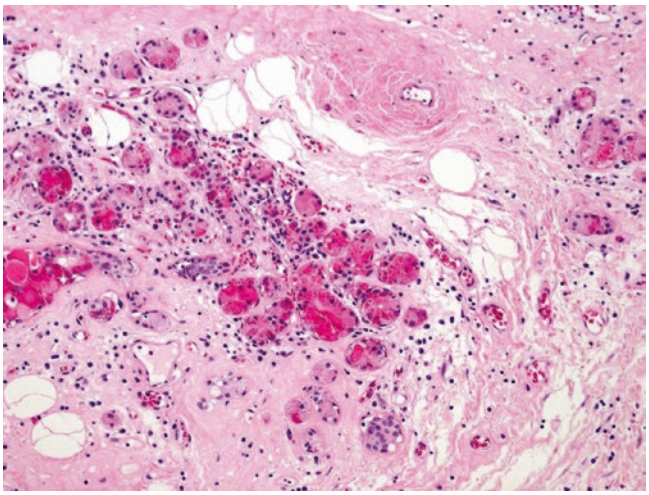

Fig. 5.8 Sclerosing polycystic adenosis. The hallmark is a presence of large acinar cells with numerous coarse eosinophilic cytoplasmic granules

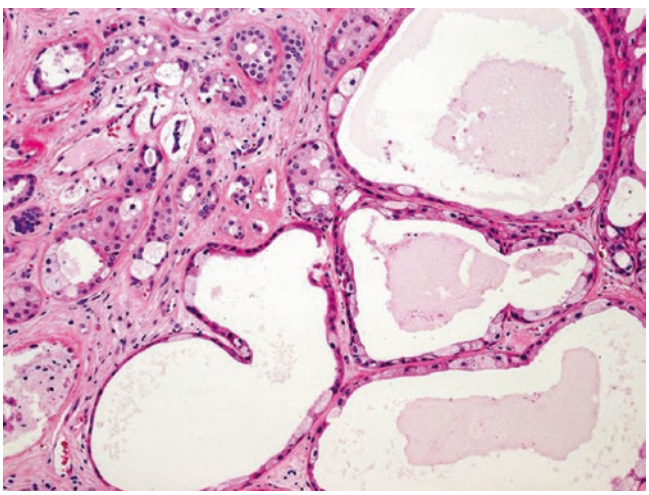

Fig. 5.7 Sclerosing polycystic adenosis. Multiple dilated ducts are often lined by a flattened bilayered epithelium. The ductal cells comprise a spectrum of vacuolated, foamy, apocrine and mucous appearances

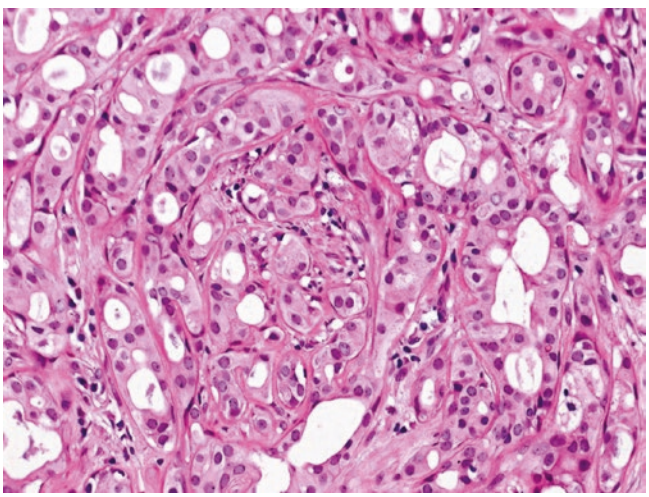

Fig. 5.9 Sclerosing polycystic adenosis. Nuclear polymorphism is present, ranging in severity from mild up to severe dysplasia and then amounting to low-grade ductal carcinoma in situ

acinar cells are positive for cytokeratin (AE1/AE3 and CAM5.2); variably positive for epithelial membrane antigen (EMA), S-100 protein and antimitochondrial antibody; and negative for CEA, p53 and HER2/neu. The acinar cells with coarse eosinophilic cytoplasm stain positively for GCDFP-15. Oestrogen and progesterone receptors are detected focally in some cases [54, 55]. Ducts, filled with hyperplastic and dysplastic epithelium, are surrounded by an intact myoepithelial layer, positive for αSMA, p63 and calponin. Polymerase chain reaction (PCR)-based analysis of patterns of X chromosome inactivation using a HUMARA locus demonstrated that SPA is composed of a clonal population [53].

Differential diagnosis Most cases of SPA were initially misdiagnosed as tumors, such as mucoepidermoid and acinic cell carcinomas, cystadenocarcinoma and pleomorphic adenoma. Major microscopic clues to a correct diagnosis include maintenance of the lobular architecture of the gland, ductal ectasia, scar-like hyalinised fibrous sclerosis and a spectrum of foamy, apocrine, granular and mucous cells, in addition to the presence of tubuloacinar structures composed of large acinar cells with prominent brightly eosinophilic zymogen-like cytoplasmic granules. Intraductal hyperplasia in some cases of SPA, particularly if associated with dysplasia, may cause suspicion of a low-grade malignancy, but clues to the benign nature of SPA are that it is well circumscribed, that it lacks an invasive growth pattern and that mitotic/proliferative activity is low. Major benign differential diagnoses include pleomorphic adenoma, polycystic dysgenetic disease (see Sect. 5.7.1) and chronic sclerosing sialadenitis (see Sect. 5.2.2). Definitive lobular growth pattern and large Paneth cell-like acinic cells of SPA are not seen in pleomorphic adenomas. In contrast, SPA lacks a prominent myoepithelial cell component and chondromyxoid stroma typical of pleomorphic adenoma. Chronic sclerosing sialadenitis lacks nodular pattern and typical structural heterogeneity of SPA, though both lesions share prominent fibrosis. Moreover, large acinic cells with coarse PAS-positive zymogen-like cytoplasmic granules are not seen in chronic sclerosing sialadenitis.

Treatment and prognosis Treatment is primarily surgical with complete conservative local excision with good margins and facial nerve preservation followed by prolonged surveillance. Recurrences, sometimes multiple, have been reported quite frequently (29 %) as summarised by Gnepp et al. [56]. The proposed mechanism behind this is either incomplete surgical resection and/or multifocal disease [55, 57]. Although rare cases of invasive carcinoma developing in SPA have been described to date [58], no patient has developed metastases or died due to disease.

5.7.6 Miscellaneous Other Cysts

Other salivary cysts include dermoid, keratocystoma and a variety of epithelial and non-epithelial cysts including parasites and gas cysts in glass blowers [59].

Dermoid cyst of the parotid is very rare. A review of the literature has shown <10 cases reported [60], one in a child [61]. The cyst had the characteristic squamous lining with sebaceous glands and hair follicles in its wall as seen in dermoid cysts occurring in the orbit and floor of mouth.

Keratocystoma is also a rare, recently described, benign parotid tumor characterised by multicystic keratin-filled spaces lined with stratified squamous epithelium with no atypical features [62, 63].

5.8 Benign Tumors

5.8.1 Pleomorphic Adenoma

Definition Pleomorphic adenoma (PA) is a tumor composed of a mixture of epithelial and modified myoepithelial cells in varying proportions. Most authors accept that PA is part of a spectrum of salivary gland adenomas with benign myoepithelioma, which is composed almost entirely of myoepithelial cells representing one end and basal cell adenoma and canalicular adenoma at the other end [64–66]. The particular morphology of any particular tumor reflects the different proportions of the constituent cells (Fig. 5.10). (Pleomorphic adenoma spectrum. Reproduced with permission from Zarbo [65]).

Epidemiology PA is the most common tumor of the salivary glands. PA arises de novo in healthy salivary glands where it accounts to approximately 60 % of all salivary gland tumors. Although most often found in young to middle-aged women, PA can occur in either sex and at any age. Up to 80 % occur in the superficial lobe of the parotid gland.

Etiology and pathogenesis Not known.

Clinical aspects It typically presents as a painless swelling.

Macroscopy PAs are usually well-circumscribed masses of 20–40 mm. The cut surface is white, and grey glistening areas are commonly seen. Recurrent PA occurs after incomplete surgical excision and is usually composed of multiple nodules completely separate from each other. In the first recurrence the nodules are usually seen within salivary gland tissue, but in further recurrences tumors are found in the soft tissue of the surgical bed.

Microscopy Histologically, PA is 'a tumor of variable capsulation characterised microscopically by architectural

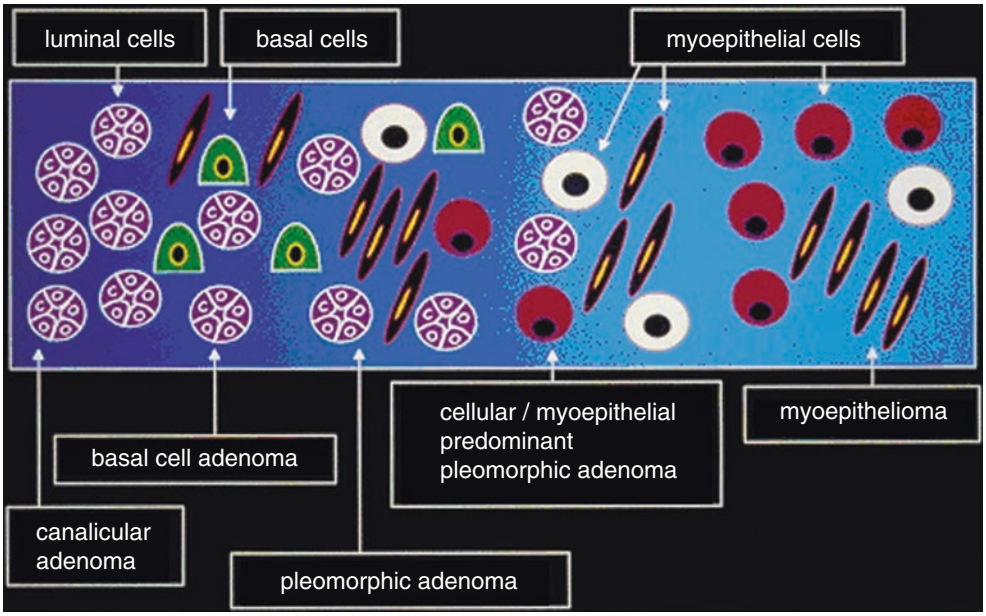

Fig. 5.10 Pleomorphic adenoma spectrum (Reproduced with permission from Zarbo et al. [65])

rather than cellular pleomorphism. Epithelial and modified myoepithelial elements intermingle most commonly with tissue of mucoid, myxoid or chondroid appearance [67]. The pattern varies from case to case and also from area to area within any individual tumor. All are composed of a mixture of ductal epithelial cells, basal and myoepithelial cells and variable amounts of stroma, both hyaline and chondromyxoid. Attempts have been made to subclassify PA based on the proportions of cell types and stroma [68], but because of the variation in any tumor, this is difficult and probably has no prognostic value. Ducts are lined with flat, cuboidal or columnar epithelial cells, with little or no atypia. The ducts are usually small tubules but can be cystically dilated and also arranged in a cribriform-basaloid pattern, resembling adenoid cystic carcinoma, but mitotic figures are rare and the proliferation index low. Squamous metaplasia with or without keratinization is seen in up to 25 % of PAs [69] (Fig. 5.11). Squamous plus mucinous metaplasia in PA resembling mucoepidermoid carcinoma is rarely present (Fig. 5.12). Myoepithelial cells are arranged in sheets, smaller islands and trabeculae and also surround epithelium-lined spaces. As in benign myoepithelioma, neoplastic myoepithelial cells may take several forms – epithelioid, spindle, plasmacytoid, clear and oncocytic, as well as transitional forms with features of two or more of these types (Fig. 5.13). The stroma varies in amount and is either densely eosinophilic hyaline material or chondromyxoid tissue (Fig. 5.14). The former is composed of basement membrane material and stains with PAS diastase and collagen type IV; the chondromyxoid material only rarely resembles true cartilage and is Alcian blue positive. Calcification and bone formation can occur in long-standing tumors. Occasionally, collagenous spherules and crystalloids are seen (Fig. 5.15), particularly in tumors rich in myoepithelial cells of the plasmacytoid type [70]. Nuclear atypia is not common but can be seen in tumors where epithelial or myoepithelial cells display oncocytic features [69, 71]. Occasional myoepithelial cell nuclei are enlarged and bizarre, somewhat analogous to 'ancient' change in schwannomas. Mitotic figures are generally sparse but can occur as part of the repair process after FNA. Similarly, areas of necrosis or haemorrhage may follow surgical manipulation, FNA or other trauma, and these neoplasms should also be sampled thoroughly. Tumor cells in lymphatics ('vascular invasion') are occasionally seen in benign PAs (Fig. 5.16), but this does not indicate malignancy [72]. None of the reported cases were followed by metastases. PAs are often completely or partly surrounded by a fibrous capsule of variable thickness, but it can be absent, especially in tumors of the minor glands. Neoplastic elements may extend into and even through the capsule in the form of microscopic pseudopodia or apparent satellite nodules. They may be the cause of future recurrence after apparent surgical removal (Fig. 5.17) [73], and their presence should be noted in the surgical pathology report. Special stains and immunohistochemistry are not necessary for the diagnosis in most cases, but can be used to identify the different cell types and also early malignant change.

More than half of PAs can be shown to have breakpoints affecting chromosomes, 8q12 (>50 % of cases) and 12q14-15 (10–15 %). The involved genes are *PLAG1* and *HMGA2*.

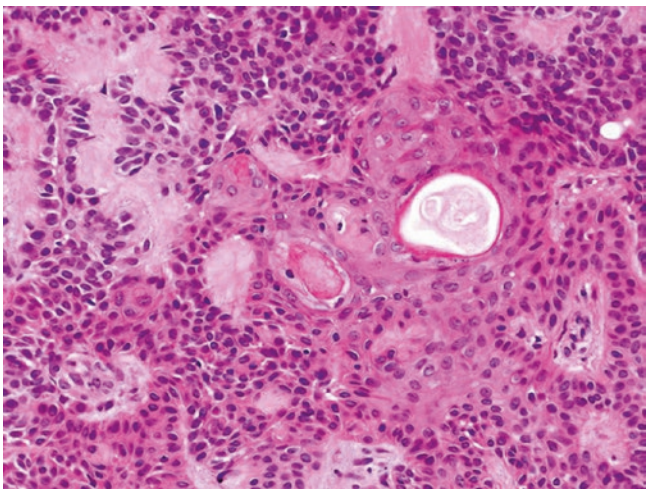

Fig. 5.11 Pleomorphic adenoma: myoepithelial cells with an epithelioid cytomorphology. These cells may also be spindle shaped and plasmacytoid (hyaline) or have clear cytoplasm. Note also a small duct and a focus of squamous metaplasia. Keratinizing squamous metaplasia is seen in up to a quarter of pleomorphic adenomas

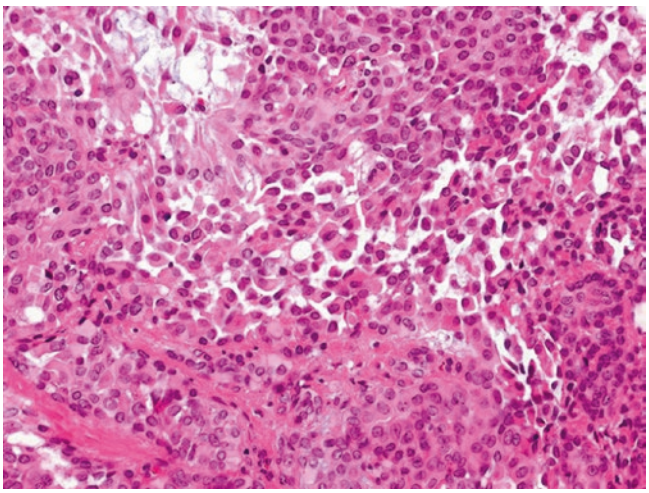

Fig. 5.13 Pleomorphic adenoma: myoepithelial cells showing an epithelioid and plasmacytoid appearance

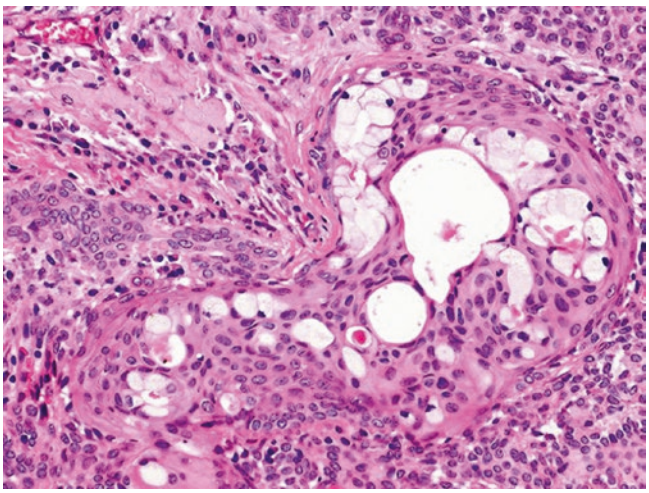

Fig. 5.12 Pleomorphic adenoma with squamous and focal mucinous metaplasia resembling mucoepidermoid carcinoma

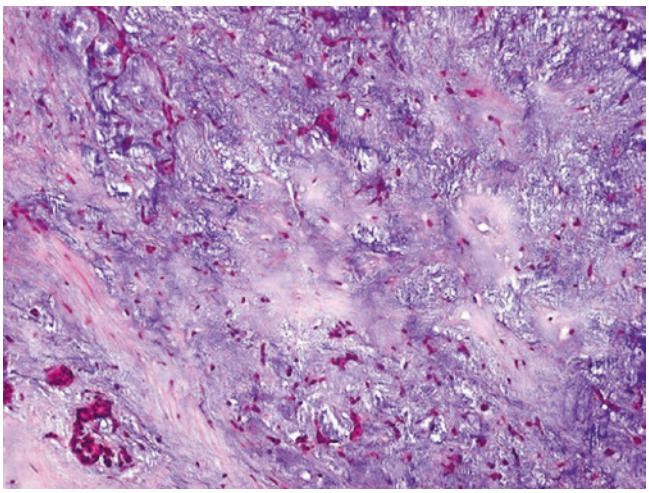

Fig. 5.14 Pleomorphic adenoma: chondromyxoid stroma containing isolated small ducts and small aggregates of myoepithelial cells

Differential diagnosis PA should be distinguished from other adenomas of salivary gland such as myoepithelial and basal cell adenoma (see Sects. 5.8.2 and 5.8.3).

Treatment and prognosis Complete surgical excision with a rim of normal salivary gland tissue is the treatment of choice. Incomplete excision leads to a local recurrence in the surgical bed. The long-term prognosis is excellent providing excision is complete.

5.8.1.1 Salivary Gland Anlage Tumor ('Congenital Pleomorphic Adenoma')

Definition Salivary gland anlage tumor (SGAT) is a pedunculated polypoid lesion of the nasopharynx that presents with respiratory distress syndrome at birth or within first few weeks of life.

Epidemiology Very rare tumor with fewer than 30 cases reported in literature [74–76].

Etiology and pathogenesis The morphology of SGAT is the same as the normal salivary glands in early weeks of their development. The pathogenesis is most likely hamartomatous.

Clinical aspects Respiratory distress usually starting at about 6 weeks after birth. There is a predilection for males. Radiological investigation helps to identify the tumor in the posterior septum.

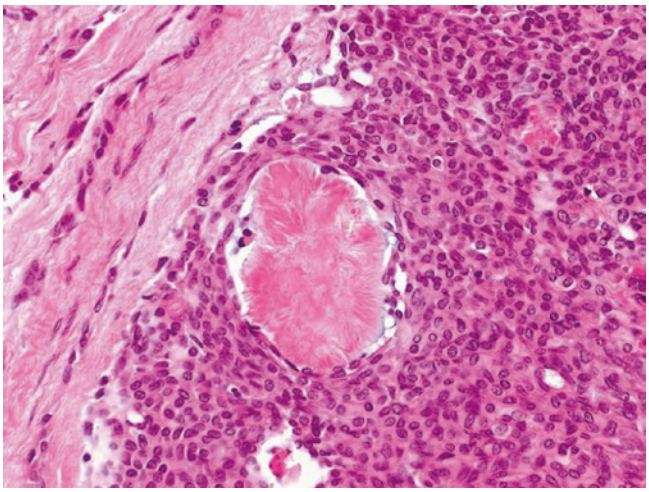

Fig. 5.15 Collagenous crystalloids can be seen in some benign myoepitheliomas and myoepithelium-rich pleomorphic adenomas

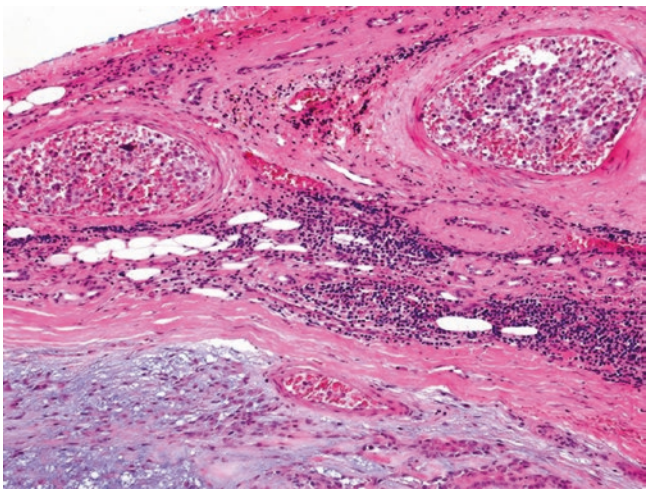

Fig. 5.16 Vascular permeation is a rare finding in benign pleomorphic adenoma, due to displacement of neoplastic cells into vascular spaces. It is not indicative of malignancy

Macroscopy Well-circumscribed nodule usually solid but may contain necrotic and cystic areas. The gross findings are those of bosselated surface and polypoid pedunculated mass measuring between 1.3 and 3.0 cm in greatest dimension. The tumors are attached by a thick stalk to the nasopharynx, and they are soft in consistency and white to pink in colour. The mucosal surface is intact in most cases.

Microscopy SGAT is characterised by solid cords and branching duct-like structures that appear to originate from the surface mucosa. Some of the duct-like structures have a focal squamous lining resembling sialometaplasia. The tumors are divided by variously thick septa into nodules composed of fascicles of spindle-shaped and ovoid cells with

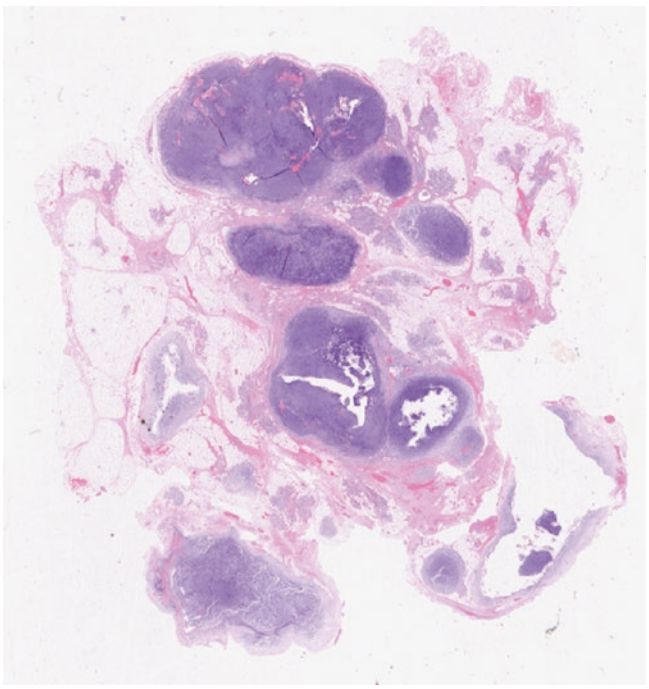

Fig. 5.17 Recurrent pleomorphic adenoma. Multiple and often well-separated tumor nodules of different sizes are seen in periglandular soft tissue

indistinctive borders, eosinophilic cytoplasm and bland nuclei. Within these nodules, the cells focally form glands, cystic spaces and squamous cell nests.

Differential diagnosis The biphasic multinodular growth pattern and solid nodules composed of mesenchyme-like spindle-shaped cells can mimic synovial sarcoma. The presence of budding epithelium from the surface mucosa in SGAT, its actin positivity and lack of numerous mitotic figures are major distinguishing features from synovial sarcoma. Low-grade mucoepidermoid carcinoma can be distinguished because of presence of spindled actin-positive stromal cells and keratinizing squamous epithelium in SGAT.

Treatment and prognosis Although potentially fatal due to its location causing respiratory obstruction, prognosis after surgery is good. SGAT is a benign lesion characterised by non-recurring clinical behaviour.

5.8.2 Benign Myoepithelioma

Definition Myoepithelioma (myoepithelial adenoma) is a benign neoplasm composed almost exclusively of myoepithelial cells. It represents one end of the spectrum of benign salivary gland tumors which also includes PA and basal cell adenoma [67].

Epidemiology The incidence of myoepithelioma depends on how strictly criteria of myoepithelial predominance are applied for diagnosis; thus, percentages vary from 0.3 to 5.7 [77]. Men and women are equally affected. The most common sites include parotid gland (48 %) and the palate (35 %), but any salivary gland may be affected [77]. Patients have ranged from 6 to 98 in age with a mean in the early to mid-40s [77].

Etiology and pathogenesis Whether or not it is truly a separate biological entity is debatable, but most commentators believe that it represents one end of a spectrum that also includes pleomorphic and at least some basal cell adenomas.

Clinical aspects Most cases present as a well-circumscribed mass, usually 10–50 mm in diameter, in either major or minor salivary glands.

Macroscopy Grossly, myoepitheliomas are usually well circumscribed and encapsulated and have yellow-tan colour and glistening cut surface.

Microscopy There are several typical appearances, reflecting the different forms that neoplastic myoepithelial cells can take. Solid, myxoid and reticular growth patterns may be seen, and the component cells may be spindle shaped, plasmacytoid (hyaline), clear, epithelioid or oncocytic (Fig. 5.18) and occasionally mucinous. Many tumors show more than one growth pattern or cell type, but myoepitheliomas of the minor glands are more often composed of plasmacytoid cells and those of the parotid spindle cells [78]. Although most authors accept the plasmacytoid cells as myoepithelial, it has been suggested that these cells originate from luminal and not from myoepithelial cells [79], and thus the tumors should possibly be reclassified as plasmacytoid adenomas [79]. A clear cell variant can occur in both major and minor glands [80] but is relatively rare. The stroma of most myoepitheliomas is usually scanty, fibrous or myxoid, and it may very occasionally contain chondroid material or mature fat cells [81]. Extracellular collagenous crystalloids are seen in 10–20 % of plasmacytoid cell-type myoepitheliomas (as well as sometimes in myoepithelial-rich PAs); these structures are about 50–100 μm in diameter and consist of radially arranged needle-shaped fibres composed of collagen types I and III, which stain red with the van Gieson method (Fig. 5.15) [70]. Immunohistochemically, there may be considerable variability in staining within the same tumor and between different tumors. However, almost all tumors express S-100 protein and broad-spectrum cytokeratins (AE1/AE3) and some cytokeratin subtypes, mostly CK14 and CK 5/6. αSMA and muscle-specific actin are expressed in spindle-shaped myoepithelial cells, but they are usually absent in epithelioid and plasmacytoid cells. Staining for CD10, calponin, smooth muscle heavy chain and maspin is inconsistent, but p63, vimentin and GFAP are positive in most benign myoepitheliomas.

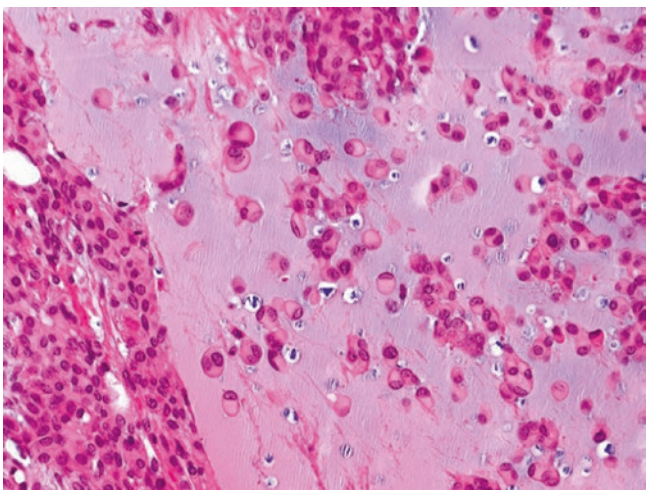

Fig. 5.18 Benign myoepithelioma composed of plasmacytoid (hyaline) and epithelioid cells with areas of myxoid stroma. Plasmacytoid cells have eccentric nuclei and dense eosinophilic cytoplasm

Differential diagnosis Spindle cell myoepithelioma should be distinguished from schwannoma, solitary fibrous tumor, synovial sarcoma and spindle cell sarcomas. The clear cell variant must be distinguished from other clear cell tumors of salivary glands both primary and secondary, in particular from metastatic renal cell carcinoma. Immunohistochemistry is valuable in demonstration of myoepithelial phenotype in these tumors. Myoepithelial carcinoma, in contrast to benign myoepithelioma, shows invasive growth, necrosis and high proliferative index (MIB1). Scanty small ducts may be present (usually less than 10 % of the tumor tissue) in otherwise typical myoepitheliomas, but if more numerous, the tumor should be considered as a myoepithelial cell-rich PA.

Treatment and prognosis The behaviour of myoepithelioma is similar to that of PA, and complete excision is curative. Neither growth pattern nor cell type appears to carry prognostic significance. Malignant change in a benign lesion has been described [82], but too little information is available about the percentage of cases involved.

5.8.3 Basal Cell Adenoma

Definition A tumor composed of basal cells without myxoid component (previously called monomorphic adenoma).

Epidemiology Basal cell adenoma (BCA) is a rare tumor representing about 3 % of all tumors of the salivary glands.

Etiology and pathogenesis BCA represents the end of a spectrum of PAs; it is usually a solitary tumor, but occasionally the membranous subtype may be multifocal and associated with dermal cylindromas and trichoepitheliomas [67].

Clinical aspects A painless tumor mass usually clinically diagnosed as PA.

Macroscopy Well-circumscribed nodule most often found in the parotid. Occurrence in submandibular or minor glands is rare [83, 84].

Microscopy Four histological subtypes are recognised subtypes – solid, tubular, trabecular and membranous – but it is likely that, in reality, there are only two separate biological entities [67], non-membranous BCA (Fig. 5.19) and membranous BCA (Fig. 5.20).

Non-membranous BCAs have an equal sex incidence and arise mostly in the major glands. They probably represent part of the spectrum of myoepithelioma and PA [65, 85]. The tumors are ovoid, well-circumscribed masses in which islands, nests and trabeculae of basaloid cells are each surrounded by a distinct thin PAS-positive basement membrane. The component cells may take two forms – small with scanty cytoplasm and a round, dark nucleus and larger with amphophilic or eosinophilic cytoplasm and an ovoid paler staining nucleus. These two types are intermixed, but the smaller cells tend to be arranged around the periphery of the nests and trabeculae, giving the appearance of palisading. Ductal differentiation may or may not be apparent but can be highlighted by EMA. There is little pleomorphism and mitotic figures are rare. The stroma varies in amount and cellularity, but S-100 protein-positive spindle cells may be numerous. S-100-positive cells are also present within the islands of epithelial cells, which react strongly with cytokeratins [66].

Membranous BCA (dermal analogue tumor) occurs predominantly in men and can be multicentric. Most arise in the major glands, including within intraparotid lymph nodes [86]. Microscopically, they are not encapsulated and appear multinodular, often with a jigsaw-like pattern. The most characteristic feature is the deposition of large amounts of hyaline basement membrane material, which is brightly eosinophilic and PAS positive. It surrounds the epithelial cell islands in a similar manner to a dermal cylindroma and blood vessels and is present within the islands as small droplets. There is little pleomorphism or mitotic activity. In about 40 % of cases, the salivary adenoma is associated with synchronous and often multiple skin appendage tumors of sweat gland or hair follicle origin, usually cylindromas or eccrine spiradenomas.

Differential diagnosis The most important differential diagnosis of all types of BCA is adenoid cystic carcinoma.

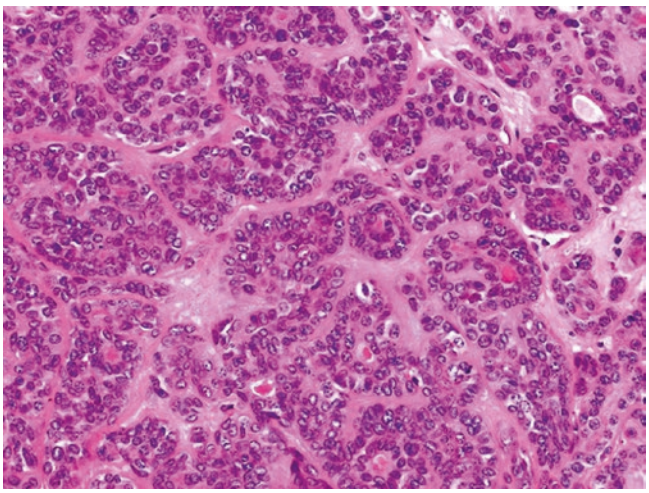

Fig. 5.19 Basal cell adenoma. The tumor is arranged in nests, islands and trabeculae of basal cells without cytological abnormalities. Ductal differentiation is also noted

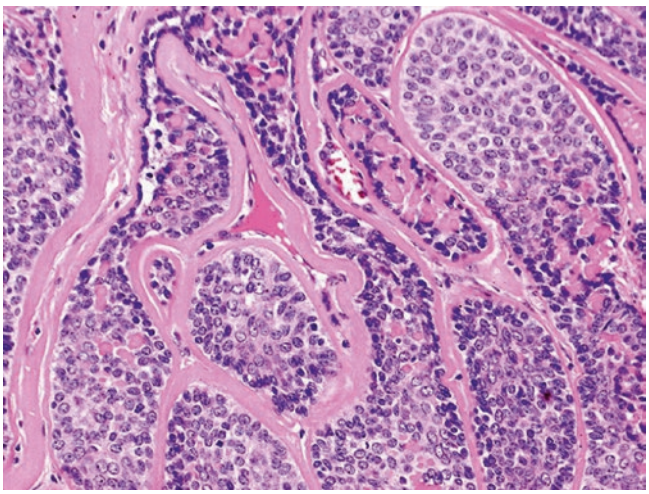

Fig. 5.20 Membranous basal cell adenoma: jigsaw-like pattern: multiple epithelial islands surrounded by large amounts of basal membrane-like material. The latter is also present within the cytoplasm of some of the small dark hyperchromatic basal cells. There is little cellular pleomorphism

Useful pointers to adenoma include lack of invasiveness and cytological pleomorphism, low mitotic and proliferative activity and whorled eddies of epithelial cells. S-100 protein positivity of spindled stromal cells may help, as this does not occur in adenoid cystic carcinoma [64]. A more difficult differential diagnosis is BCA and basal cell adenocarcinoma which may lack cytological pleomorphism and mitotic figures, the diagnosis then depending principally on the presence of genuine invasion (see Sect. 5.9.9), which it may not be possible to assess on small biopsy sample.

Treatment and prognosis The recurrence rate for non-membranous BCA is extremely low (0 out of 102 patients in

one series) [85], and local excision with clear margins is sufficient treatment. There is a low rate of malignant transformation (about 4 %) into BCA [87]. In contrast, up to 24 % of membranous BCAs recur after surgery [85] probably reflecting multicentricity, and, in addition, malignancy (also as basal cell adenocarcinoma) is said to develop in 28 % [87]. Surgery for this subtype needs to be more extensive.

5.8.4 Warthin's Tumor

Definition Warthin's tumor is composed of oncocytic columnar epithelial and basal cells arranged in papillary architecture and embedded in lymphoid stroma. The term adeno- and cystadenolymphoma should be discouraged for the possible confusion with malignant lymphoid neoplasms [88].

Epidemiology It is the second most common tumor of the salivary gland after PA. It occurs almost exclusively in the parotid gland and occasionally in peri-parotid lymph nodes.

Etiology and pathogenesis There is a known association with smoking; radiation exposure and a history of preoperative trauma such as FNA may play a role in the development of the metaplastic subtype. It is still not certain whether Warthin's tumor is a true neoplasm or a non-neoplastic tumor-like lesion. Honda et al. examined the clonal status of epithelial cells of Warthin's tumor by using a PCR method based on trinucleotide repeat polymorphism of the HUMARA and on random inactivation of the gene by methylation. The pattern was non-clonal, suggesting that Warthin's tumor is a non-neoplastic mass lesion [89].

Macroscopy Circumscribed oval encapsulated mass. The cut surface usually shows a cystic appearance containing mucoid grey and brown fluid.

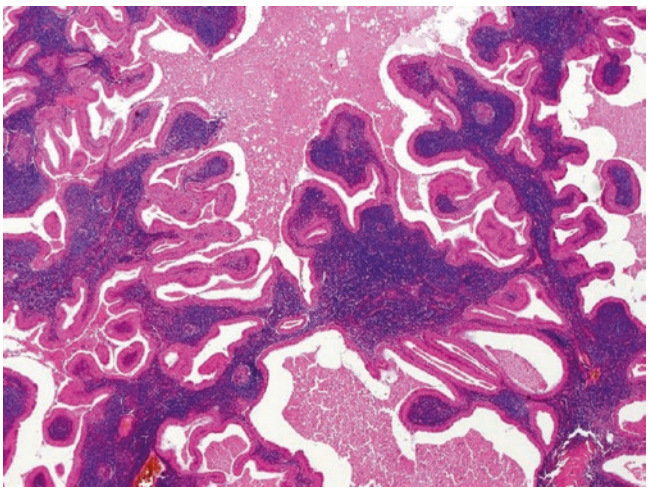

Fig. 5.21 Warthin's tumor. Cystic and slitlike spaces with papillary infoldings lined with oncocytic cells. Lymphoid tissue occupies the cores of most papillae

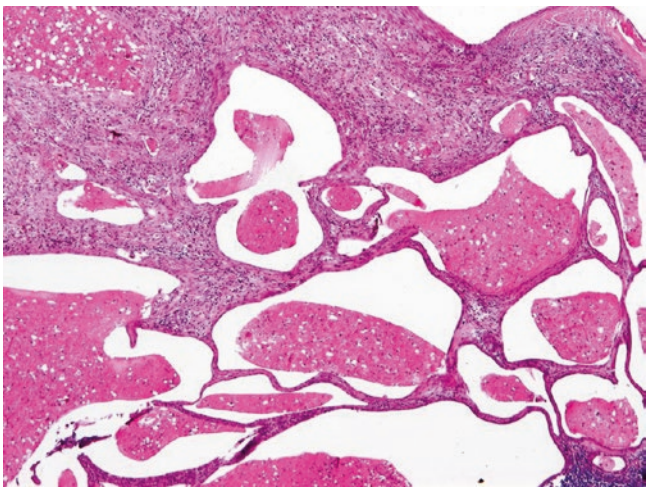

Fig. 5.22 Metaplastic (infarcted) Warthin's tumor. There is extensive necrosis and inflammation

Microscopy The combination of oncocytic epithelium arranged in papillary structures and embedded in lymphoid tissue is characteristic. The light and dark oncocytic cells, which are usually columnar in shape, lie on basal-type cells and are arranged in a palisade. There is usually no cytological atypia or mitotic activity. The stroma comprises lymphoid tissue with germinal centres (Fig. 5.21). Occasional mucinous and squamous metaplastic changes may be seen but are extensive in the metaplastic subtype [32].

Metaplastic subtype This subtype variously termed infarcted, infected or metaplastic accounted for 6.2 % (20/323) of Warthin's tumors in one series [32] and 7.5 % (21/275) in another [90]. The histopathological definition is a Warthin's tumor in which much of the original oncocytic epithelium has been replaced by squamous cells (hence the term metaplastic) resembling a ruptured epidermoid or lymphoepithelial cyst (Fig. 5.22) [88].

Other microscopic features include extensive necrosis, in which a ghost architecture of papillary structures may be retained. Non-keratinizing squamous metaplasia is prominent, consisting of tongues and cords of often spongiotic squamous cells extending into surrounding tissues in a pseudo-infiltrative pattern. Cytological atypia can be prominent and mitotic figures numerous, although none is abnormal. Goblet cells may also be seen, but should not be numerous. At the periphery of the lesions, there is extensive fibrosis, with dense hypocellular collagen and myofibroblastic spindle cell proliferation. There is often a heavy mixed inflammatory infiltrate, comprising neutrophils, chronic inflammatory cells, as well as sheets

of macrophages, some with foamy cytoplasm. Lipogranulomas, with or without cholesterol clefts, are not uncommon [67]. The definition of metaplastic Warthin's tumor does not encompass minor microscopic foci of inflammation, necrosis and fibrosis, as these findings can be commonly seen in any Warthin's tumor [32]. The diagnosis is straightforward if residual tumor is present, but it will not always be so, particularly if there is complete necrosis. Several cases have been reported following FNA acting on an ordinary Warthin's tumor to produce the infarcted subtype [34, 91]. The most likely mechanism would be direct injury of a blood vessel by the needle, as Warthin's tumors tend to contain few blood vessels within the substance of the tumors [32]. Therefore, they could be at risk of a needle harming a limited number of feeder arteries. Another possible important factor is cell type; in the well-documented injuries from FNA in other organs, tumors rich in oncocytic cells, such as Hürthle cell adenoma of the thyroid, feature prominently. Not surprisingly, similar infarction has been reported in salivary oncocytoma [92].

Differential diagnosis The characteristic appearance means that Warthin's tumor is generally the easiest salivary tumor to diagnose by microscopy [32], although difficulty may arise with the metaplastic subtype, particularly when there is total necrosis of the original tumor.

Treatment and prognosis Most cases are treated with surgery. For example, with a definite cytological diagnosis, the treatment may be conservative. Malignancy occurs in fewer than 1 % of cases, involving either epithelial or lymphoid elements leading to carcinoma or lymphoma.

5.8.5 Oncocytoma

Definition A tumor composed of oncocytic cells only, with no features of another neoplasm such as a PA.

Epidemiology Oncocytoma accounts for approximately 1 % of salivary gland tumors and may be associated with MNOH (see Sect. 5.6.3) [69]. The mean age is 60 years; it occurs in both sexes with a slight male predominance (67 %) [93]. Parotid is the preferred site with both glands involved in cases associated with bilateral MNOH [94].

Etiology and pathogenesis Some patients with oncocytic tumors have a history of previous radiation exposure.

Clinical aspects Most commonly a painless mass.

Macroscopy They are encapsulated tumors 5–30 mm.

Microscopy Usually solid, composed of oncocytic cells with characteristic 'light and dark' cytoplasm arranged in sheets and duct-like and trabecular structures (Fig. 5.23). The tumor can be composed entirely of clear cells [95] or have the features of an oncocytic lipoadenoma (Figs. 5.24, 5.25 and 5.26) for the presence of variable amount of fatty tissue [96].

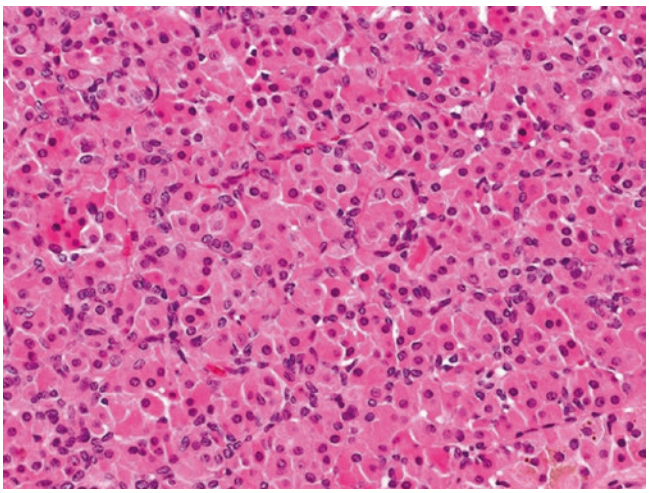

Fig. 5.23 Oncocytoma. Light and dark oncocytic cells are arranged in a solid, trabecular and tubular configuration

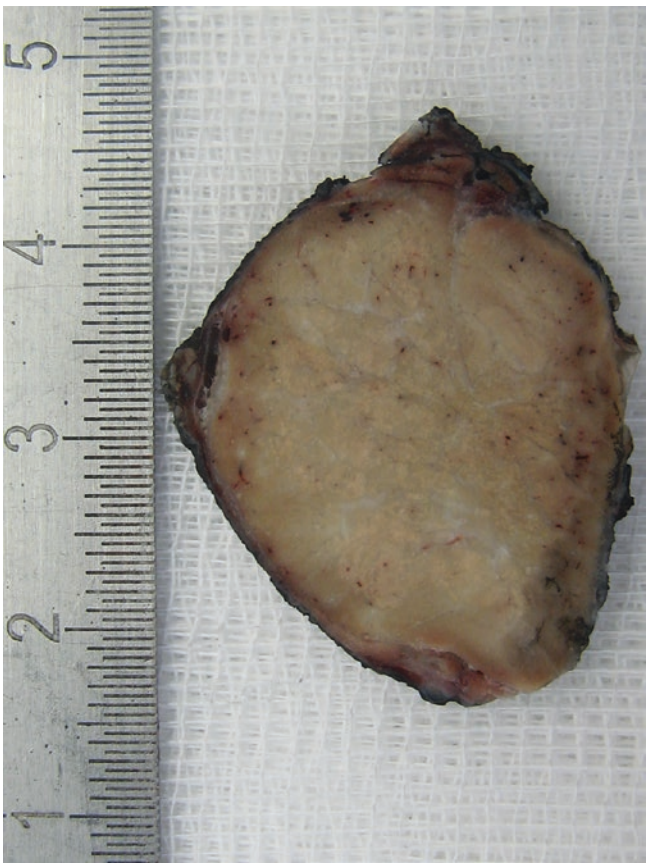

Fig. 5.24 Oncocytic lipoadenoma: the tumor is encapsulated and has a tan cut surface (Courtesy of Dr. Abbas Agaimy)

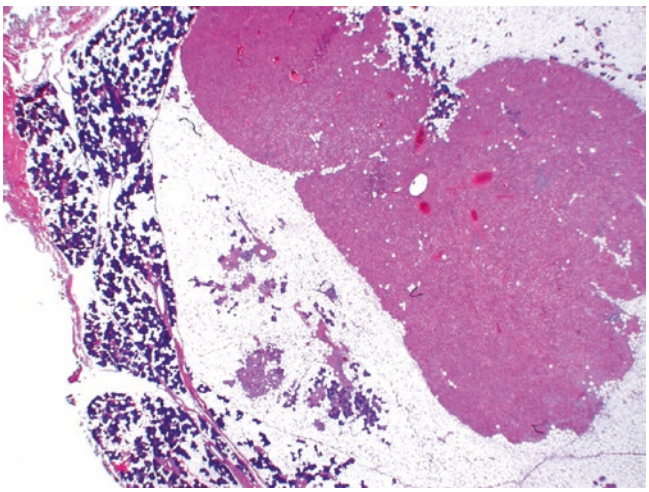

Fig. 5.25 Oncocytic lipoadenoma. The tumor is formed by mature fatty tissue and oncocytic cells (Courtesy of Dr. Abbas Agaimy)

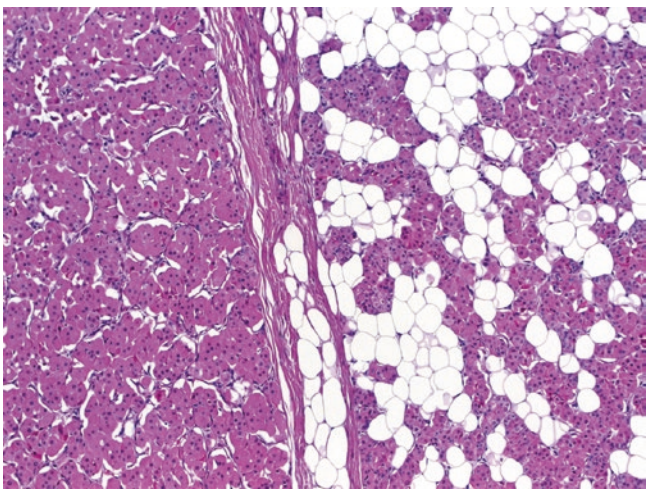

Fig. 5.26 Oncocytic lipoadenoma. The lesion is formed by of adipose tissue and oncocytic cells with eosinophilic granular cytoplasm (Courtesy of Dr. Abbas Agaimy)

Differential diagnosis The granular cytoplasm is PAS diastase (PASD) negative, unlike in acinic cell carcinoma. Oncocytomas composed of clear cells must be distinguished from renal cell carcinoma and other clear cell tumors. p63 immunostaining has been used to differentiate oncocytoma (and oncocytic carcinoma) from metastatic renal cell carcinoma [97].

Treatment and prognosis With surgery, the prognosis is good. Recurrences occur when oncocytoma is associated with MNOH (see Sect. 5.6.2).

5.8.5.1 Striated Duct Adenoma

Definition Striated duct adenomas (SDA) are ductal tumors that recapitulate normal striated ducts, which are lined by only a single layer of epithelial cells with absent (or at most, very occasional) basal or myoepithelial cells.

Epidemiology SDA is rare with only six cases reported so far, four in the parotid gland and two in the palate [98].

Etiology and pathogenesis Not known.

Clinical aspects Patients complain of painless tumor mass in the parotid gland or palate.

Macroscopy An encapsulated circumscribed mass.

Microscopy Composed of back-to-back ducts with virtually no stroma. The ducts vary in size with some showing cystic dilatation, up to 1 mm in diameter. The cells are eosinophilic and bland, and prominent cell membranes reminiscent of 'striations' of normal striated ducts are characteristic. All tumors express keratins, and S-100 positivity is present in most. Occasional tumors show focal bilayered ducts with calponin or SMMHC, but in general SDAs are unilayered. Only isolated cells in some tumors are positive with p63 – a pattern identical to normal striated ducts, in contrast to normal excretory and intercalated ducts which demonstrate diffuse bilayering with basal (p63 positive) or myoepithelial (SMA, calponin, SMMHC +) cells, respectively [98].

Treatment and prognosis SDA is part of the spectrum of benign salivary adenomas, and complete excision should be curative.

5.8.6 Canalicular Adenoma

Definition A tumor composed of basal-type epithelial cells embedded in oedematous and highly vascular stroma.

Epidemiology Canalicular adenoma accounts for 1 % of salivary gland tumors almost exclusively intraoral, particularly affecting the upper lip [67] and less often the palate [99].

Clinical aspects Most tumors present when they are small, rarely more than 20 mm in diameter.

Macroscopy Soft and well-circumscribed nodule with pale cut surface.

Microscopy It has a characteristic morphology of branching and interconnecting bilayered strands of darkly staining epithelial cells set in a loose vascular stroma (Fig. 5.27). There is no pleomorphism or significant mitotic activity. The cells express cytokeratins and S-100 protein [100]. Not infrequently, they are bilateral [101] or multifocal [102] and can thus mimic invasion.

Differential diagnosis Multifocal canalicular adenoma needs to be distinguished from cribriform adenoid cystic carcinoma.

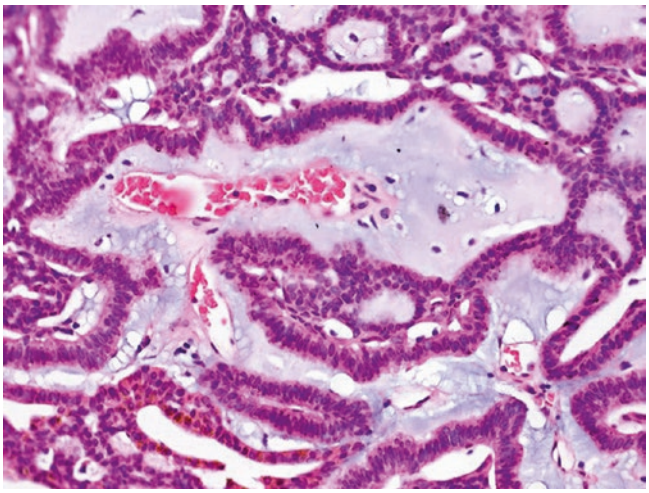

Fig. 5.27 Canalicular adenoma of the upper lip. It is composed of bilayered strands of basal-like cells embedded in a loose oedematous stroma

The lack of destructiveness, cytological atypia and low mitotic and proliferative activity together with the presence of oedema and blood vessels in the cribriform spaces are good guides to canalicular adenoma, which is completely benign.

Treatment and prognosis The tumor is benign, but occasional recurrences can occur as a result of multifocality [102].

5.8.7 Sebaceous Adenoma

Definition Tumors composed of nests of sebaceous cells mixed with metaplastic squamous cells and cysts [103].

Epidemiology It represents <1 % of salivary gland tumors with a predilection for the parotid gland, buccal mucosa and retromolar trigone [103].

Clinical aspects Painless tumor mass with long duration of symptoms.

Macroscopy Well-circumscribed nodule with a median size of 20 mm.

Microscopy Histologically there is a mixture of sebaceous and squamous cells without cytological atypia. Fibrosis and foreign body-type giant cells are also noted [103].

Differential diagnosis In small biopsies the presence of squamous cell in a sebaceous adenoma can be mistaken for squamous cell carcinoma.

Treatment and prognosis They do not recur after complete surgical excision.

5.8.8 Sebaceous Lymphadenoma

Definition Tumor composed of sebaceous glands mixed with lymphoid stroma and epithelial cells of various types (squamous, mucinous, ductal and basal types) [103].

Epidemiology Sebaceous lymphadenoma is rare. In the largest series of sebaceous and non-sebaceous lymphadenoma, it comprised <1 % of parotid tumors. It occurs mainly in older adults [103, 104], but children can occasionally be affected [105].

Etiology and pathogenesis Altered immune system may be a predisposing factor. EBV, HPV and HHV-8 do not seem to play any role. It is possible that, like Warthin's tumor, sebaceous lymphadenoma develops from salivary inclusions within lymph nodes and shows sebaceous rather than oncocytic metaplasia [103, 104].

Clinical aspects Patients complain of a painless mass in the parotid gland, rarely in the neck.

Macroscopy A well-circumscribed and encapsulated tumor with multicystic cut surface.

Microscopy This lesion comprises irregularly proliferating nests of epithelium. Oncocytic papillary changes, mucus-secreting cells and keratinization can be seen. The cysts contain eosinophilic and sebaceous-like material. The sebaceous cells are seen inside a layer of basal cells. The tubules and glands have an outer layer of basal cells and an inner layer of luminal glandular cells. The lymphoid infiltrate has a mixed population of B and T lymphocytes, with some germinal centre formation (Fig. 5.28).

A foreign body reaction to keratin and fat material complicated 11/22 cases in one study [104].

Differential diagnosis The most important differential diagnosis is with nodal metastases of squamous cell carcinoma, and this can be particularly difficult in preoperative core biopsies performed on neck or intraparotid nodes. The lack of atypia and mitoses favour lymphadenoma. Mucoepidermoid carcinoma is excluded by the absence in lymphadenoma of mucous and intermediate cells together with the presence of sebaceous cells.

Benign conditions such as lymphoepithelial cysts and lymphoepithelial sialadenitis (see Sect. 5.10.1) also lack the prominent sebaceous component.

Treatment and prognosis These are benign tumors which are cured with surgical excision.

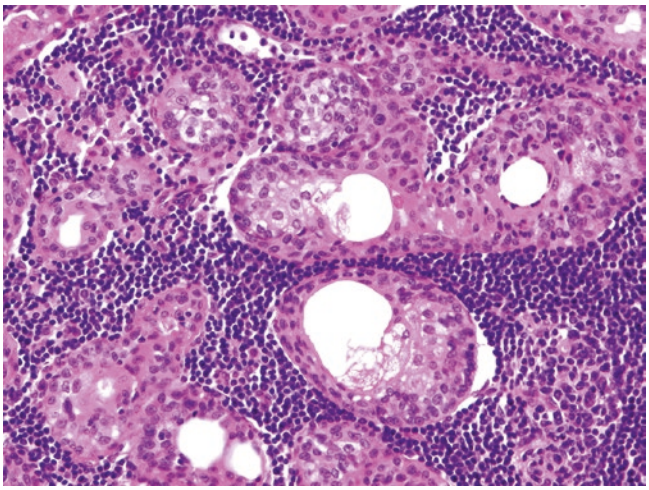

Fig. 5.28 Sebaceous adenoma composed of a mixture of sebaceous and squamous cells without cytological atypia

5.8.9 Ductal Papilloma

Definition An adenoma arising mainly in the excretory duct [106]. Ductal papilloma has a fibrovascular core lined with myoepithelial and ductal cells, and it is usually seen in a dilated duct.

There are three subtypes, all rare, inverted ductal papilloma (similar to analogous sinonasal Schneiderian tumors), intraductal papilloma and sialadenoma papilliferum (similar to skin syringocystadenoma papilliferum [106]. Their main characteristic is described below.

5.8.9.1 Inverted Ductal Papilloma

Rare examples have been reported in the minor salivary glands of the palate, floor of mouth, buccal mucosa and lower lip [106]. Histologically, the squamous epithelium shows a nodular proliferation not surrounded by a capsule. The papillary islands comprise a mixture of squamous and basal cells covered by columnar cells, together with single or small groups of mucus-secreting cells.

The most important differential diagnosis is with mucoepidermoid carcinoma (see Sect. 5.9.3) especially in small-sized biopsies.

5.8.9.2 Intraductal Papilloma

This papillary-cystic tumor arises in an excretory or interlobular duct of a salivary gland. With the exception of scattered cases reported in parotid, submandibular and sublingual glands, intraoral minor salivary glands are most affected [106].

Histology shows a dilated duct containing a papillary growth. The papillae comprise fibrovascular cores lined with myoepithelial and ductal cells without cytological atypia or mitotic activity.

5.8.9.3 Sialadenoma Papilliferum

This is an exophytic papillary lesion associated with an endophytic proliferation of squamous or ductal epithelium from the mucosal surface and excretory salivary duct. It is histologically identical to syringocystadenoma of the skin. It arises in minor salivary glands of the palate, buccal mucosa, upper lip, retromolar area and exceptionally in the parotid [106].

5.8.10 Cystadenoma

Definition Cystadenoma is a rare, benign neoplasm composed of one or usually more cystic spaces often with intraluminal papillary projections. The epithelial lining may be oncocytic, apocrine, epidermoid and mucous. Cystadenoma occurs in two major variants, as papillary oncocytic type and mucous cell type [67].

Epidemiology The frequency of cystadenoma is between 0.7 and 8.1 % of all benign salivary tumors, but it is probably underestimated, as some examples are classified as 'monomorphic adenomas' [67]. The average age is about 50 years of age (range 8–89). Cystadenomas occur more frequently in females, with a female-to-male ratio 3:1. Most are located in major glands with almost 58 % arising in parotid and 7 % in submandibular gland. Cystadenoma of minor glands affect mostly the lip and buccal mucosa.

Macroscopy Cut section reveals multiple small cystic spaces of variable sizes with intraluminal proliferations. The tumors are well circumscribed and encapsulated.

Microscopy Most cystadenomas are multilocular with individual cystic spaces separated by limited amounts of intervening stroma [107]. The lumina often contain eosinophilic material with scattered epithelial, foamy or inflammatory cells. Rarely, psammoma bodies and crystalloids have been described within the luminal secretion [108]. The lining epithelium of the cystic spaces is mostly columnar and cuboidal. Oncocytic, mucous and apocrine cells are sometimes present focally or may predominate. An oncocytic variant of papillary cystadenoma is composed of oncocytes present in unilayered or bilayered papillary structures (Fig. 5.29). Squamous epithelium may be present but rarely predominates. Mucinous cystadenoma is composed of multiple cystic spaces lined by mucus-secreting columnar cells without atypia (Fig. 5.30).

Differential diagnosis Differential diagnosis consists of simple cyst, duct ectasia, polycystic dysgenetic disease, intraductal papilloma, low-grade mucoepidermoid carcinoma and striated duct adenoma [98]. Both duct ectasia and simple cysts have simple epithelial lining without papillary

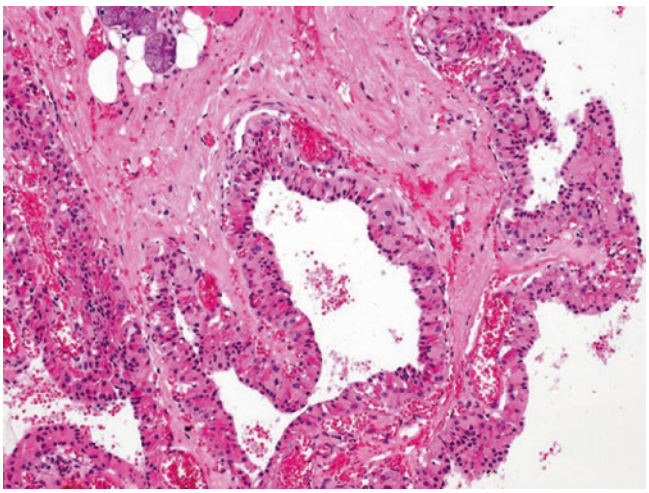

Fig. 5.29 Oncocytic (papillary) cystadenoma of the larynx. Cystically dilated ducts are lined with oncocytic cells

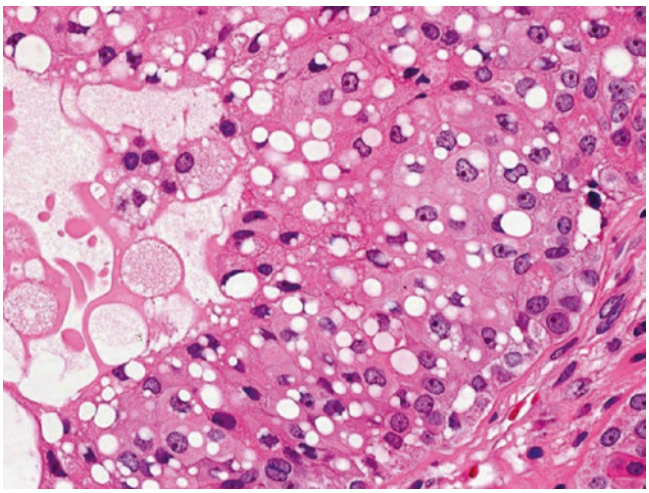

Fig. 5.31 Mucinous cystadenoma with malignant transformation. Cellular pleomorphism and signet ring cell appearance

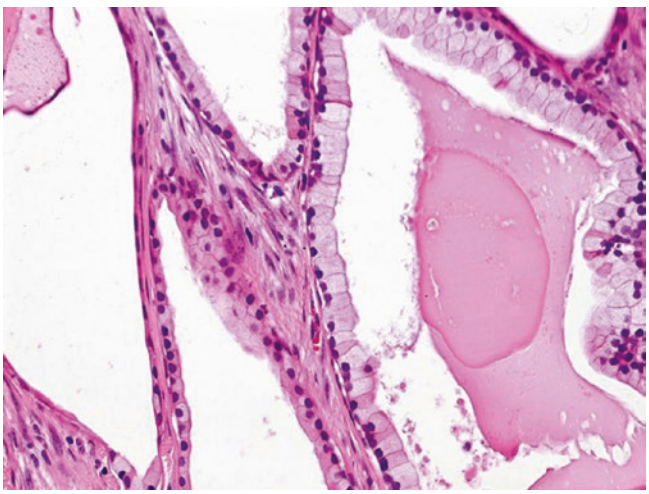

Fig. 5.30 Mucinous cystadenoma. Cysts are lined with mucus-secreting cells without atypia

intraluminal projections. In addition, duct ectasia, in contrast to cystadenoma, is often associated with fibrosis and chronic inflammatory infiltration. Polycystic dysgenetic disease is extremely rare and involves the whole gland [43]. Intraductal papilloma has overlapping histological features with papillary cystadenoma; however, intraductal papilloma is a unicystic lesion with prominent intracystic papillary proliferation, whilst cystadenomas are multicystic tumors [77]. Low-grade mucoepidermoid carcinoma shares some histological features with the mucous cell variant of cystadenoma, but solid islands composed of intermediate cells are characteristic of mucoepidermoid carcinoma. Furthermore, cystadenoma is well circumscribed without invasive growth. Striated duct adenoma is composed mostly of closely packed unilayered ducts without a myoepithelial layer and with minimal intervening stroma and variable cystic ductal spaces [98]. In contrast, cystic formations dominate the picture in cystadenoma.

Treatment and prognosis Cystadenomas are benign tumors; complete surgical excision is curative. The tumors are unlikely to recur, but rare cases of mucinous cystadenomas with malignant transformation have been reported (Fig. 5.31) [109].

5.9 Malignant Epithelial Tumors

5.9.1 Acinic Cell Carcinoma

Definition Acinic cell carcinoma (AciCC) is defined as a malignant epithelial neoplasm in which at least some of the neoplastic cells demonstrate serous acinar cell differentiation [67].

Epidemiology AciCC accounts for about 2–4 % of salivary gland tumors and approximately 5 % of malignancies; AciCC is the second most common salivary gland carcinoma occurring in childhood [77]. The reported age range is 3–91 years, with female-to-male predilection of approximately 2:1 [77]. The mean age at presentation is 38–46 years. The parotid is involved in at least 92 % of cases (3 % bilateral), with only occasional examples in the submandibular or minor glands [77].

Clinical aspects The typical clinical history is of a slowly enlarging mass (for as long as 40 years), but occasionally patients present with pain and facial nerve weakness.

Macroscopy Most tumors are partly circumscribed, with an average diameter of 1–3 cm, although larger tumors can be encountered.

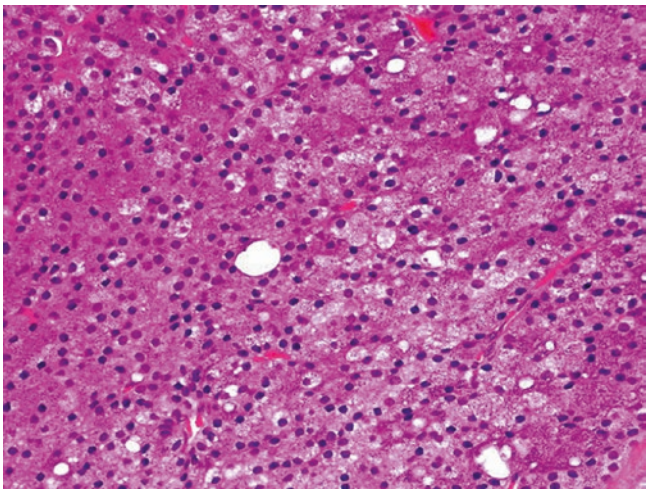

Fig. 5.32 Acinic cell carcinoma solid variant. The cells show granular cytoplasm and acinar differentiation similar to normal salivary gland acini

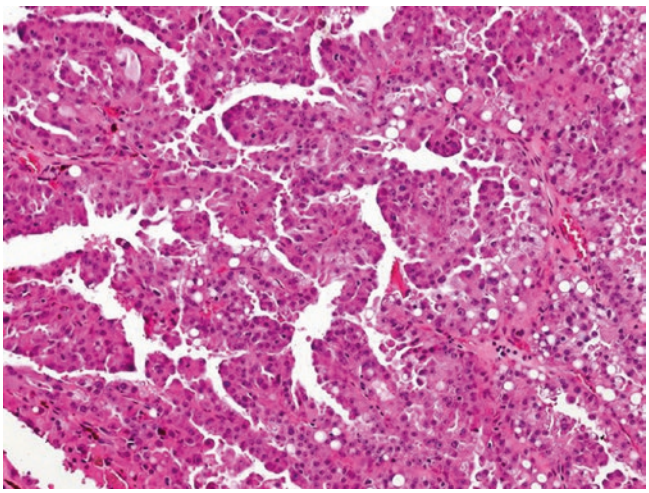

Fig. 5.34 Acinic cell carcinoma, papillary subtype: papillae are lined with intercalated duct-like cells, some containing microvesicles, others showing a hobnail/clear cell appearance

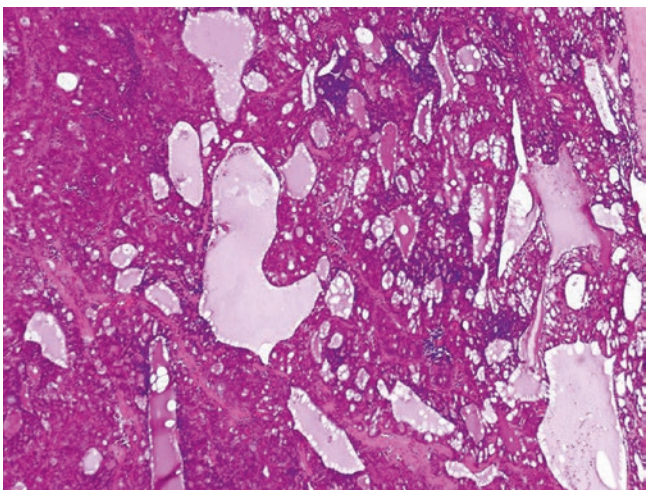

Fig. 5.33 Follicular variant of acinic cell carcinoma: the tumor is composed of follicle-like spaces of varying sizes lined with cuboidal intercalated duct-type cells

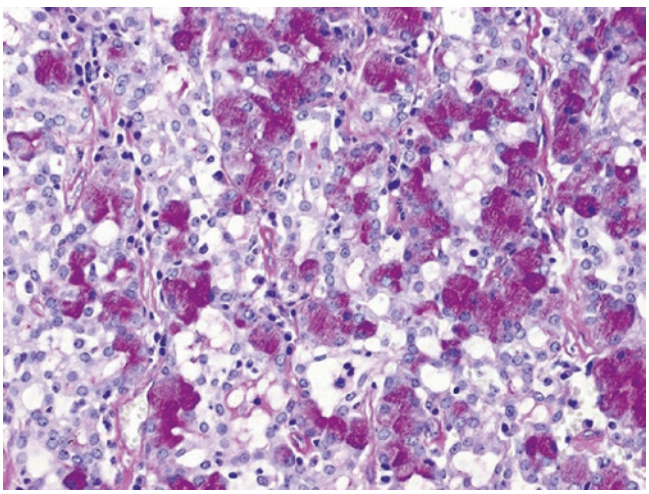

Fig. 5.35 Acinic cell carcinoma. Periodic acid-Schiff with diastase staining emphasises coarse zymogen granules in the cytoplasm of the tumor cells

Microscopy By definition, AciCC is characterised by the presence of well-differentiated serous acinar cells with abundant cytoplasmic PAS-positive zymogen granules, resistant to diastase digestion (Fig. 5.32). However, several other cell types, such as intercalated ductal, vacuolated, clear and non-specific glandular cells are recognised. Solid/lobular and microcytic growth patterns are most commonly seen in AciCC, but macrocytic, follicular (thyroid gland-like) (Fig. 5.33), papillary-cystic patterns are also recognised (Fig. 5.34). The most useful special stain in AciCC is PASD which highlights cytoplasmic zymogen granules (Fig. 5.35). With one possible exception, the immunoprofile is not specific – positivity is seen with cytokeratin, amylase and CEA, but mammaglobin and myoepithelial markers are negative. Up to now, immunohistochemistry has been considered of limited diagnostic value, but a new potentially useful marker, DOG1, shows intense apical membranous staining around lumina as well as complete membranous and variable cytoplasmic staining in AciCC (Fig. 5.36) [110].

Differential diagnosis The differential diagnosis depends on the subtype: solid variant, composed of prevalent serous cells, resembles normal parotid acini, but with an abnormal architecture. The papillary-cystic type bears a close similarity to the controversial entity cystadenocarcinoma. A follicular pattern suggests metastatic thyroid carcinoma but is thyroglobulin negative. The clear cell variant must be differentiated from other neoplasms composed of clear cells, but there are always some cells with PASD-positive granules in

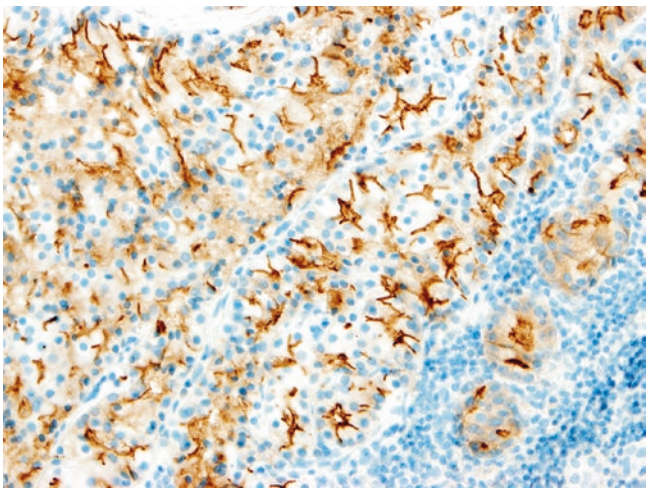

Fig. 5.36 Acinic cell carcinoma: DOG1 antibody shows an intense apical membranous staining around lumina as well as complete membranous and variable cytoplasmic staining

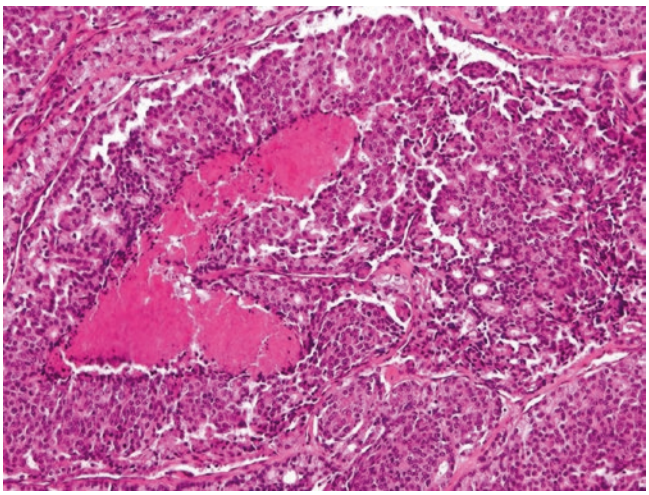

Fig. 5.37 Acinic cell carcinoma with high-grade transformation

AciCC. The most common tumor entity in salivary gland pathology that mimics AciCC is a recently recognised new tumor entity, mammary analogue secretory carcinoma [111] (see Sect. 5.9.2). The neoplastic cells of mammary analogue secretory carcinoma resemble intercalated duct cells, but serous acinar cells with cytoplasmic PAS-positive granules typical of AciCC are completely absent in mammary analogue secretory carcinoma . In contrast to mammary analogue secretory carcinoma, AciCC shows an intact *ETV6* gene and is mammaglobin and (usually) S-100 protein negative, as well as positive with DOG1.

Treatment and prognosis AciCC is characterised by a protracted clinical course, but it is a genuine malignancy capable of killing the patient. Reported rates of recurrence, distant metastasis and mortality with treatment are 30 %, 13 % and 13 %, respectively [77]. Prediction of prognosis of AciCC based on grading is uncertain, although there are two studies demonstrating that Ki-67 (MIB1) is an independent prognostic indicator [112, 113]. Skálová et al. found that tumors with a proliferation MIB1 index <5 % were cured by complete excision, whereas more than half of AciCCs with indices above this either recurred or metastasised [113]. Clinical stage also gives the most prognostic information [77]. High-grade (HG) transformation of AciCCs (Fig. 5.37) has been reported; these tumors are aggressive with rapid progression and a poor outcome [114]. In contrast, Michal and colleagues have reported a well-differentiated variant that is surrounded completely by heavy lymphoid stroma which has a better prognosis than conventional AciCC [115]. Overall, the most effective treatment for AciCCs is complete surgical excision. Radiation may have a role in treatment of patient with HG-transformed AciCC [114].

5.9.2 Mammary Analogue Secretory Carcinoma

Definition Mammary analogue secretory carcinoma (MASC) is a recently described distinctive salivary gland tumor with the *ETV6* gene rearrangement [111]. As the name implies, MASC is characterised by histological and immunohistochemical resemblance to secretory carcinoma of the breast [116]. Moreover, MASC of salivary glands, like secretory carcinoma of the breast, harbours a recurrent balanced chromosomal translocation t(12;15) (p13;q25) which leads to a fusion gene between the *ETV6* gene on chromosome 12 and the *NTRK3* gene on chromosome 15 [111, 116].

Epidemiology The exact incidence of this recently described entity is not yet known, but it occurs frequently enough to be encountered regularly in routine diagnostic practice, and it is most probably much more common than originally realised [117, 118]. Oddly, its incidence is much higher than its breast equivalent.

Clinical aspects In contrast to AciCC, MASC has a slight male predilection [111, 119]. The parotid is the usual site, but it is also found in extra-parotid sites, much more commonly than AciCC [111, 117, 119].

Macroscopy Grossly, the tumors are rubbery, with a white-tan to grey cut surface. Occasionally, on cut surface the tumors may appear cystic, containing yellow-whitish fluid. The borders of the tumors are usually circumscribed but not encapsulated (Fig. 5.38), and invasion within the salivary gland is often present.

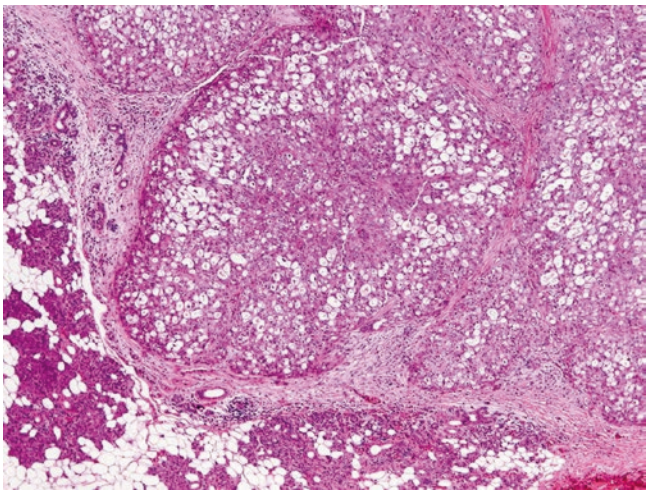

Fig. 5.38 Mammary analogue secretory carcinoma. The borders of the tumors are usually circumscribed but not encapsulated

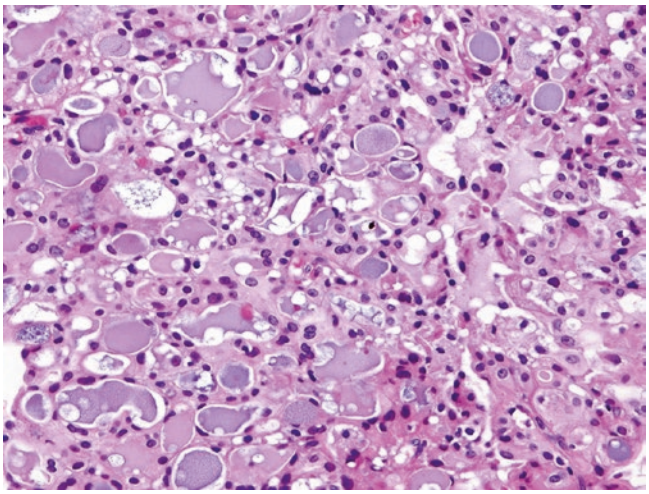

Fig. 5.40 Mammary analogue secretory carcinoma with abundant eosinophilic homogeneous or bubbly secretory material.

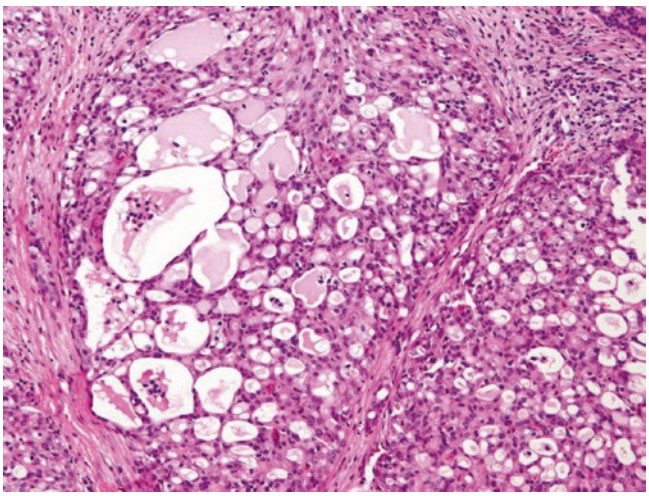

Fig. 5.39 Mammary analogue secretory carcinoma. The tumor often has a lobulated growth pattern divided by fibrous septa, and it is composed of microcystic/solid and tubular structures

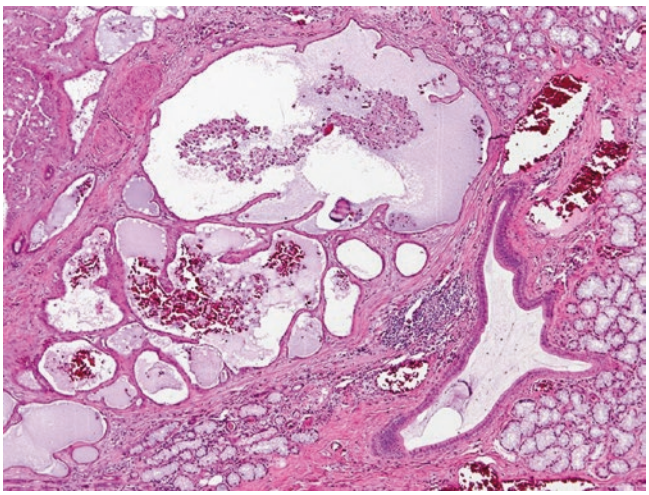

Fig. 5.41 Less commonly, mammary analogue secretory carcinoma is formed by one large cyst with multilayered lining

Microscopy MASC often has a lobulated growth pattern divided by fibrous septa (Fig. 5.39). In most cases, MASC is composed of microcystic/solid and tubular structures with abundant eosinophilic homogeneous or bubbly secretory material (Fig. 5.40). Less commonly, the tumors are dominated by one large cyst with multilayered lining, which can display tubular, follicular, macro- and microcystic or papillary architecture, with occasional solid areas (Fig. 5.41). The tumor cells have low-grade vesicular round-to-oval nuclei with finely granular chromatin and distinctive centrally located nucleoli (Fig. 5.42). The cytoplasm is pale to pink with a granular or vacuolated appearance. Cellular atypia is usually mild, and mitotic figures are in most cases sparse. Perineural invasion can be sometimes present, but the tumors usually do not have evidence of lymphovascular invasion. Necrosis is typically not identified. Abundant bubbly secretion is present within microcystic and tubular spaces (Fig. 5.43). This secretory material stains positive for PAS before and after diastase digestion and for mucicarmine and Alcian blue (Fig. 5.44). In contrast to AciCC, serous acinar differentiation is not a feature of MASC, and the cells of MASC are devoid of PAS-positive secretory zymogen cytoplasmic granules. The immunohistochemical profile of MASCs shows diffuse and strong expression of cytokeratins (AE1/AE3 and CAM 5.2), CK7, CK8, CK18, CK19, epithelial membrane antigen (EMA), S-100 protein and vimentin. The tumor cells also show strong positive expression of STAT5a (signal transducer and activator of transcription 5a) and mammaglobin (secretory material is also positive) in all cases. In addition, in most cases, there is

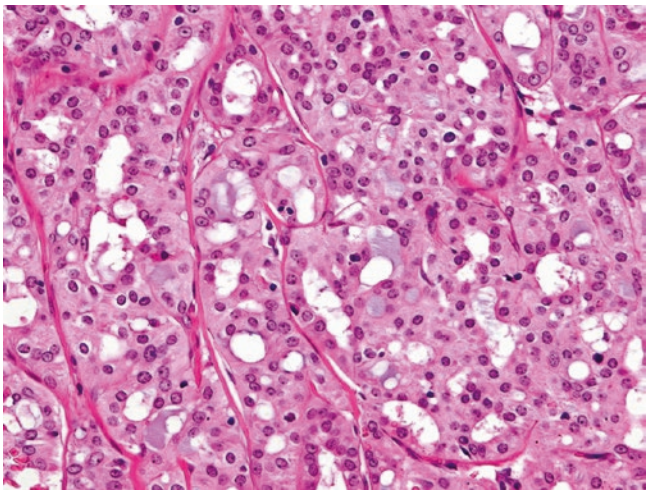

Fig. 5.42 Mammary analogue secretory carcinoma. The tumor cells have low-grade vesicular round-to-oval nuclei with finely granular chromatin and distinctive centrally located nucleoli

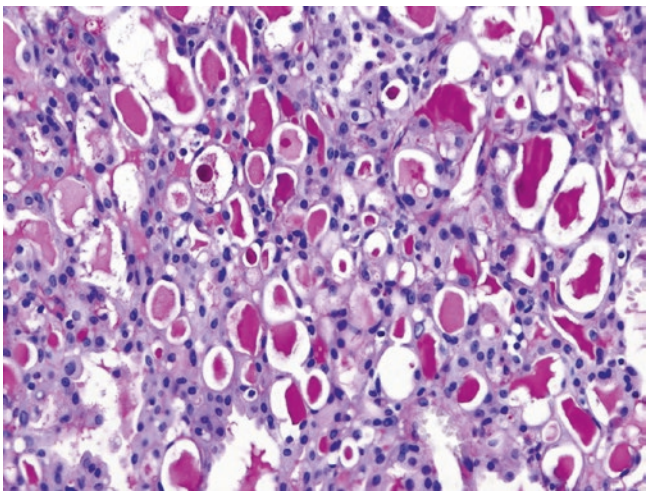

Fig. 5.44 Mammary analogue secretory carcinoma. Secretory material stains positive for periodic acid-Schiff

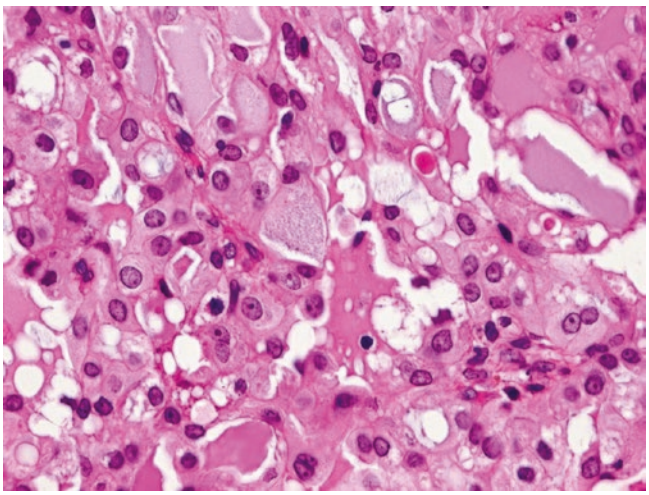

Fig. 5.43 Mammary analogue secretory carcinoma. Abundant bubbly secretion is present within microcystic and tubular spaces

significant positivity for gross cystic disease fluid protein 15 (GCDFP-15) (particularly secretory material is stained) and EMA. Basal cell/myoepithelial cell markers, such as p63, calponin, CK14, αSMA and CK5/6, are virtually negative as described in original report [111]; however, we have recently identified few cases of MASC, which showed focal expression of high-molecular-weight keratin (34betaE12) and p63 protein-positive cells [118, 120]. Most cases of MASC are DOG1 negative, whilst AciCCs demonstrate intense apical membranous staining around lumina and variable cytoplasmic positivity in most cases [110]. At the molecular level, MASC harbours a distinctive t(12;15) (p13;q25) translocation resulting in *ETV6-NTRK3* fusion product. This defines the entity, as it has not been demonstrated in any other salivary gland tumor type [111].

Differential diagnosis The most common salivary tumor entity that mimics MASC is AciCC (AciCC) (see Sect. 5.9.1). Classic AciCC is readily distinguishable from MASC even at the morphological level. In contrast, zymogen granule poor AciCC shows considerable overlap with MASC, and in one series more than half of such cases diagnosed morphologically turned out to be MASC when examined by FISH for *ETV6* gene rearrangements [111, 119]. In general, AciCC is characterised by cytological and structural diversity, being composed of a mixture of serous acinar, intercalated duct-like, hobnail, vacuolated, clear and non-specific glandular cells arranged in solid/lobular, microcystic, papillary-cystic and follicular growth patterns. In contrast, MASCs are structurally homogenous, uniformly composed of microcystic and slightly dilated glandular spaces with secretory material in lumina. The major differential diagnostic feature of MASC is the absence of acinar cells. Furthermore, MASCs display a characteristic immunohistochemical profile (S-100 protein+, mammaglobin+, vimentin+ and DOG1 absent), largely different from AciCC. Definitive separation of these entities is by the demonstration of the *ETV6-NTRK3* translocation by FISH which is diagnostic of MASC and absent in AciCC.

Adenocarcinoma, not otherwise specified (NOS), is a poorly defined category of otherwise unclassifiable salivary gland carcinomas, and it usually represents a diagnosis of exclusion. Description of the MASC with a diagnostic *ETV6-NTRK3* translocation allows reclassification of some cases of adenocarcinomas NOS as MASC.

Low-grade cribriform cystadenocarcinoma (see Sect. 5.9.11.1) must be considered in the differential diagnosis of MASC as well. Although it shares with MASC strong

diffuse S-100 protein expression, low-grade cribriform cystadenocarcinoma possesses a complete intact myoepithelial rim around tumor nests, unlike MASC. However, ductal involvement consisting of a morphologically apparent connection of tumor to medium-sized ducts in the major salivary glands and/or immunohistochemical evidence (p63) of a basal layer surrounding a portion of the tumor nests or cysts was noted in several cases of MASC [118, 120].

The immunohistochemical demonstration of high-molecular-weight cytokeratins (HMWK) and focal mucinous differentiation of MASC raises a differential diagnosis with mucoepidermoid carcinoma [118, 121]. The distinction can be made by the lack of a cobblestone-like appearance with intercellular bridges, true squamoid areas or basal-like intermediate cells in mucoepidermoid carcinoma. In addition, MASC typically lacks p63 staining and shows diffuse S-100 positivity in most cases, which would be distinctly unusual in mucoepidermoid carcinoma, as would papillary formations and hobnailing in the lining of the cysts. The positivity for HMWK in MASC is also less intense than is typically seen in MEC in our experience. Moreover, more than 50 % of mucoepidermoid carcinomas are characterised by a t(11;19) translocation coding for a *CRTC1-MAML2* fusion protein (see Sect. 5.9.3). This is distinct from the t(12;15) translocation encoding *ETV6-NTRK3* of MASC.

Treatment and prognosis Generally, the clinical course of conventional MASC is characterised by a moderate risk of local recurrence (15 %) and lymph node metastases (20 %) and low risk of distant metastases (5 %) [111, 119]. Clinical stage at the time of diagnosis is the most powerful predictor of prognosis. Based on few cases with follow-up data, MASC is currently regarded as a low-grade carcinoma, and its prognosis seems to be favourable overall [111, 117, 119]. However, compared to AciCC, MASC has a slightly higher risk of regional lymph node involvement [119]. Distant metastatic dissemination and tumor-related deaths were reported in three patients so far [111, 119]. Clinical stage at the time of diagnosis is the most powerful predictor of prognosis [119].

Recently, a few cases of HG transformation in MASC have been reported (Fig. 5.45). This is a much more aggressive neoplasm that follows an accelerated clinical course resulting in local recurrences, cancer dissemination and death of all three of our patients reported so far [122]. In view of the aggressive nature of HG-transformed MASC, radical surgery and adjuvant radiotherapy are recommended. Unlike conventional MASC, HG-transformed MASC seems to have a high propensity for cervical lymph node metastases suggesting the need for neck dissection in the management of such patients [122].

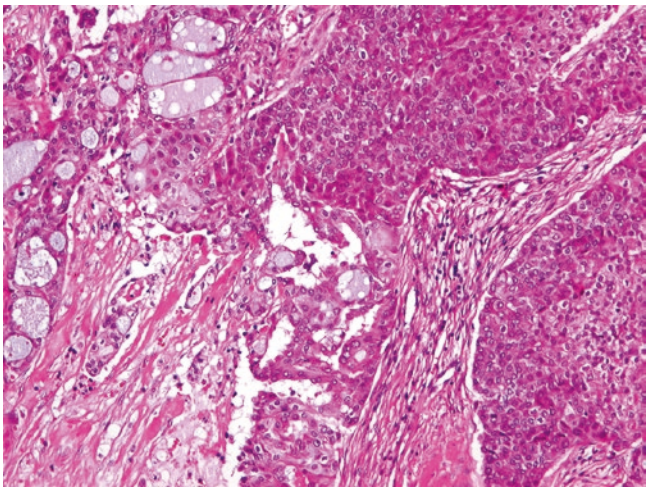

Fig. 5.45 High-grade transformation of mammary analogue secretory carcinoma

5.9.3 Mucoepidermoid Carcinoma

Definition Mucoepidermoid carcinoma (MEC) is a malignant tumor showing mucous, squamous and intermediate cell differentiation.

Epidemiology MEC demonstrates a wide age distribution with a mean of 45 years. It is the most frequent malignant salivary gland tumor in most series [48]. It is also the commonest salivary malignancy in childhood and can be seen even in small children [123]. There is a slight female predominance (3:2). MEC occurs in major and minor glands with roughly equal frequency. In minor glands, MEC is seen most often in the palate, but it may arise in any location. Rarely, it is seen in intra-bony locations in the mandible and the maxilla as the most frequent central salivary gland tumor.

Etiology and pathogenesis The most common aetiologic factor associated with MEC is radiation, and MEC is the most frequent salivary malignancy arising in survivors of childhood cancers treated with radiotherapy. A majority of MECs harbour a balanced translocation t(11;19)(q21;p12-13) and a corresponding fusion gene *CRTC1-MAML2* with alterations in cAMP and Notch signalling pathways suggesting pathogenetic significance for the fusion [124]. Correlating the occurrence of *CRTC1-MAML2* fusion, patterns of genomic changes and follow-up information of MEC cases, tumors negative for *CRTC1-MAML2* fusion appear to represent adenocarcinomas other than MEC [121, 125]. Occurrence of rarer fusions such as *CRTC3-MAML2* and *EWSR1-POU5F1* has been reported in minor subsets of MEC [124].

Clinical aspects A slowly growing tumor mass is the most common presentation.

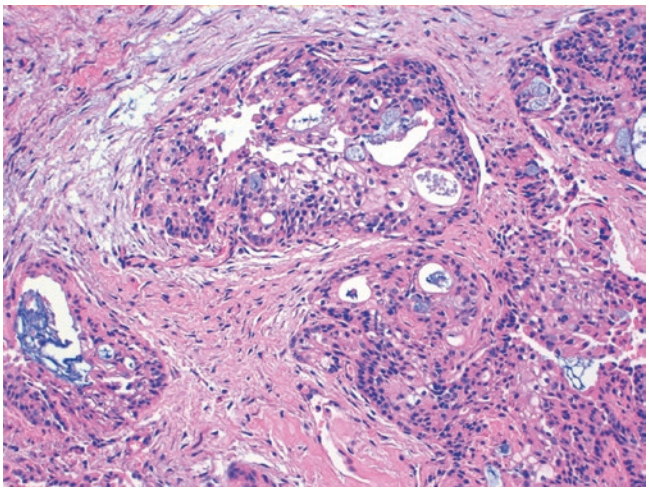

Fig. 5.46 Mucoepidermoid carcinoma: the tumor is formed by epidermoid, intermediate and mucin-producing cells

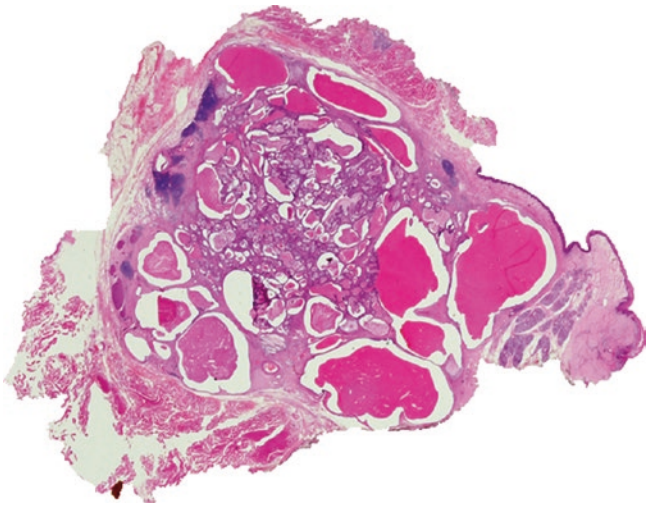

Fig. 5.47 Low-grade mucoepidermoid carcinoma: typical cystic and solid pattern

Macroscopy A cystic and solid tumor which can be circumscribed and mimic a benign tumor.

Microscopy MEC is composed of varying proportions of mucous (mucus-secreting) cells, epidermoid cells, clear cells and cells of intermediate differentiation. The proportion of the different cell types and their architectural configuration (including cyst formation) vary between tumors of differing grades (Fig. 5.46). Mucous cells tend to be more numerous in MECs with cyst formation. Mucous cells are cuboidal, columnar or goblet-like and may form solid proliferations or lining of cysts in single or multiple layers. Their cytoplasm is foamy or reticular and variably basophilic. Cytoplasmic mucins stain with Alcian blue and mucicarmine, which are particularly useful if there are only a few mucous cells. Epidermoid cells have large eosinophilic cytoplasm and may have intercellular bridges, but it should be noted that the term *epidermoid* only indicates squamous-*like* appearance and not necessarily squamous differentiation. Intracellular keratinization or extracellular keratin pearls are very rare in MEC, and they are much more frequent in squamous metaplasia in connection with PA or in a metastatic squamous cell carcinoma from the skin or upper aerodigestive tract. Whilst epidermoid cells may be sparse in MEC, they can be identified using immunohistochemical stains for p63 and high-molecular-weight cytokeratins. Intermediate cells are small basal-like cells with dark-staining nuclei, and they often form the lining of cysts beneath the mucous cells. Clear cells representing clear cell change in squamous or intermediate cells may be frequent in MEC.

Occasionally MEC may mimic a clear cell carcinoma [80]. Sometimes, MEC may also have extensive oncocytic change involving most of the tumor [126]. A rare sclerosing variant of MEC is associated with increased numbers of IgG4 plasma cells and increased fibrosis, but there is no relationship to systemic IgG4-related sclerosing disease [127].

MECs are histologically classified as low-, intermediate- and high-grade malignancies. All MECs are regarded as malignant although only rare cases of low-grade MEC will metastasise. Low-grade MECs typically have a prominent cystic component lined by mucous cells juxtaposed to intermediate and epidermoid cells (Fig. 5.47). Nuclear atypia and mitotic activity are not usually features of low-grade MEC.

Intermediate-grade MECs are less cystic with higher frequency of solid nests of squamous and intermediate cells. A minor degree of nuclear atypia and mitotic activity may be present. High-grade tumors are predominantly solid and infiltrative and show nuclear atypia (Fig. 5.47). They may resemble squamous cell carcinomas with a minor mucous component. Grading of MEC is subjective, and no universally accepted grading system exists yet. However, histological features including extent of the cystic component, mitotic activity, neural invasion, tumor necrosis and cytological pleomorphism have been widely used. Additional features including vascular/lymphatic invasion, bony invasion and invasion of tumor front in small nests have been combined to increase prognostic accuracy [128]. Assessment of MIB1 cell proliferation index has also been shown to be of value [129] (Fig. 5.48).

Differential diagnosis Squamous cell carcinoma, clear cell carcinoma and mucous secreting lesions are the main differentials. Occasionally MEC may mimic a clear cell carcinoma [80]. Sometimes, MEC may also have extensive oncocytic change involving most of the tumor [126].

Treatment and prognosis The treatment is surgical. Grading assessment of MEC has considerable prognostic

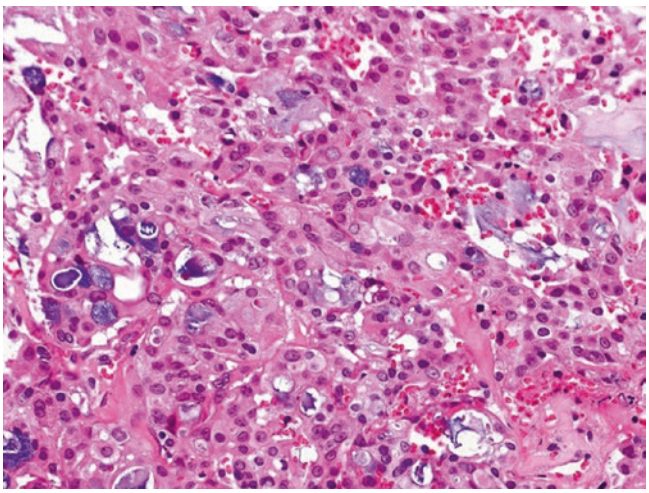

Fig. 5.48 High-grade mucoepidermoid carcinoma: epidermoid cells arranged in a solid pattern also show nuclear pleomorphism. Mucus-secreting cells may be scarce

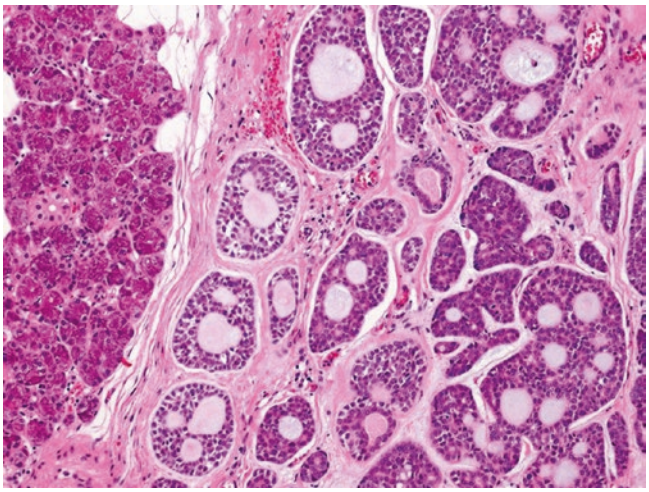

Fig. 5.49 Adenoid cystic carcinoma, cribriform variant: multiple cribriform spaces composed of basaloid cells, with hyalinised material surrounded by small hyperchromatic cells

significance, with death rates due to disease of 3.3, 9.7 and 46.3 % for low-grade, intermediate-grade and high-grade cases, respectively [130].

5.9.4 Adenoid Cystic Carcinoma

Definition Adenoid cystic carcinoma (AdCC) is a malignant tumor composed of a dual population of basal/myoepithelial and luminal cells.

Epidemiology Approximately 10 % of malignant tumors of salivary glands are AdCC. It can occur in any gland, but most often in the submandibular or minor salivary glands, particularly the palate. Other non-salivary anatomic sites include the breast, skin, tracheobronchial, sinonasal and female genital tract [67].

Clinical aspects It presents itself as a slow growing but aggressive tumor mass often resulting in multiple local recurrences and metastases.

Macroscopy Generally it is a solid tumor with hyalinised areas which may be extensive. Margins are usually ill defined, but rarely it can be partly circumscribed.

Microscopy The cellular component is arranged in three main growth patterns:

Cribriform: This is the most characteristic microscopic feature, dominated by multiple cribriform structures, composed of epithelial and basal/myoepithelial cells. The nuclei are usually dark, hyperchromatic and angulated. Mitotic figures are easy to find and may be abundant. The contents of the spaces can be loose and basophilic or dense and eosinophilic (Figs. 5.49 and 5.50).

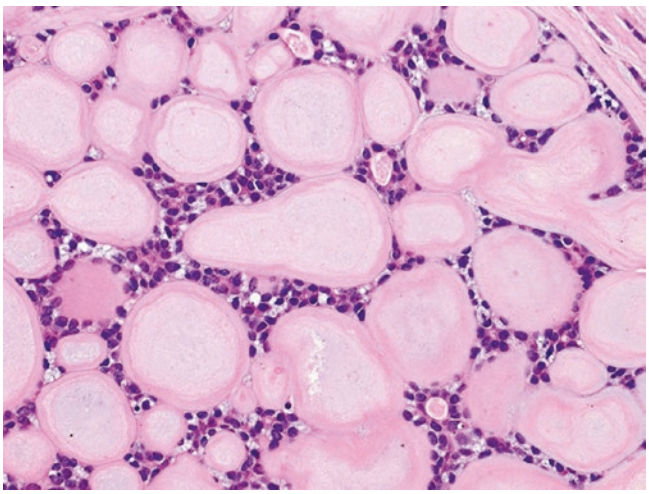

Fig. 5.50 Adenoid cystic carcinoma, cribriform variant. Extensive hyalinisation with compression of tumor cells. Nuclear pleomorphism may be difficult to appreciate, leading to a false diagnosis of pleomorphic adenoma

Tubular: This is composed of small tubules lined with one or two cell types, luminal and abluminal without significant cytological atypia. Because of this bland cytological appearance with well-differentiated tubules, it may be difficult to diagnose on small biopsies, but it poses no problems in surgical specimens due to the presence of infiltration.

Solid (*basaloid*): This is dominated by large solid sheets of tumor cells, sometimes with comedo-like central necrosis (Fig. 5.51). Within the solid masses of tumor cells, there are small duct-like spaces surrounded by a definite layer of epithelial cells (Fig. 5.52).

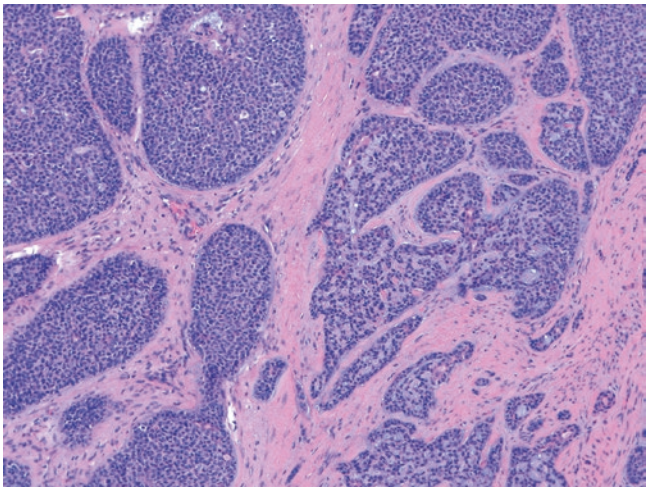

Fig. 5.51 Adenoid cystic carcinoma, solid variant. This is composed of multiple solid nodules; some might display central comedo-like necrosis

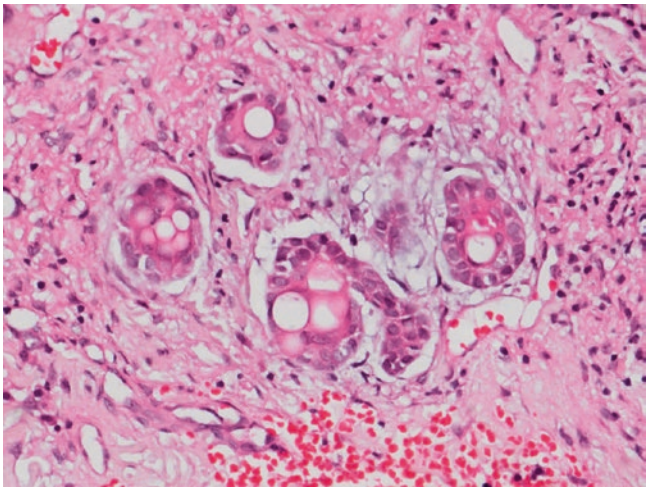

Fig. 5.53 Adenoid cystic carcinoma. Signet ring cell morphology can rarely be identified (Courtesy of Dr. Albina Altemani)

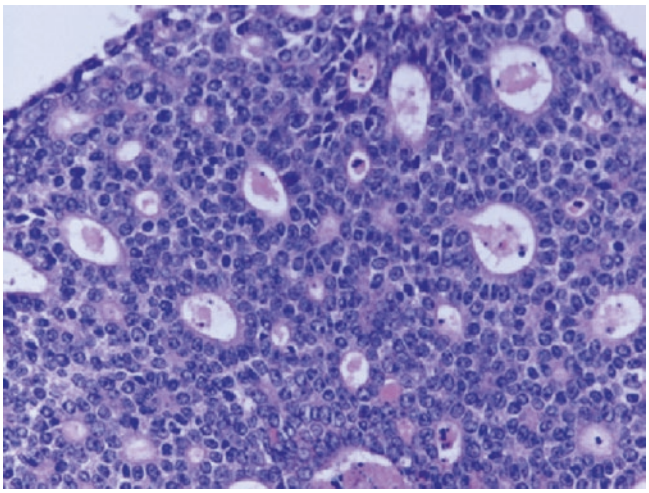

Fig. 5.52 Adenoid cystic carcinoma, solid variant. Tumor islands contain small ducts lined with a layer of epithelial cells. In the absence of characteristic cribriform structures, the latter feature is diagnostic

A rare finding in all types of AdCC is squamous metaplasia, either as single cells or with keratin pearl formation. Another uncommon phenomenon is the presence of signet ring cell changes in AdCC of salivary glands. This is not due to the presence of mucous substance in the signet ring cells as no mucous is shown by PAS staining (Fig. 5.53) [131].

On immunohistochemistry AdCC can display considerable variability in staining for epithelial and myoepithelial markers. Reactivity for broad-spectrum keratins (AE1/AE3, CAM5.2) and myoepithelial markers αSMA, p63 protein, CD10, GFAP, calponin and SMMHC is helpful for the diagnosis. In particular, the presence of p63 positivity in the peripheral rim of tumor islands is helpful. S-100 protein staining is usually weak or patchy. The MIB1 proliferation index almost always exceeds 10 % [132]. AdCC has been reported to show expression of the proto-oncogene and therapeutic target c-KIT [133], but this finding has been questioned by other researchers who have not been able to demonstrate activating KIT mutations in AdCC of various anatomical sites [134].

On the other hand, AdCCs regardless of the anatomical site are characterised by similar molecular features. The majority harbour a specific chromosomal translocation t(6;9) leading to the fusion gene *MYB-NFIB* and overexpression of the oncogene *MYB* [135] which can be a diagnostically useful biomarker for primary and metastatic AdCC [136].

Differential diagnosis The histological features of AdCC are well known, and the cribriform pattern is easily recognised by most pathologists. However, the *tubular* and *cribriform* patterns may be confused with other benign and less aggressive tumors of salivary glands such as PA/BCAc, canalicular adenoma and polymorphous low-grade adenocarcinoma. PA may contain adenoid cystic-like areas, but myxochondroid matrix and plasmacytoid or spindle-shaped myoepithelial cells are usually present [137]. Differentiation is mainly on H&E, but immunohistochemistry for MIB1 which is <5 % in PA/BCA has some value [138].

The most important histological differential diagnosis is between AdCC and polymorphous low-grade adenocarcinoma (see Sect. 5.9.5). Both are diffusely infiltrating neoplasms displaying morphological diversity but can be distinguished cytologically: the former typically have closely packed dark, angular, atypical nuclei and frequent mitotic figures, in contrast to the uniform bland nuclei of the latter. There are also some immunohistochemical guides but no absolute discriminants, e.g. S-100 staining is usually more diffuse and stronger in polymorphous low-grade adenocarcinoma, and p63 typically reacts with cells at the periphery of the islands in AdCC. This latter finding distinguishes solid variant AdCC from the relatively low-grade basal cell adeno-

carcinoma and with the aggressive basaloid squamous cell carcinoma, which, in addition, often shows intraepithelial dysplastic changes. CD117 is of uncertain significance and diagnostic usefulness [134].

Treatment and prognosis The average 5- and 10-year survival rates are about 60 % and 40 %, respectively, but most patients eventually die of disease. The main prognostic factors are site (e.g. submandibular worse than parotid), histological pattern including HG transformation, perineural infiltration, resection margins and clinical TNM stage. A system of three grades based on the presence of tubular, cribriform and solid pattern [139] has shown that outcome is better in tubular AdCC, whilst the worst prognosis is seen when the solid component exceeds 30–50 % of the tumor. Rarely AdCC can undergo high-grade transformation (also referred to as dedifferentiation) characterised by nuclear enlargement and irregularity, higher mitotic counts and the loss of the biphasic ductal-myoepithelial differentiation AdCC with HG transformation that tends to be more advanced at presentation [140]. The pathological stage for this histologic subtype however is prognostically and therapeutically relevant [141]. Another unfavourable feature of AdCC is the frequent involvement of resection margins in the surgical specimen, particularly as the result of extensive perineural infiltration. As complete excision of AdCC is difficult, patients often require postoperative radiotherapy [142].

Nevertheless, clinical stage appears to be a better predictor than grade [141]. Unlike other salivary gland malignancies, when AdCC metastasises, it tends to involve distant organs such as the lung, bone, liver and more rarely the skin rather than local lymph nodes [136].

Interestingly the clinical behaviour of salivary gland and breast AdCC differs significantly; whereas salivary gland AdCC have a high proclivity to recur locally and metastasise, patients with breast AdCCs have an excellent outcome [133].

5.9.5 Polymorphous Low-Grade Adenocarcinoma

Definition Polymorphous low-grade adenocarcinoma (PLGA) is an infiltrating tumor with cytological uniformity and architectural diversity [67]. Previously called terminal duct or lobular carcinoma, PLGA is now a well-established entity characterised by its broad variety of histological patterns [143].

Epidemiology It is the second most common malignant tumor of minor salivary glands of the palate after MEC [48].

Clinical presentation It is more frequent in women, and the average age at presentation is 59 years (range 21–94) [48, 144, 145]. Most cases characteristically arise in intraoral minor salivary glands, particularly the palate, with only rare examples in the parotid [146], sometimes developing from a PA [147].

Macroscopy PLGA is often a well-circumscribed tumor which contrasts with the microscopic infiltrative growth. The median size is 25 mm.

Microscopy The characteristic histological picture of PLGA is of an infiltrating tumor with cytological uniformity and diverse histological patterns [67]. The architectural patterns include tubular, solid, trabecular, fascicular, cribriform and papillary. Perineural infiltration is often seen (Fig. 5.54). Diffuse infiltration of tumor cells with Indian filing and concentric growth around nerves is reminiscent of lobular carcinoma of the breast (Fig. 5.55). The cells each have single regular round, ovoid or fusiform bland nuclei, sometimes with intranuclear vacuoles and absent or small nucleoli [148]. Variably present are oncocytic, clear or mucous cells. Mitotic figures are scanty and never atypical. The stroma varies from fibromyxoid to densely hyaline, but the chondroid matrix of a PA is not seen.

Immunohistochemistry shows positivity with epithelial markers (cytokeratins, EMA), S-100, bcl-2 and sometimes CEA, αSMA and vimentin [149]; MIB1 proliferation is low, mean 2.4 % (range 0.2–6.4) in one study [132].

Differential diagnosis The most important histopathological differential diagnoses are AdCC and PA [150]. Difficulties are particularly the case in small biopsies. Although both

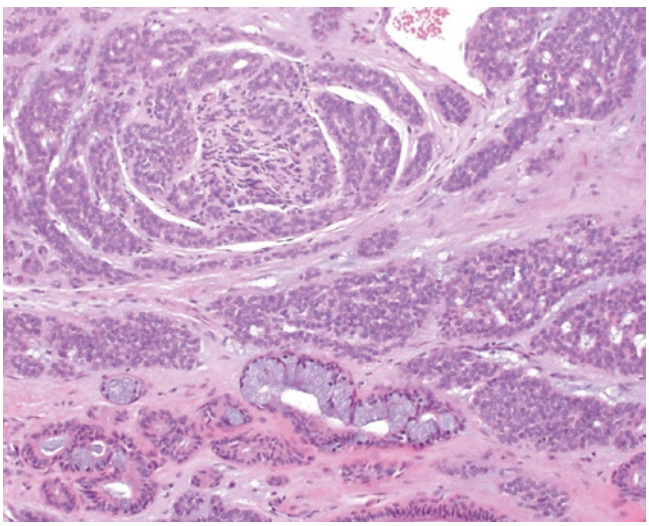

Fig. 5.54 Polymorphous low-grade adenocarcinoma. Perineural infiltration. Tumor cells show bland cytonuclear abnormality

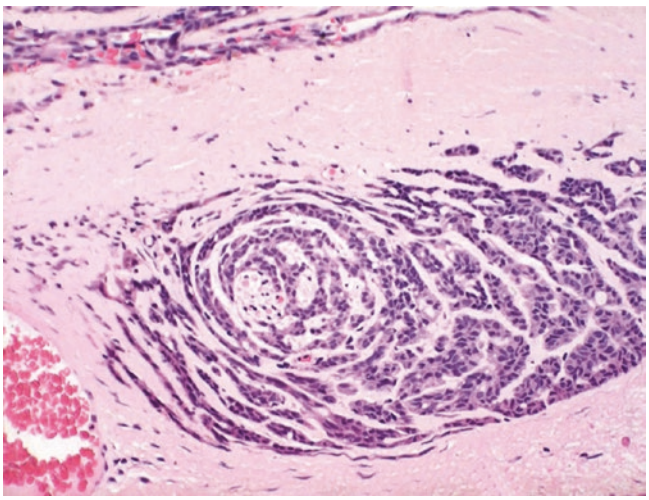

Fig. 5.55 Polymorphous low-grade adenocarcinoma. Indian filing appearance resembling lobular carcinoma of the breast

PLGA and AdCC are diffusely infiltrating carcinomas, separation is based on architectural and especially cytological features. The nuclei in AdCC are hyperchromatic, angulated, pleomorphic and densely packed with more frequent mitotic figures. In contrast, the nuclei in PLGA are uniform with finely speckled chromatin. The tubules of AdCC are surrounded by an outer layer or myoepithelial cells, whereas in PLGA the tubules generally have a single layer of epithelial cells. The cribriform architecture is more rigid in AdCC than in PLGA. In addition, staining with S-100 protein is usually more diffuse and stronger in PLGA than AdCC [138]. Other markers such as c-kit (CD117) are of little use in practice, as staining can be seen in AdCC and most PLGAs [151]. A much more reliable marker is the MIB1 proliferation index, which is almost always significantly lower in PLGA [132]. The differential diagnosis with PA, which in minor salivary glands can be poorly circumscribed, is based on the presence of chondroid matrix and circumscription of PA, but it is sometimes not possible to distinguish these tumors, particularly on a small biopsy. One other differential diagnosis is with cribriform adenocarcinoma of the tongue and other minor salivary glands, the most useful discriminant being the presence of clear nuclei resembling those of papillary thyroid carcinoma (see Sect. 5.9.6).

Treatment and prognosis The treatment of choice is surgical with safe (15 mm) margins [143]. Postoperative radiation and chemotherapy have little place. PLGA behaves as a low-grade malignancy; a literature review found a recurrence rate of 21 %, regional nodal metastasis in 6.5 %, distant metastasis in 1.8 % and death due to cancer in 0.9 % [152]. However, after 10 years late recurrences and metastases can appear. In a series of 19 cases of PLGA, 5 patients had local recurrences as late as 15 years after the initial treatment. One patient had regional lymph node metastases 20 years after surgery, and another patient developed lung metastasis after local recurrence [145]. These data confirm that of the previous larger series [145, 149], but there is still debate in literature as to whether recurrences are due to incompleteness of excision – none of the 22 excised tumors recurred or caused death [153] – or whether they can occur even with histologically clear margin but after many years [145]. In a larger series of 164 PLGA, more than 95 % of the patients had no evidence of disease after a long-term follow-up [149]. Papillary structures form part of the spectrum of growth patterns seen in PLGA [154], but when extensive, there is evidence that such tumors are more aggressive [144] or may represent the newly described entity of cribriform adenocarcinoma of tongue and other minor salivary glands (see Sect. 5.9.6) although they do not seem to affect long-term survival. Perineural infiltration does not confer a worse prognosis. Genuine HG transformation can occur rarely, as either a poorly differentiated PLGA or as a salivary duct carcinoma [155].

5.9.6 Cribriform Adenocarcinoma of the Tongue and Other Minor Salivary Glands

Definition Cribriform adenocarcinoma of the tongue (CATS) and other minor salivary glands is a distinctive hitherto poorly recognised low-grade adenocarcinoma, with several histological features reminiscent of papillary carcinoma of the thyroid and which mostly but not exclusively occurs in the tongue [67, 156]. This tumor was recognised by the latest issue of the WHO classification as a possible variant of polymorphous low-grade adenocarcinoma (PLGA), but it was noted then that it was not yet clear whether this represented a genuine separate entity [157].

Epidemiology In 1999, Michal et al. published a series of a distinctive type of adenocarcinoma occurring in the tongue characterised by synchronous metastases in lateral neck lymph nodes, but no distant spread [156]. Of the so far 31 published cases in the literature [156–161], 21 tumors were located in the tongue (usually the base), 3 in the soft palate, 2 in the retromolar buccal mucosa, 3 in the lingual tonsils, 1 in the upper lip and 1 in the floor of the mouth. One tumor located in the tongue was described to have a pedunculated configuration [160]. The sex was known in 27 cases: the tumors occurred in 15 women and 12 men. The age of the patients ranged from 21 to 85 (mean 56.8 years).

Clinical aspects The majority of patients (19 of 31) present with metastases in the cervical lymph nodes, mostly at

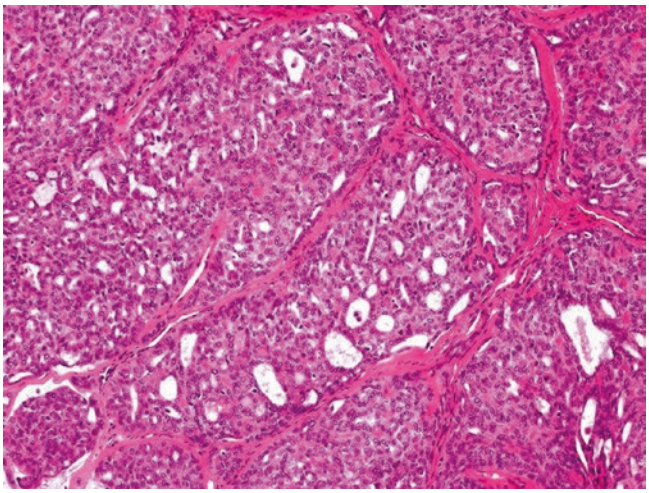

Fig. 5.56 Cribriform adenocarcinoma of the tongue. A vaguely nodular growth pattern is composed of solid nests with tubular structures

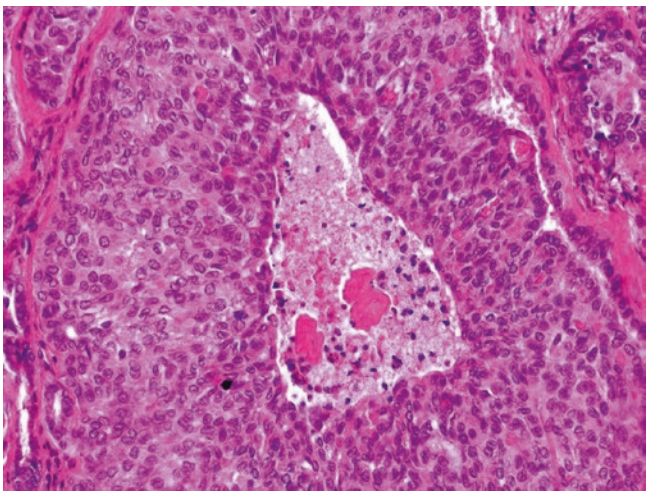

Fig. 5.57 Cribriform adenocarcinoma of the tongue. In solid areas, peripheral epithelial layer is detached from the surrounding fibrous stroma by (presumably artefactual) clefts and often displays hyperchromatic nuclei in a palisaded pattern

approximately the same time as the diagnosis of the primary (bilateral in three cases) and one after an interval of 8 years. The primary sites in the lymph node-positive cases were in most cases posterior tongue and retromolar mucosa. However, cervical lymph node metastasis appeared also in extralingual cases including primary of tonsils (two cases) and the palate (two cases).

Macroscopy The tumor size ranges from 3 to 8 cm in greatest dimension. Grossly, the tumors are covered by intact mucosa devoid of ulceration. They are unencapsulated, white-tan to grey in colour, hard in consistency with no areas of haemorrhage or necrosis.

Microscopy Histologically, the tumors have invasive margins, in most cases with infiltration of the muscular layer of the tongue and/or adjacent tissues. Lymphovascular invasion is observed in about half of cases. There are often deposits of haemosiderin in focally hyalinised interstitial stroma close to the invasive border of the lesions. The tumors are composed predominantly of cribriform and solid structures in variable proportions (Fig. 5.56). In most instances the tumor architecture consists mainly of a solid mass, often divided by fibrous septa into irregularly shaped and sized nodules composed of solid, cribriform and microcystic structures (Fig. 5.56). In the solid areas, the tumor nests may become detached from the surrounding fibrous stroma by (presumably artefactual) clefts, giving a glomeruloid appearance. The peripheral layer of such solid tumor nests often display hyperchromatic nuclei in a somewhat palisaded pattern (Fig. 5.57). Typically, the tumors also include intermingled tubular, solid and cribriform growth patterns. The tubules are approximately all of the same size, and they consist of one cell layer (Fig. 5.58).

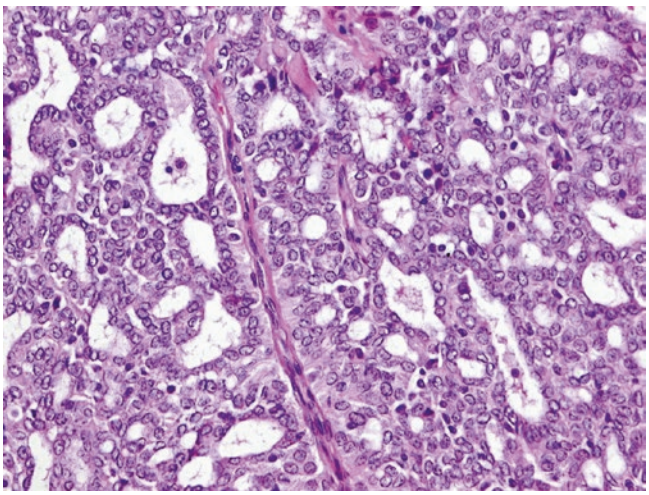

Fig. 5.58 Cribriform adenocarcinoma of the tongue includes intermingled tubular, solid and cribriform growth patterns. The tubules were approximately all of the same size, and they consisted of one cell layer

The most prominent feature of the tumors, however, is the appearance of the nuclei. These overlap one another and are pale, optically clear and vesicular with a ground glass appearance, so that the tumors cytologically strongly resemble papillary carcinoma of the thyroid gland (Fig. 5.59). Cellular atypia is usually mild, and mitotic figures are in most cases rare. The cytoplasm is clear to eosinophilic and often abundant. Cytologically, all the tumors are composed of one cell type. The overall morphology of the tumor, particularly with focal papillary growth and with overlapping clear 'Orphan Annie eye-like nuclei', is remarkably similar to the solid variants of papillary thyroid carcinoma. The cervical lymph node metastases have identical appearances to the primary tumors.

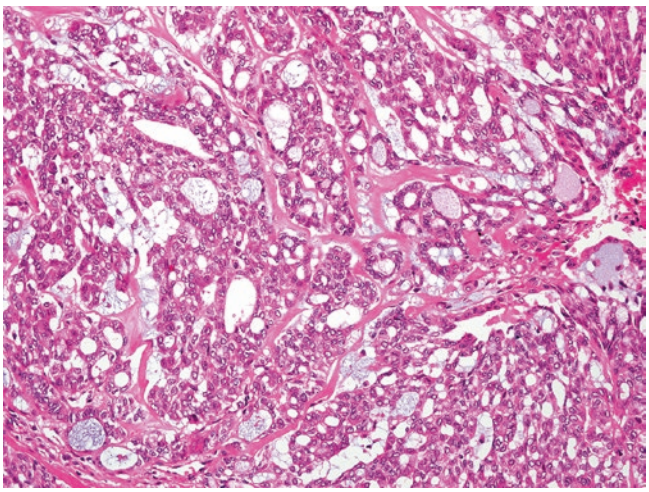

Fig. 5.59 Cribriform adenocarcinoma of the tongue. The most prominent feature is the appearance of the nuclei. They overlap one another and are optically clear and vesicular with a ground glass appearance resembling thus papillary carcinoma of the thyroid gland

On immunohistochemistry CATS is characterised by co-expression of cytokeratins (AE1/AE3, CAM5.2, CK7, CK8 and CK18), S-100 protein and vimentin. Basal and myoepithelial cell markers, such as p63, calponin, CK14, αSMA and CK5/6, are positive in all tumors with variable proportions up to 60%. Often the palisaded cells surrounding the glomeruloid structures were positive for these markers. Expression of CK19 was variable with mild to moderate focal staining of membranes and cytoplasm in few cases (range 3–5% of cells, mean 3%), other cases being completely devoid of staining, but no case was diffusely positive. Proliferative activity was low. EMA, EGFR and HER2/neu were negative in all cases. More importantly all the tumors were completely devoid of any staining for TTF1 and thyroglobulin. No somatic mutations of *BRAF*, *K-RAS*, *H-RAS*, *N-RAS*, c-kit and *PDGFRa* genes were found in any of the analyzable cases in two papers [157, 161]. However, in *RET* proto-oncogene, heterozygous polymorphism Gly691Ser in exon 11 (one case), heterozygous polymorphism p. Leu769Leu in exon 13 (one case), heterozygous polymorphism Ser904Ser in exon 15 (one case) and intronic variant p.IVS14-24 G/A of exon 14 (two cases) were found in one study [161].

Differential diagnosis The most important differential diagnosis of CATS is PLGA. This neoplasm typically has a wide range of architectural appearances, including tubule and fascicle formation, as well as solid, cribriform and sometimes small papillary structures. A particularly characteristic feature of PLGA is the occurrence of streaming columns of single file or narrow trabeculae of cells forming concentric whorls, thereby creating a target-like appearance [67]; perineural invasion is often seen. At the cellular level, PLGA quite often contains clear cells and, less frequently, mucous cells. Perhaps most importantly, the most striking feature of CATS is the great nuclear similarity to papillary carcinoma of the thyroid, and this is not seen to any great extent in PLGA. Consequently, the other most significant differential diagnosis of CATS is from metastatic papillary carcinoma of the thyroid, particularly if nodal disease is the first presentation. CATS is always thyroglobulin and TTF1 negative and devoid of colloid. However, both CATS and papillary thyroid carcinoma may show variable expression of galectin-3, CK 19 and HBME-1 [161]. In contrast to thyroid cancer, all cases of CATS stain strongly for S-100 protein, and focal myoepithelial differentiation with calponin and actin positivity is common.

Treatment and prognosis The tumors were treated by surgical excision often accompanied by neck lymph node dissection. Of the 31 patients, 14 individuals additionally received radiotherapy and 1 adjuvant chemotherapy. Clinical follow-up was known in 21 cases. All patients with available follow-up (range 2 months to 13 years; mean 4.3 years) were alive without signs of metastasis [157].

5.9.7 Epithelial-Myoepithelial Carcinoma

Definition Epithelial-myoepithelial carcinoma (EMC) is a malignant tumor composed throughout of both epithelial and myoepithelial cells ('biphasic tumor') in varying proportions.

Epidemiology EMC has a wide age range (8–103, mean 60 years) and a slight female predominance [67]. Most cases occur in the parotid, less often in other salivary glands. Analogous neoplasms have been described in the breast (adenomyoepithelioma) and elsewhere.

Etiology and pathogenesis EMC usually arises de novo but occasionally develops in a pre-existing PA [162].

In addition, cases of EMC (and other tumors such as BCA) have been associated with multiple nodules of intercalated duct adenomas and hyperplasia in the surrounding parotid gland [27–29]. This suggests a ductal origin of EMC and also perhaps why in hybrid carcinomas of the salivary glands, themselves very rare, the most frequent combination is that of two typical biphasic tumors, EMC and AdCC [28].

Clinical aspects The usual clinical presentation is a slow-growing mass, with ulceration in mucosal minor gland tumors. The duration of symptoms before diagnosis ranges from a few months to many years.

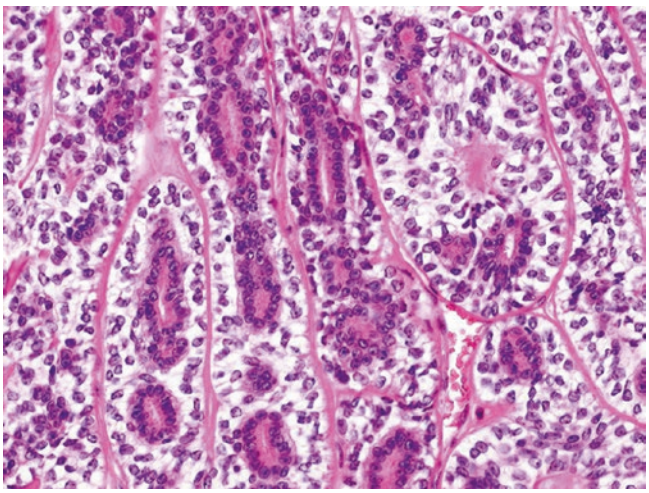

Fig. 5.60 Epithelial-myoepithelial carcinoma: characteristic biphasic appearance with an inner layer of ductal cells and outer layer of clear myoepithelial cells. Basal membrane-like material surrounds the outer cells

Macroscopy The typical macroscopic appearance of EMC is that of a multinodular, unencapsulated mass, mainly solid, although cystic change is seen in 30 %. The size is usually 20–30 mm but can be as large as 120 mm.

Microscopy EMC is composed of lumina lined by two distinct layers of cells, epithelial and myoepithelial, beyond which is a basement membrane of variable thickness. The inner epithelial cells are generally small, cuboidal to low columnar and composed of scanty pale to eosinophilic cytoplasm and a round-to-oval nucleus; they can be identified by low-molecular-weight cytokeratin. The surrounding myoepithelial cells typically are larger and polygonal in shape but at times can be spindled or plasmacytoid; their cytoplasm is usually clear, but not always (Fig. 5.60). Immunohistochemically, they express αSMA, SMMHC, calponin and p63 and S-100. CD10 and cytokeratin 14 are less specific, sometimes reacting with both cell layers. Cytological pleomorphism in EMC is usually mild but was classed as severe in 6.6 % of cases [163]; schwannoma-like 'ancient change' has also been described. Mitotic figures vary in number and can be numerous; similarly, Ki-67 proliferative activity has a wide range 0–50 and a mean of 17 %. The biphasic pattern of EMC is reproduced throughout most of the tumor (and, indeed, is retained in cell cultures [164]) though each element may vary in prominence between cases as well as within any given lesion [165]. Myoepithelial cells with clear cytoplasm usually dominate the picture, but more than 20 % of EMCs lack clear cells altogether [163], and immunohistochemistry is necessary to demonstrate the double cell population. Variants involving the epithelial cells include apocrine [166] and sebaceous differentiation [167]. Either or both cell types can show oncocytic change [163]. The stroma of EMC is usually a fine network of PAS-positive basement membrane material surrounding the bilayered ducts, but on occasions, it is much thicker with relatively inconspicuous bilayered ducts overall resembling a PA [168]. Although most EMCs are minimally invasive often in a 'pushing' pattern, one study identified perineural infiltration in 34 % and involvement of lymphovascular spaces in 11 %; there was extensively infiltrating growth in 13 % [163]. Areas of necrosis are found in about 20 % of EMCs.

Differential diagnosis The differential diagnosis is mainly with other biphasic tumors of salivary glands such as PA and AdCC. Bilayered ducts are not uncommon in any PA, but this appearance will always be only focal. AdCC may have occasional EMC-like areas, and extensive sampling may help to identify typical areas (see Sect. 5.9.4). AdCC has hyperchromatic and angulated nuclei with the three, *tubular*, *cribriform* and *solid*, characteristic growth pattern. Similarly myoepithelial carcinoma may contain occasional ducts resembling an EMC. Therefore, any tumor with EMC-like areas must be sampled widely in order to identify their diagnostic features. When EMC is composed of clear cells, the differential diagnosis includes other clear cell lesions, e.g. other clear cell tumors [169].

Treatment and prognosis Surgical excision with or without neck dissection is the treatment of choice. The behaviour of EMC is low grade, typically with recurrences in 31 %, cervical node metastases in 18 %, distant metastases and death due to tumor in 7 % [169], although more recent studies suggest a lower rate of distant metastases, probably a consequence of better clinical management [166]. Higher rates of recurrence (50 %) and death (40 %) in a series from a large referral centre in Portugal likely reflected a patient population with advanced disease [170]. Morphological features found to correlate with a poor prognosis include positive margin status, angiolymphatic invasion, necrosis and myoepithelial anaplasia [163]. EMC can occasionally dedifferentiate as a HG myoepithelial neoplasm [171] or adenocarcinoma [172].

5.9.8 Hyalinizing Clear Cell Carcinoma

Definition Hyalinizing clear cell carcinoma (HCCC) is a malignant tumor composed of epithelial cells usually with clear cytoplasm. Until recently, the diagnosis required the exclusion of other salivary tumors with a clear cell component [67], but it is now recognised that HCCC is a distinct neoplastic entity. Most cases harbour a unique (in salivary glands) molecular genetic abnormality [173]. As such, it is expected to be included as a distinct tumor in the coming WHO classification rather than as part of clear cell carcinoma, not otherwise specified (NOS) [67] as it was in the 2005 WHO classification.

Epidemiology The sex incidence of HCCC is equal or with a slight female predominance [173] and the age range wide. Most cases arise in minor salivary glands, mainly the palate [48, 67, 173–176] and much less frequently elsewhere such as the parotid [174].

Etiology and pathogenesis Ultrastructural studies have demonstrated desmosomes and tonofilaments, suggesting squamous differentiation [177]. Most cases of HCCC harbour a recurrent and consistent rearrangement of the *EWSR1* gene, usually a *EWSR1-ATF1* fusion [178, 179]. This is not found in any other primary salivary carcinoma but is seen in clear cell odontogenic carcinoma, suggesting the two entities may be related or even identical [180].

Clinical aspects Patients typically present with a painless mass in the palate or, more rarely, in the parotid gland. Exceptionally, cervical lymph node metastases are seen at initial presentation.

Macroscopy A circumscribed tumor usually <30 mm in diameter with pale cut surface.

Microscopy HCCC is composed of invasive nests, sheets and trabeculae of polygonal glycogen-rich cells characteristically separated by dense hyaline basement membrane material and fibrocellular stroma [67]. The predominance of clear cells is seen in a minority of cases, and the tumor cells in most cases have pale eosinophilic cytoplasm rather than clear cytoplasm, or they may have a mixture of both (Fig. 5.61). HCCC is composed of nests, sheets and trabeculae of polygonal cells with clear cytoplasm separated by fibrocellular stroma (Fig. 5.62). Occasional HCCCs demonstrate squamous or ductal differentiation. Mucin is often present in small amounts and occasionally is readily apparent [173]. The nuclei display generally mild pleomorphism and inconspicuous nucleoli; mitotic figures are rare. In most cases, HCCCs express epithelial markers, including cytokeratin 7 (but not 20), EMA and 34βE12 as well as p63 (Fig. 5.63) [181], but other myoepithelial markers (e.g. S-100 protein, actin) are consistently negative.

Differential diagnosis Includes other primary salivary neoplasms composed of clear cells [169, 174] (Table 5.2) as well as metastases principally from renal cell carcinoma, which can present as a parotid mass.

Treatment and prognosis The treatment of HCCC is surgical excision. The prognosis is generally good; occasional

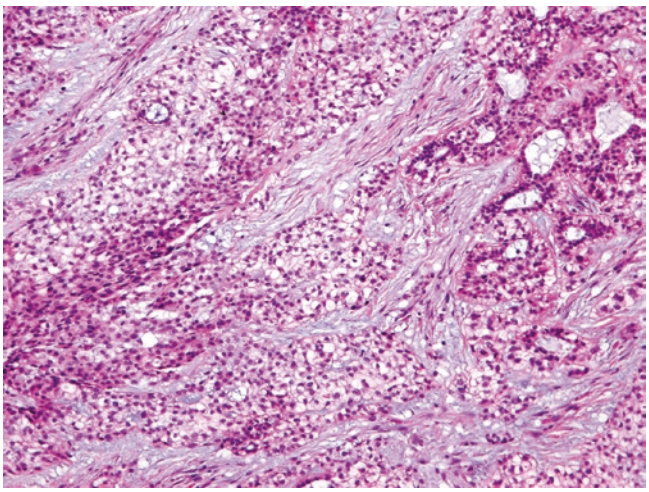

Fig. 5.61 Hyalinizing clear cell carcinoma. Cells in which the cytoplasm appears weakly eosinophilic rather than clear are usually present and may predominate in some tumors

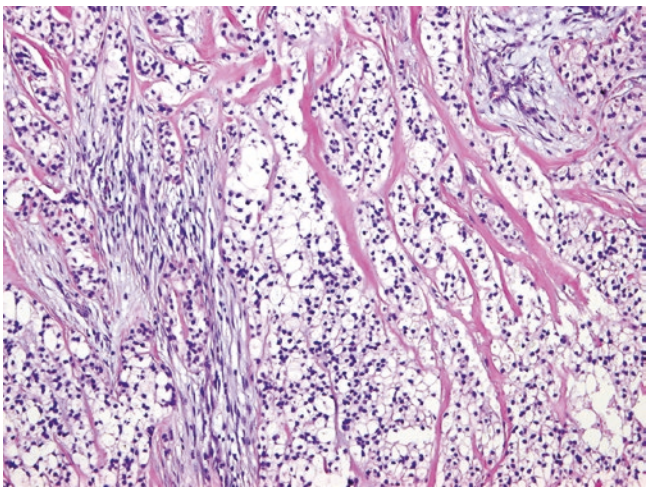

Fig. 5.62 Hyalinizing clear cell carcinoma is composed of nests, sheets and trabeculae of polygonal cells with clear cytoplasm separated by fibrocellular stroma

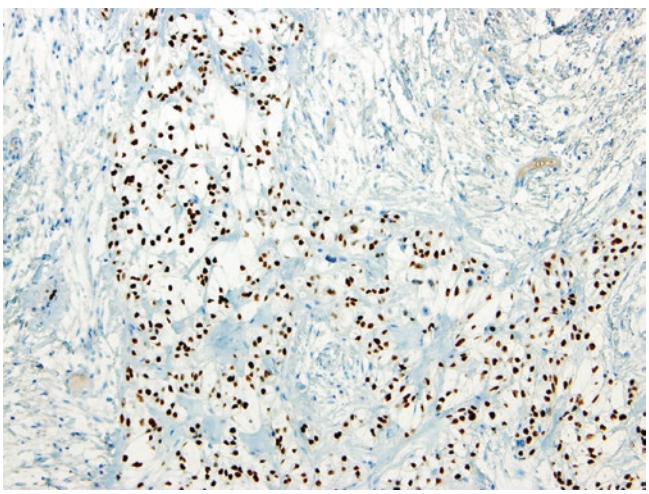

Fig. 5.63 Hyalinizing clear cell carcinomas express epithelial markers and p63 in all tumor cells

Table 5.2 Classification of clear cell tumors of the salivary glands

Benign	
Pleomorphic adenoma, myoepithelioma, sebaceous adenoma, oncocytoma and oncocytic hyperplasia (MNOH)	
Malignant, primary	
(a) Carcinomas not usually characterised by clear cells but with rare clear cell variants, e.g. mucoepidermoid, acinic cell, myoepithelial carcinomas	
(b) Carcinomas usually characterised by clear cells:	
(i) Dimorphic	Epithelial-myoepithelial carcinoma
(ii) Monomorphic	Hyalinizing clear cell carcinoma
(iii) Sebaceous carcinoma	
Malignant, metastatic	
Carcinomas, especially the kidney, thyroid. Also melanoma	

patients have developed metastases in the neck nodes and rarely the lungs [67, 174], but recently a few higher-grade tumors have been identified with the *EWSR1* gene rearrangement suggesting that not all HCCCs behave indolently [173].

5.9.9 Basal Cell Adenocarcinoma

Definition Basal cell adenocarcinoma (BCAc) is a malignant tumor composed of basal-type cells with an infiltrative pattern and potential for metastasis. The WHO classification of salivary gland tumor in 1991 [88] named this tumor 'adenocarcinoma' to separate BCAc from the more common basal cell carcinoma of the skin.

Epidemiology BCAc is rare. It accounts for approximately 2 % of salivary gland carcinomas. Most cases arise in patients over 50 years of age, and there is an equal sex incidence [85]. The usual site is the parotid gland, but they have been described in the submandibular [182], sublingual [183] and minor glands [184, 185].

Etiology and pathogenesis BCAc can arise de novo, but about 25 % develop in a pre-existing BCA usually of the membranous type [186] (see Sect. 5.8.3). The association between dermal cylindroma and BCA is well known, but BCAc can also arise in association with malignant dermal cylindroma [187].

Macroscopy Usually unencapsulated tumor mass ranging from 7 to 70 mm, infiltrating the surrounding salivary gland, rarely can be circumscribed.

Microscopy Microscopically, the general morphological and cytological appearances are almost identical to BCA, and likewise, four growth patterns are recognised – *solid*, *tubular*, *trabecular* and *membranous* – although these are not thought to have prognostic significance. The tumor islands contain a mixture of large, paler and small basaloid cells, with the latter usually demonstrating peripheral palisading, though this is less marked than in the benign counterpart. The large cells sometimes form 'eddies', and the tumor islands may also contain small tubules and foci of squamous metaplasia. The amount of basement membrane material varies but can be marked, especially in the membranous variant. Occasional cases show cytological pleomorphism, but generally this is absent, and mitotic figures are usually sparse (Fig. 5.64).

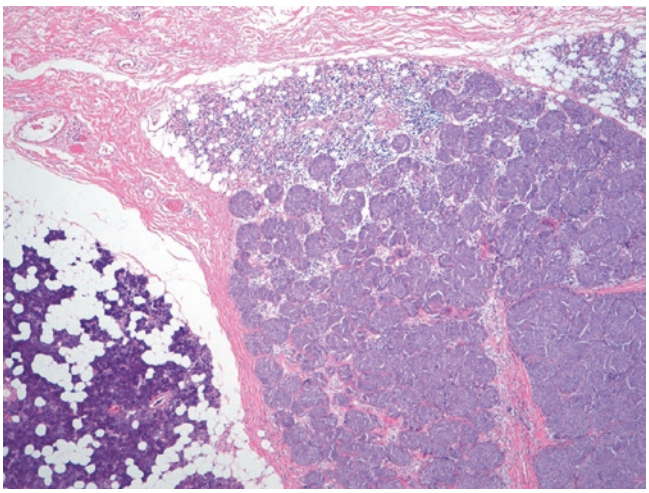

Fig. 5.64 Basal cell adenocarcinoma. In spite of the lack of significant cellular atypia, the infiltrative pattern is diagnostic of malignancy

Differential diagnosis This is mostly with BCA and AdCC.

The most reliable indicator of malignancy is infiltration of the surrounding gland and less frequently of blood vessels and nerves [186]. In addition, the Ki-67 proliferation index is usually higher in BCA than its benign counterpart (>5 % vs. <2.7 %) [188]. More than half the BCAcs in one study expressed p53, and 3 out of 11 cases were positive for epidermal growth factor receptor (EGFR); in contrast, all the adenomas were negative [188]. The differential diagnosis of BCAc includes solid forms of AdCC (see Sect. 5.9.4), which are much more aggressive neoplasms with cytological pleomorphism and plentiful mitotic figures; these are generally associated with other growth patterns such as small luminal structures. Although distinction between AdCC and BCAc is usually obvious on morphology, the p63 pattern may help, in that it stains particularly the peripheral cells of tumor islands in the former and most cells in the latter. In the rare case of minor salivary gland BCAc and multiple malignant cylindromas of the skin, immunocytochemical studies showed positive staining for high- and low-molecular-weight keratins and S-100 with negative staining for CEA in both skin and salivary gland tumors [187].

Treatment and prognosis BCAc is treated with surgery of the primary tumor with clear margins.

The behaviour of most BCAcs is that of a low-grade malignant tumor. A review found an incidence of local recurrence of 37 %, cervical lymph node metastasis of 8 % and distant metastases of 4 %, and one patient died of disseminated disease [186]. Rarely BCAc can show aggressive behaviour with cerebral and pulmonary metastases and death of the patient [189].

5.9.10 Myoepithelial Carcinoma

Definition Myoepithelial carcinoma is a neoplasm composed almost exclusively of cells with myoepithelial differentiation showing infiltrative growth and metastatic potential. The terms myoepithelial carcinoma and malignant myoepithelioma are interchangeable, but the former is preferred by the 2005 WHO classification [67].

Epidemiology Myoepithelial carcinoma accounts for about 1–2 % of malignant salivary neoplasms, but it may not be as rare as supposed earlier. The mean age of patients at presentation is about 55 years (range 14–86 years), and the sex incidence is approximately equal [77]. Most cases arise in the parotid gland, but they also occur in submandibular and minor glands, usually the palate; rarely they are found in the base of the tongue, maxillary sinus and larynx [77, 190].

Etiology and pathogenesis Myoepithelial carcinomas may arise de novo, but at least half of them develop in pre-existing PAs or benign myoepitheliomas particularly recurrences [190].

Clinical aspects The commonest complaint is that of a mass in the parotid region. When myoepithelial carcinoma arises in recurrent PA, the patient often gives a history of previous surgical resections.

Macroscopy Grossly, myoepithelial carcinomas are unencapsulated but may be well defined with nodular surfaces. The cut surface is grey-white and can be glassy. Some tumors reveal areas of haemorrhage, necrosis and pseudocystic degeneration.

Microscopy The architecture is often multinodular with infiltration into adjacent tissues (Fig. 5.65). The nodules comprise solid and sheet-like growths of tumor cells often with plentiful myxoid or hyaline material and sometimes displaying central necrosis (Fig. 5.66). The range of cell types reflects that seen in benign myoepitheliomas and includes epithelioid cells (the most frequent) often arranged in trabecular or pseudo-acinar structures with cleft-like spaces.

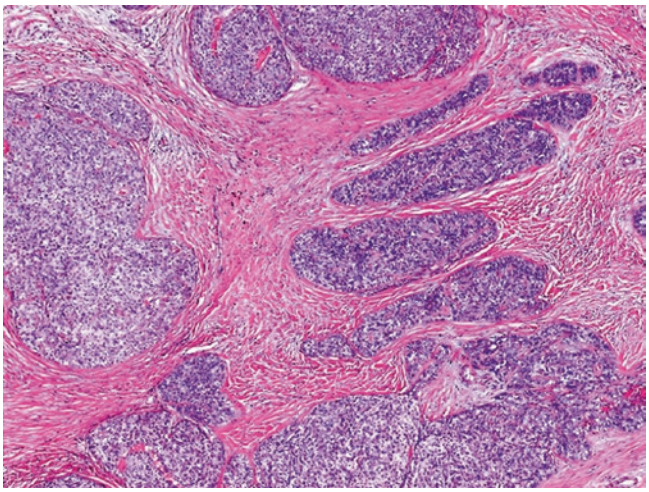

Fig. 5.65 Myoepithelial carcinoma. Multiple nodules infiltrate dense fibrous tissue

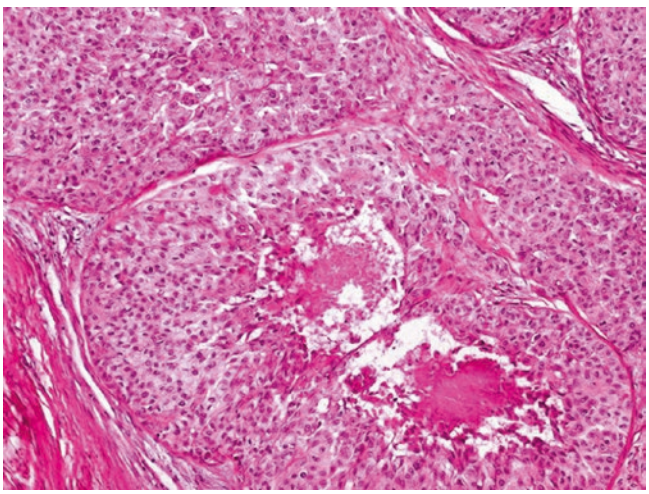

Fig. 5.66 Myoepithelial carcinoma, solid growth pattern with central necrosis in one of the nodules. This finding may mimic salivary duct carcinoma with comedo-like necrosis

Cells with clear cytoplasm or vacuolation (resembling lipoblasts) and cells with hyaline (plasmacytoid) and spindle to stellate forms are also seen (Fig. 5.67). In most myoepithelial carcinomas, one cell type predominates, but there is usually a minor component of other cell types. Some authors (including ourselves) feel that a few true glands or lumina are allowable in otherwise typical myoepithelial carcinomas, but others feel they preclude the diagnosis [77]. The nuclei vary from relatively uniform, small with finely distributed chromatin, lacking obvious nucleoli, to markedly enlarged and pleomorphic, showing chromatin clumping and large nucleoli. Mitotic figures may be plentiful (range 3–51 per 10 HPF) and include atypical forms [190]. Multinucleated and bizarre tumor giant cells may occasionally be present. The

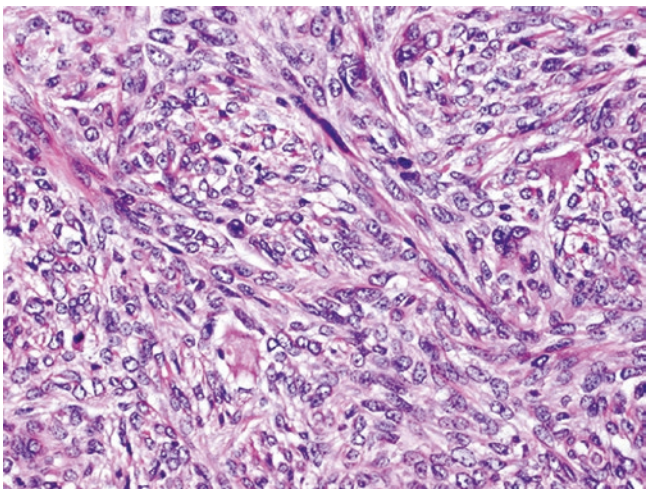

Fig. 5.67 Myoepithelial carcinoma. The spindle cell component shows nuclear pleomorphism resembling a soft tissue sarcoma. A helpful diagnostic pointer is that other types of myoepithelial cell are usually identified elsewhere

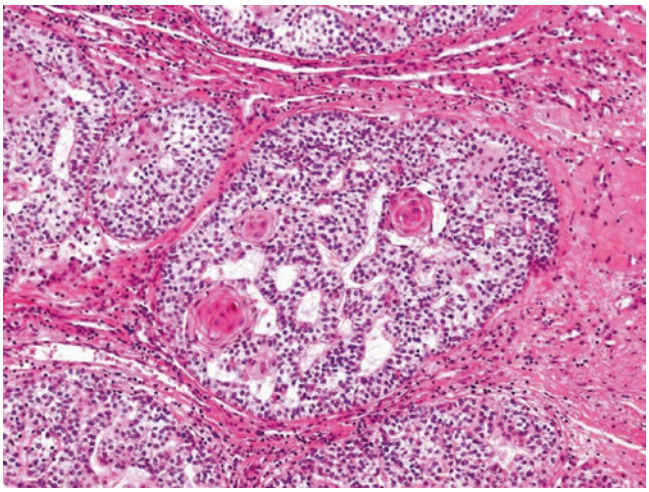

Fig. 5.68 Myoepithelial carcinoma: focal squamous metaplasia with keratin pearl formation

tumor-related matrix is generally prominent and is hyalinised or myxoid. Special stains show no mucicarmine positive mucus, but plentiful glycogen is found in clear cells, and the myxoid matrix is positive with Alcian blue. Metaplastic changes are frequent and include areas showing squamous differentiation, often with keratinization (Fig. 5.68). Perineural invasion is seen in 44 % and vascular invasion in 16 %. In one series, 40 % of tumors were categorised as HG and 60 % as low grade [190].

Immunohistochemically, there may be considerable variability in staining within the same tumor and between tumors [77]. Reactivity for broad-spectrum cytokeratins (AE1/AE3, CAM 5.2 or MNF116) and at least some of myoepithelial markers, including αSMA, GFAP, CD10, p63 protein, calponin and SMMHC, is required for diagnosis [64]. All myoepithelial carcinomas display diffuse or patchy staining for S-100 protein and vimentin; about half of cases are positive for cytokeratins CK14 and CK5/6 [67]. In contrast, CK7 is often absent. The mean MIB1 (Ki-67) index in one series was 35 % (range 15–65), with any count above 10 % said to be diagnostic of malignancy in a myoepithelial neoplasm [191]. Recent molecular genetic data suggest that *EWSR1* gene rearrangement is a common event in myoepithelial tumors arising outside the salivary glands, irrespective of anatomical location [192]. In general, salivary myoepithelial carcinomas have been shown to have an intact *EWSR1* gene [193, 194], but recent data suggests that a subset of salivary myoepithelial carcinomas composed mainly of clear cells exhibit *EWSR1* gene rearrangements [195].

Differential diagnosis The variable appearance of myoepithelial carcinoma results in a wide differential diagnosis [64], including salivary duct carcinoma, a spectrum of clear cell tumors [80] and MEC. Nodular structures with central necrosis in myoepithelial carcinomas mimic salivary duct carcinoma, but there is more stromal myxoid and hyaline material in myoepithelial carcinomas; and also S-100 protein and other myoepithelial markers are absent in salivary duct carcinoma. There is no expression of androgen receptors and HER2/neu in myoepithelial carcinoma. The spindle cell variant of myoepithelial carcinoma can mimic various soft tissue sarcomas, and plasmacytoid cell variant should be differentiated from melanoma and plasmacytoma. The clear cell variant of myoepithelial carcinoma [196] resembles a variety of clear cell neoplasms primary and metastatic, including EMC, clear cell carcinoma NOS, HCCC and metastatic renal cell carcinoma (see Table 5.2). In almost every case, immunohistochemistry is helpful in excluding these neoplasms.

Treatment and prognosis Treatment consists of wide surgical excision combined with radiation, but any role of chemotherapy is not yet established. Whether low-grade and low-stage tumors can be treated only with wide excision needs to be further studied.

The prognosis of myoepithelial carcinoma is variable. Approximately one third of patients die of disease, another third have multiple recurrences, and the remaining third are disease-free [67]. Tumors arising in conventional PAs behave in the same way as those that arise de novo, but it has been suggested that neoplasms developing in recurrent PAs may pursue a prolonged clinical course [197]. Marked cellular pleomorphism, high mitotic rate and high proliferative activity (MIB1 index) correlate with poor prognosis [190].

5.9.11 Salivary Duct Carcinoma

Definition Salivary duct carcinoma (SDC) is an adenocarcinoma which histologically resembles ductal carcinoma of the breast [67].

Epidemiology It accounts for about 4–6 % of all salivary carcinomas [198]. Most patients are over 50 years old with an at least 4:1 male-to-female ratio. It arises mainly in the parotid gland, less often in the submandibular and only occasionally in the minor glands. SDC can arise de novo, but recent evidence suggests that approximately half develop as the malignant component of carcinoma ex pleomorphic adenoma [199]. A few others represent HG transformation of low-grade cribriform cystadenocarcinoma SDC, and a single case has been reported arising in (or in association with) a polymorphous low-grade adenocarcinoma of the palate [155].

Etiology and pathogenesis There are no known aetiological factors, although one case was reported in a patient with long-standing chronic obstructive sialadenitis [200] and another in a patient with IgG4-related sclerosing disease of the parotid [201].

Clinical aspects Patients complain of a rapidly growing often painful tumor mass sometimes with facial nerve palsy. A long history of a mass with rapid growth is described in SDCs arising from PAs.

Macroscopy Poorly circumscribed tumor infiltrating the salivary gland and surrounding soft tissues with scar-like cut surface.

Microscopy All histological studies on SDC have confirmed the strong morphological resemblance to in situ and invasive ductal carcinoma of the breast (Fig. 5.69). The former component comprises expanded salivary ducts with solid, papillary, 'Roman bridge', cribriform and comedo patterns, and the infiltrating element can include small ducts, cribriform structures, small nests of cells and trabeculae, all accompanied by stromal desmoplasia; perineural and lymphovascular invasion are frequent. SDC is composed mainly of cells with eosinophilic cytoplasm and often vesicular nuclei containing prominent central nucleoli. Frequently, there is marked nuclear pleomorphism, also apparent on FNA cytology. In better differentiated areas, cells may show definite apocrine features, such as luminal snouts [202]. Other than in the genuine mucin-rich variant [203], mucus is scanty at most, and goblet cells are absent (Fig. 5.70). Usually, mitotic figures are numerous and the Ki-67 index over 25 %.

Relatively uncommon morphological variants of SDC include papillary [204], micropapillary [205], mucin-rich [203] and oncocytic [206] subtypes (Fig. 5.71), as well as sarcomatoid SDC, a composite of usual salivary duct and spindle cell carcinomas; this may account for some tumors previously classified as carcinosarcoma ('true malignant mixed tumor') [207].

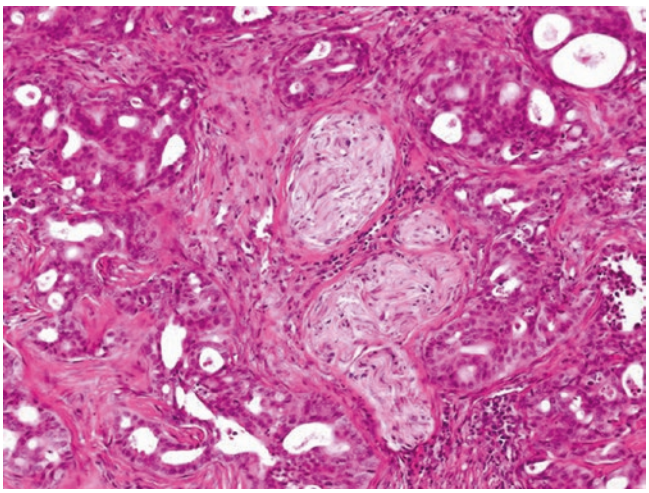

Fig. 5.69 Salivary duct carcinoma: invasive irregular ducts and cribriform structures strongly resemble ductal carcinoma of the breast

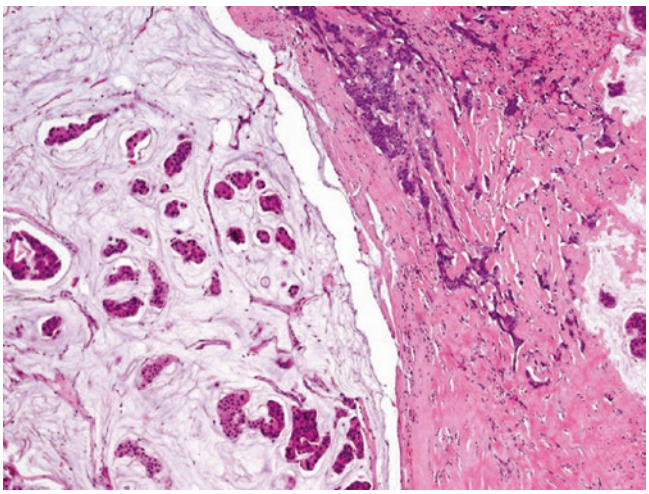

Fig. 5.70 Salivary duct carcinoma, mucin-rich variant. This is composed of a mixture of usual-type salivary duct carcinoma and lakes of mucinous adenocarcinoma

Pure salivary duct carcinoma in situ (SDCIS) is occasionally encountered in either major or minor glands, the diagnosis requiring strict criteria, particularly the absence of local invasion, determined by adequate sampling of the whole lesion and the presence of an intact myoepithelial layer around all tumor islands [208]. Immunohistochemically, SDC expresses broad-spectrum and low-molecular-weight cytokeratins and EMA (EMA). It is also strongly and diffusely positive with CK7 and occasionally and focally with CK20 [209]; GCDFP-15 is found in more than 80 % of SDCs

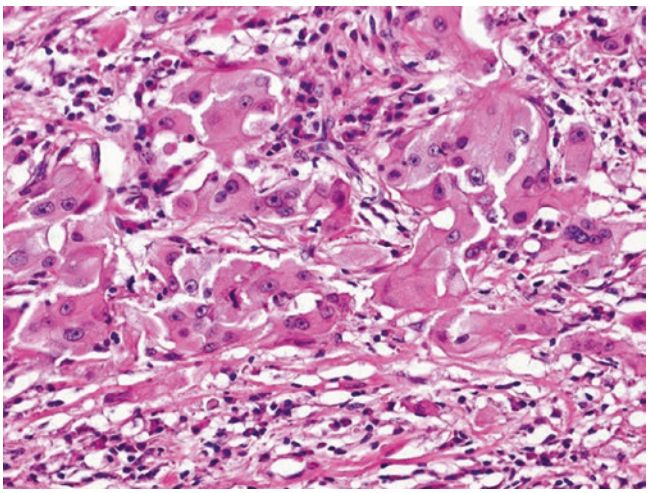

Fig. 5.71 Salivary duct carcinoma with oncocytic differentiation. The cells have ample granular cytoplasm with vesicular nuclei and prominent nucleoli. A clear distinction between oncocytic salivary duct carcinoma and true oncocytic carcinoma may not be possible, as they may not be separate entities

[210]. S-100 protein and myoepithelial markers (e.g. CK14, calponin and SMMHC) are negative, other than in the non-neoplastic cells of in situ lesions; CK5/6 and p63 patterns are similar, except in basal phenotype SDC [*see below*]. HER2 protein overexpression has been reported in up to 90 % of cases, due to considerable variation between different antibody clones and scoring systems [211]. However, when stricter criteria are used or when HER2 positivity is defined by fluorescence or chromogenic in situ hybridisation amplification, HER2 positivity is identified in only 15 % to a maximum of 40 % [211–213]. Oestrogen receptor α (ERα) and progesterone receptor expression is extremely rare [210, 214], although 73 % of SDCs react with the oestrogen receptor β isoform (ERβ) [213]. More importantly, most SDCs express androgen receptor (AR) – 67–83 % [210, 212, 213] – this is often used as a diagnostic marker due to its near absence in other tumor types [215]. Prostatic marker staining has been described [216], but other studies and personal experience suggest this is exceptional [212, 217, 218].

It has recently been proposed that, similar to schemes in the breast, SDC could be divided into three molecular subtypes: luminal-AR+, HER2 positive and basal phenotype. AR expression in SDC is analogous to ERα reactivity in breast carcinoma and can be used as a marker of the luminal phenotype. HER2 positivity is established by a combination of immunohistochemistry and FISH/SISH for the HER2/neu protein or amplified gene. The basal type is identified by markers such as high-molecular-weight cytokeratins [212]. In that study the relative percentages for each subtype were 69 % luminal-AR+, 17 % HER2, 5 % basal and 10 % indeterminate. There was no correlation between nuclear grade and subtype, except that both basal subtype SDCs were HG [212].

Differential diagnosis The range of differential diagnosis for both in situ (SDCIS) and invasive SDC includes low-grade cribriform cystadenocarcinoma, myoepithelial carcinoma and metastatic adenocarcinomas to the salivary gland. The relationship of SDCIS to low-grade cribriform cystadenocarcinoma remains unclear. They could perhaps be separate entities with significant immunohistochemical differences, but more probably, low-grade cribriform cystadenocarcinoma represents the extreme low-grade end of the spectrum of salivary DCIS. In favour of the latter is the overlap of architectural patterns with SDCIS, together with cases showing apparent progression to HG invasive SDC [199]. Thus, the entity of low-grade cribriform cystadenocarcinoma may better be termed low-grade intraductal carcinoma [219]. Myoepithelial carcinoma with prominent comedo-like necrosis may simulate SDC. Immunohistochemistry is required to distinguish between primary and metastatic carcinoma with ductal architecture (see Sect. 5.9.20).

Treatment and prognosis The standard treatment at present is complete surgical excision with radical neck dissection followed by radiotherapy to the tumor bed and possibly chemotherapy [198]. In the future, the subdivision of SDCs into molecular subtypes could lead to new approaches for patients with these cancers. As luminal-AR+ SDCs by definition consistently express AR, anti-androgen therapy may be of value, and preliminary studies on limited numbers of patients have shown a positive result in some patients [220]. Rare examples of patients with HER2-subtype SDCs benefitting from targeted therapies with anti-HER2 monoclonal antibodies (trastuzumab, pertuzumab) or HER2 tyrosine kinase inhibitors (lapatinib) have been reported [221, 222]. Further studies are warranted to determine whether basal-like SDCs, in a way akin to basal-like breast cancers, are sensitive to platinum salts and inhibitors of the poly (ADP) ribose polymerase (PARP). Finally, it has recently been found that some SDCs harbour *PIK3CA* mutations and/or *PTEN* loss; it is possible that this may lead to therapeutic targeting of the *PI3K* pathway [223].

Overall, SDC is one of the most aggressive salivary malignancies. At present, about a third develops local recurrence and 50 % distant metastases; death occurs in 60–80 % of patients, usually within 5 years. Amongst possible prognostic indicators, tumors smaller than 30 mm may have a better outlook [224], but nevertheless several fatal lesions of 20 mm have been reported [204, 218, 225, 226]. More recently, it was found that SDCs negative for both AR and ERβ were more aggressive than tumors which expressed one or both markers, and in addition, carcinomas which were HER2 protein 3+ had a worse outcome than those which were HER2 protein 0–2+ [213]. The outcome for pure SDCIS should be good, provided it is completely excised.

5.9.11.1 Low-Grade Cribriform Cystadenocarcinoma

Definition Low-grade cribriform cystadenocarcinoma (LGCCC) [67] is a neoplasm of ductal origin composed of cytologically low-grade cells arranged in solid, cribriform or cystic structures surrounded by a rim of myoepithelial cells. It was previously named 'low-grade salivary duct carcinoma' [227, 228] but is perhaps better termed low-grade intraductal carcinoma [219].

Epidemiology The average age of patients is 64 years (range 32–93), with an equal sex incidence. Most cases arise in the parotid glands, rarely at other sites.

Etiology and pathogenesis The relation between LGCCC and SDC is discussed in Sect. 5.9.11.

Macroscopy It is unencapsulated and generally displaces rather than truly invades salivary tissue.

Microscopy Microscopically, it is formed of multiple cysts and solid structures of varying sizes, which are composed of regular small, bland ductal cells with uniform nuclei and clear to eosinophilic cytoplasm (Fig. 5.72). They proliferate to form papillae or cribriform structures, but no necrosis or comedo-like appearance are seen. Occasional cells contain lipofuscin pigment. Mitotic figures are sparse and the Ki-67 index usually <1 %. At the periphery of the tumor islands, there is usually a population of flattened myoepithelial cells.

The epithelial cells express low-molecular-weight cytokeratin and CK7 as well as S-100 protein, but not GCDFP-15 or HER2/neu; variable results have been found with ARs [228, 229].

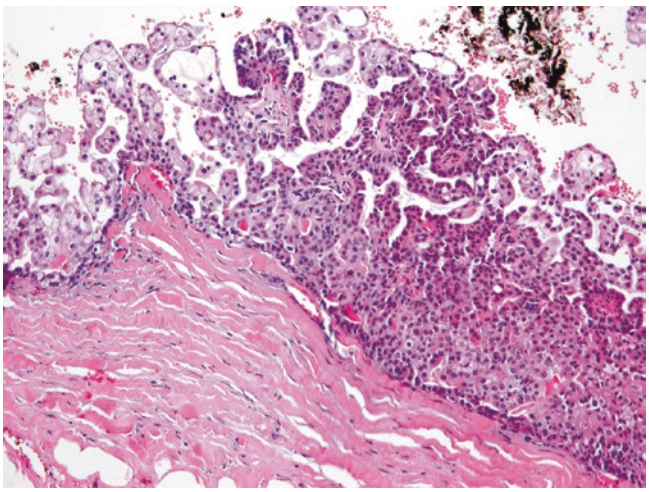

Fig. 5.72 Low-grade cribriform carcinoma is composed of regular small, bland ductal cells with uniform nuclei and clear to eosinophilic cytoplasm

Differential diagnosis The relationship of LGCCC to HG SDCIS [208] remains uncertain, as the immune-profile of both lesions often differs, particularly S-100 protein expression, suggesting two distinct entities. However, Weinreb et al. have described overlap cases [229], as well as transformation to high-grade salivary duct carcinoma [199]. This group proposes that the entity be named low-grade intraductal carcinoma [219].

Treatment and prognosis LGCCC is treated with surgical excision. If excision margins are clear, the prognosis is excellent.

5.9.12 Mucinous Adenocarcinoma

Definition An epithelial malignancy showing carcinoma cells lying in lakes of abundant extracellular mucus.

Epidemiology Mucinous (colloid) adenocarcinoma is a rare tumor arising most often in the major glands of adults [230].

Etiology and pathogenesis Unknown.

Macroscopy Macroscopically it is a poorly defined tumor with a gelatinous and cystic cut surface.

Microscopy Histologically it is composed of round and irregularly shaped clusters of epithelial cells floating in mucus-filled lakes, themselves separated by fibrous strands. The cells are cuboidal, columnar or irregular in shape, usually possessing clear cytoplasm and small dark nuclei; signet ring cells may be present. The mucus is PASD and mucicarmine positive. The carcinoma cells express epithelial markers, but not high molecular weight (MW) cytokeratins or actin [67].

Differential diagnosis Mucinous cystadenocarcinoma arises more often from the minor glands and is identified by mucin pools largely lined by carcinoma cells, which have larger nuclei than in colloid carcinoma [230]. The differential diagnosis of mucinous adenocarcinoma also includes other mucinous neoplasms such as MEC and mucin-rich SDC.

It is unclear whether low-grade signet ring cell (mucin-producing) adenocarcinomas of minor salivary gland are related, as they lack mucus pools [231]. Some may represent examples of the recently described mucinous myoepithelioma, in which many of the component cells contained abundant mucin in their cytoplasm, and all expressed some myoepithelial markers including S-100 protein [232, 233].

Treatment and prognosis Surgical resection with or without subsequent radiotherapy is the current treatment. Mucinous adenocarcinoma tends to recur and give metastases.

From the relatively few published cases, mucinous cystadenocarcinoma has a relatively favourable clinical behaviour [230].

5.9.13 Oncocytic Carcinoma

Definition A tumor composed of malignant oncocytic cells. However, some investigators have suggested that it is probably not a single entity but a mixture of several carcinomas showing oncocytic differentiation, most often SDC [206].

Epidemiology Several carcinoma types have variants composed of oncocytic cells, but fewer than 100 cases of pure oncocytic carcinoma have been reported. The average age is 63 years (range 29–91), and most have occurred in the parotid [39, 234].

Etiology and pathogenesis Oncocytic carcinoma can arise de novo or in association with a benign oncocytoma (up to 50 %) [235] and some have arisen in Warthin's tumors [236].

Clinical aspects Most cases present with a slow-growing swelling. The history is longer if the carcinoma has arisen from an underlying oncocytoma. Pain and facial paralysis are reported in some patients.

Macroscopy Nodular/multinodular mass with brown and soft cut surface.

Microscopy The diagnosis of a pure oncocytic carcinoma requires the identification of malignancy, oncocytic differentiation and lack of features of any other tumor type. Dark and light oncocytic cells are arranged in solid-trabecular and nested patterns. Necrosis, infiltration of periglandular and salivary tissue with infiltration of nerves and vessels is seen in aggressive cases. The tumor cells show positive staining for antimitochondrial antibodies [35].

Differential diagnosis Benign oncocytoma is a well-circumscribed tumor without signs of invasion. MNOH can simulate invasion, but the characteristic lobular pattern leads to the correct diagnosis. MEC contains other cells types such as mucous/intermediate and clear cells. The separation of oncocytic carcinoma from SDC may be difficult in absence of ductal differentiation. SDC shows characteristic DCIS-like areas.

Treatment and prognosis The tumor is treated surgically with or without neck dissection. It is likely that a pure oncocytic carcinoma is an aggressive tumor, as over half of the patients reported either died of disease or suffered recurrences [234].

5.9.14 Carcinoma Ex Pleomorphic Adenoma

Definition Carcinoma ex pleomorphic adenoma (CXPA) is an epithelial malignancy arising from a PA [67]. There is still confusion about terminology of these tumors, but based on the degree of invasion of carcinoma beyond the capsule of *maternal* PA, three main categories are recognised – widely invasive, minimally invasive and non-invasive CXPA. A proposal for two prognostically relevant main categories (widely invasive and early CXPA) is used in this chapter [237, 238]. The clinicopathological features of widely invasive CXPA and early CXPA are discussed separately.

5.9.14.1 Widely Invasive CXPA

Epidemiology This is the most common form of CXPA; it represents 3.6 % of all salivary gland tumors. 82 % of cases arise in the parotid gland and most of the rest in the submandibular gland; the minor salivary glands, particularly in the palate, can also be affected [239]. In exceptional cases the seromucinous glands of the sinonasal tract are involved [240]. In several large series [241–244] CXPA represents 3.6 % of all salivary gland tumors, 11.7 % of all salivary gland malignancies and 6.2 % of all PAs (range in different series from 1.9 % to 23.3 %).

Etiology and pathogenesis The incidence of malignant transformation increases with the length of history of the PA, from 1.5 % at 5 years to 10 % after 15 years [242]. Older patient age, larger size (and origin from submandibular site) and the presence of marked hyalinisation tend to be associated with more frequent malignant transformation [244, 245].

Clinical aspects Most patients are men over 60 years old with a typical presentation of a long history of a salivary gland nodule that suddenly increases in size. Because by definition CXPA must arise in association with PA with histological evidence of co-existent or pre-existing PA, a clinical history of long-standing parotid tumor is not sufficient evidence for a pre-existing PA, whilst a previously excised PA at the site of a carcinoma is acceptable [237, 238, 246].

Macroscopy Widely invasive CXPAs are often larger than benign PAs [242–244]. They are firm and ill-defined tumors with infiltration of adjacent tissue. A well-circumscribed

nodule may be seen representing pre-existent PA, but it may be seen only after extensive sectioning because the proportion between carcinoma and PA favours carcinoma [244].

Microscopy Histologically the dominant picture is the carcinoma. The pre-existing PA may be obscured by the carcinoma or may only show degenerate changes such as scarring, dystrophic calcification, necrosis and haemorrhage [238, 243, 244, 247]. A relevant proportion of tumors (probably a quarter of all cases) is not correctly diagnosed as CXPA, because the pre-existent PA is not identified for insufficient sampling.

When present, the contrast between maternal PA and carcinoma is usually obvious, and the recognition of widely invasive carcinoma ex PA is relatively simple. The malignant cells tend to have pronounced nuclear pleomorphism and increased number of mitoses (Fig. 5.73). Capsular, perineural and vascular invasion are easily identified as well as extension into neighbouring tissues. However, a notorious problem is the diagnosis of well-differentiated myoepithelial type of CXPA due to a lack of relevant cellular atypia, mitoses and blunt infiltration.

Several studies show SDC, adenocarcinoma NOS and frequently misdiagnosed myoepithelial carcinoma to be the most frequent histological types [238, 247], but it is not uncommon to find other differentiation, e.g. squamous, mucoepidermoid, polymorphous low-grade adenocarcinoma or AdCC (Fig. 5.74) [238, 240–244]. The immunohistochemical profile of widely invasive CXPA (excluding myoepithelial type) shows strong expression of pan-cytokeratin (AE1/AE3 and CAM5.2), CK7, CK8, CK18, CK19 and EMA. Staining for p63, αSMA, CK5/6 and CK14 may be focally present in myoepithelial cells of residual non-invasive/intraductal CXPA. MIB1 proliferative activity shows a higher (35 %) index in the malignant component in contrast to the low proliferation index in the *maternal* PA.

In addition, nuclear staining for AR and p53 can be detected in the malignant component. Similarly immunohistochemistry for HER2 protein is seen in the malignant cells of CXPA as a distinct membrane staining (3+) in cases of HG CXPA, whilst lower grades show moderate (2+), weak or absent (1+/0) membrane staining [211].

Gene amplifications of the *HER2* gene as detected by in situ hybridisation (ISH) techniques are seen mostly in cases of CXPA with HG morphology. Mutation of *TP53* gene [246] as detected by gene sequencing is often noted in the malignant cells of CXPA.

Other molecular studies have confirmed that the development of CXPA follows a multistep model of carcinogenesis with loss of heterozygosity at chromosomal arms 8q, 12q and 17p and inactivation of tumor suppressor genes [248–250]. A recent study has demonstrated that *PLAG1* and *HMGA2* are rearranged in about 50 % of SDC ex PA [199].

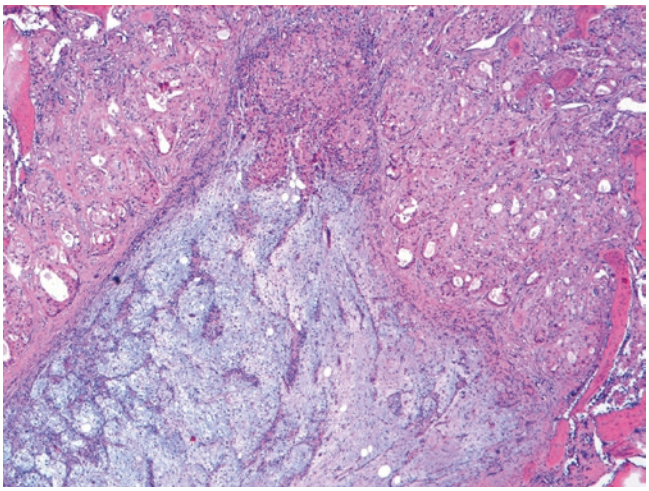

Fig. 5.73 Carcinoma ex pleomorphic adenoma. Salivary duct carcinoma with high-grade features arising in pleomorphic adenoma that is apparent at the lower part of the image

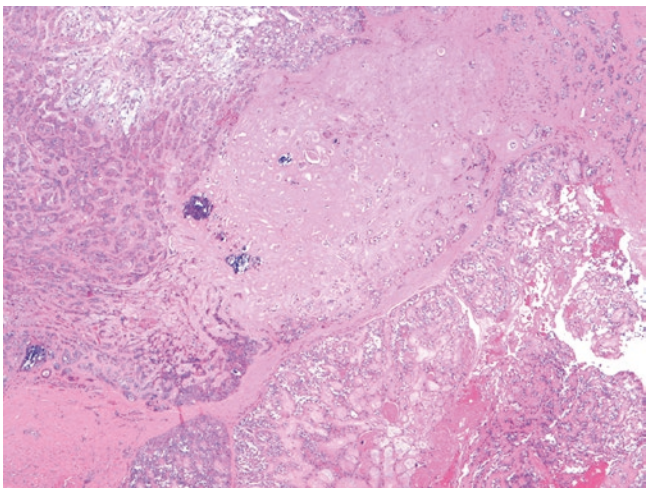

Fig. 5.74 Carcinoma ex pleomorphic adenoma. In the centre of the image, the residual pleomorphic adenoma is apparent. The malignant component has the morphology of adenoid cystic carcinoma and basal cell adenocarcinoma

Differential diagnosis Widely invasive CXPA can be confused with other primary and metastatic carcinomas of salivary glands when the maternal PA cannot be identified, but the most important differential diagnosis is the separation of widely invasive carcinoma from early CXPA.

Treatment and prognosis Widely invasive CXPA is treated with surgical resection usually associated with neck dissection. Surgery is followed by radiotherapy and in some cases chemotherapy. The prognosis depends on the degree of inva-

sion beyond the capsule of PA [237, 238, 251–253]. Histological grade, lymph node metastases and perineural invasion and extent of invasion are important factors of CXPA. Widely invasive CXPA is an aggressive tumor with local recurrences, lymph node and distant metastases. In a series of 51 patients, Zhao et al. [254] found 39.2 % developing loco-regional recurrence and 27.5 % distant metastases with an overall survival of 62.7 % at 3 years and 50.3 % at 5 years up to 70 %. Prognosis may have improved with the more recent use of targeted therapy for epidermal growth factor receptor 2 (HER2).

The goal of HER2 testing in CXPA is on one hand to assist the histological diagnosis and on the other hand to identify patients eligible for trastuzumab therapy in case of metastatic disease [221, 255]. The possibility of assessing *HER2* gene amplification by newly developed in situ hybridisation techniques may encourage the assessment of HER2 status of salivary gland carcinomas in routine diagnostic reporting [212, 221, 256].

5.9.14.2 Early Carcinoma Ex Pleomorphic Adenoma

Definition There is no agreement in the literature. In the authors' opinion, early carcinoma ex pleomorphic adenoma (ECXPA) includes both DCIS-like and micro-invasive-like carcinoma still confined in the capsule of PA and carcinomas with extension beyond the capsule of PA of no more than 6 mm (see Table 5.3).

Epidemiology, etiology and pathogenesis Are similar to that of widely invasive CXPA.

Macroscopy ECXPA are well-circumscribed and small-sized (stage T1-2) tumors without or with minor invasive growth outside the capsule of PA (Figs. 5.75, 5.76 and 5.77). Those arising in the deep parotid lobe may be larger in size.

Microscopy The *maternal* PA is usually identified in all cases. Within pre-existing ducts of PA are cytologically malignant cells, surrounded by small bland-looking actin/CK14/p63-positive myoepithelial cells. These ducts frequently show central necrosis giving a comedo-like appearance and papillary and cribriform architecture resembling intraductal carcinoma of the breast [253]. With progression of disease, intraductal carcinoma can be associated with extraductal (invasive but still intracapsular) and extracapsular invasion with invasive component of no more than 6 mm [237, 238, 246]. Immunohistochemistry for MIB1 [238, 253] and AR is suggested as useful tools to recognise microscopic foci of CXPA.

However, AR should not be used in isolation as up to 10 % of PAs express nuclear staining for AR which may lead to an overdiagnosis of CXPA [257]. The immunoprofile of ECXPA [258] is similar to that of widely invasive CXPA described in the corresponding paragraph.

Table 5.3 Proposed classification of early carcinoma ex pleomorphic adenoma (ECXPA)

Non-invasive in situ, intratubular/intraductal (DCIS-like pattern)
Invasive but still intracapsular (DCIS with microinvasion-like pattern)
Early invasive with extracapsular extension up to 6 mm

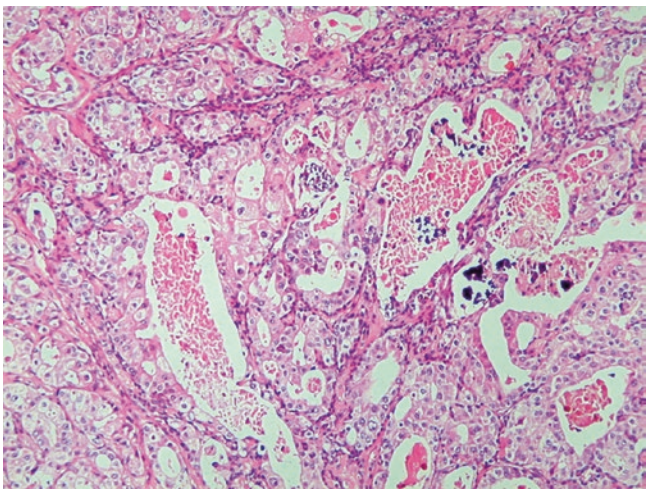

Fig. 5.75 Non-invasive carcinoma in a pleomorphic adenoma. Ducts contain cells with atypical nuclei. Focal necrosis and calcification are also present

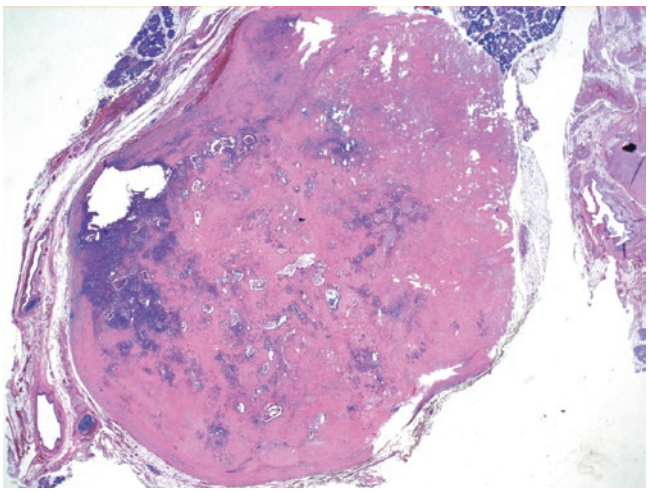

Fig. 5.76 Non-invasive carcinoma ex pleomorphic adenoma. On low-power view, hyalinisation is apparent as well as highly cellular areas

Differential diagnosis The most important differential diagnosis is between ECXPA and widely invasive CXPA, and it is based essentially on the degree of invasion of <6mm.

Treatment and prognosis Surgery with clear margin is the preferred treatment, preoperative diagnosis is rare, and the

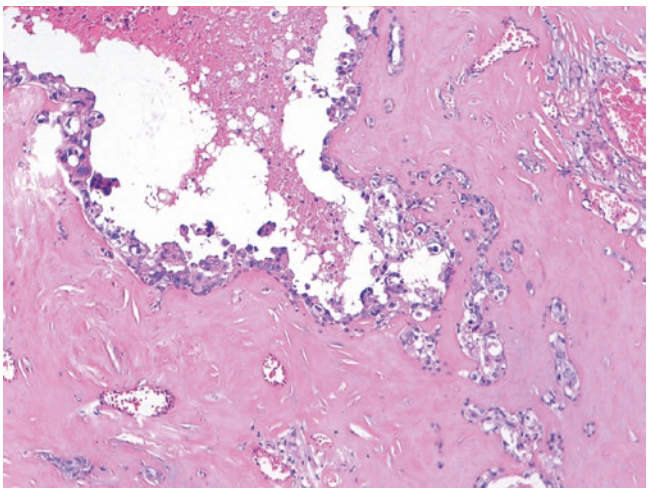

Fig. 5.77 Non-invasive carcinoma ex pleomorphic adenoma. On high-power view of the previous image, the classical appearance of salivary duct carcinoma is apparent

Table 5.4 Early carcinoma ex pleomorphic adenoma (ECXPA)

Authors	Year	Number of cases	Development of recurrence/metastases
Livosi and Perzin [241]	1977	6/47	None
Brandwein et al. [252]	1996	12	None
Olsen et al. [251]	2001	2/73	None
Felix et al. [260]	2002	1	Lymph node metastasis
Di Palma et al. [253]	2005	11	None
Altemani et al. [258]	2005	10	None
Ihrler et al. [246]	2007	8/19	None
Katabi et al. [259]	2010	13	3 developed metastases[a]
Weiler at al. [237]	2011	8/19	None
Hashimoto [256]	2012	13/31	None

[a]Not indicated if intracapsular/minimally invasive and degree of invasion

tumor is clinically diagnosed as PA. ECXPA behaves in a benign fashion after excision providing sampling is complete and diagnosis is accurate. This favourable prognosis has been proved by several studies (see Table 5.4).

As shown in Table 5.4, the concept of ECXPA – i.e. early malignant changes but still of carcinoma – was first noted in 1977 by *LiVolsi* and *Perzin* who raised the issue of non-invasive CXPA [241]. In 6/47 cases of CXPA, there was no evidence of invasion. The clinical behaviour in these patients was identical to PA with no local recurrence or distant metastases. In 1984 Tortoledo et al. reported 16 patients with extracapsular invasion ranging from 9 to 20 mm who died of their disease, whilst none of 16 patients with extracapsular invasion less than 8 mm died [147].

Brandwein et al. [252] reported 12 patients with capsular invasion of <1.5 mm. The study did not include patients with extracapsular invasion of more than 1.5 mm. No patient developed local recurrences or metastases, and this

Table 5.5 The concept of early carcinoma ex pleomorphic adenoma

Authors	Year	Extracapsular extension	Development of recurrence/metastases
Tortoledo et al. [147]	1984	<8 mm	None
Brandwein et al. [252]	1996	≤1.5 mm	None
Olsen et al. [251]	2001	2 and 3 mm	None
Felix et al. [260]	2002	0 mm	Lymph node metastasis
Di Palma et al. [253]	2005	0 mm	None
Katabi et al. [259]	2010	Not given	1/11[a] relapsed
Weiler et al. [237]	2011	<5 mm	None

[a]Degree of invasion

figure of 1.5 mm has been adopted as a threshold by the WHO classification of Salivary Gland Tumors 2005 [67]. More recently (see Table 5.5) at least five studies have appeared in literature suggesting a wider pattern of histological appearances and proposing a higher prognostic threshold of up to 5 mm of extracapsular invasion [238, 246, 253, 256, 259] to be used to separate clinically favourable from unfavourable cases of CXPA. There has been only one study [260] where lymph node metastasis developed in a properly sampled non-invasive epithelial CXPA. The explanation for these cases is uncertain but may include microinvasion not identified even with thorough sampling of the tumor edge.

5.9.14.3 Carcinosarcoma Ex Pleomorphic Adenoma

Definition A malignant tumor showing a mixture of carcinomatous and sarcomatous elements. True malignant mixed tumor (TMMT) is a common synonism for carcinosarcoma (CS).

Epidemiology CS is rare. There are fewer than 100 cases reported in literature [242, 261–263].

Etiology and pathogenesis CS can arise de novo in a healthy salivary gland or as a complication of PA. In a subset with osteoclast-type giant cells, the same mutation was found of the same allele on chromosome 17p13, a known mutation of SDC. This suggests that CSs are in reality metaplastic carcinomas, possibly sarcomatoid SDCs.

Clinical aspect The mean age at presentation is 58 years (range 14–87), and most cases are found in the parotid gland. In CS arising in a pre-existing PA, the history will usually be that of rapid growth in a long-standing salivary nodule [262].

Macroscopy Usually it is a poorly circumscribed tumor. A separate nodule can be seen in those cases where CS arises in pre-existing PA [263].

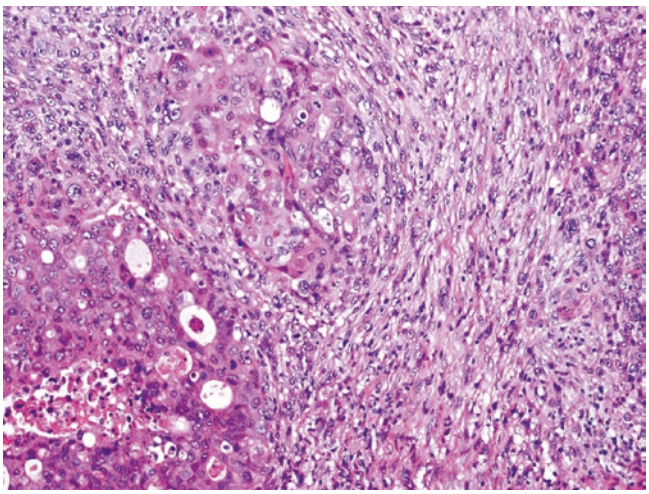

Fig. 5.78 True malignant mixed tumor/carcinosarcoma. The epithelial component is a poorly differentiated carcinoma with some features suggesting salivary duct carcinoma. The sarcomatous component is a high-grade spindle cell sarcoma, in this case, without specific differentiation

Microscopy CS is a biphasic tumor in which the mesenchymal element is usually chondrosarcoma, but osteosarcoma, fibrosarcoma, malignant fibrous histiocytoma, pleomorphic rhabdomyosarcoma and osteoclast-type giant cell neoplasms have also been described [261]. The epithelial component is generally a poorly differentiated (adeno)carcinoma with features of SDC increasingly reported (Fig. 5.78) [247]. CS can also differentiate into TTF1-positive large cell neuroendocrine carcinoma [263]. On immunohistochemistry epithelial markers are usually detected in the epithelial component and may or may not also be expressed in the sarcomatous component. Positive staining for epithelial markers has been used as proof of the fact that CSs are *carcinomas* showing divergent differentiation (possibly sarcomatoid variants of SDC) and as an indication of their monoclonal origin. However, keratin staining can be negative casting doubt onto the monoclonal-carcinomatous nature of the whole tumor. Molecular studies using comparative genomic hybridization (CGH) have revealed similar genomic profiles in epithelial and mesenchymal component, confirming their monoclonal histogenesis [264].

Differential diagnosis Includes spindle cell squamous carcinoma, primary salivary sarcomas and CXPA. The first arises from the mucosal surface and may simulate CS of minor salivary glands. Its epithelial component is epidermoid, not glandular; dysplasia of the surface squamous epithelium is diagnostic. Primary salivary sarcomas are exceptionally rare and must be well sampled to identify any minor carcinomatous component. The possibility of metastatic melanoma with spindle cell morphology should not be forgotten; immunohistochemistry shows positivity for appropriate markers.

It has not yet been established whether all or some CS are examples of the sarcomatoid variant of SDC, but any positivity for ARs would suggest that diagnosis.

The main diagnostic problem is the rarity of CS. Even specialist pathologists generally have little familiarity in reporting this tumor type.

Treatment and prognosis The treatment of CS is surgical, and the outcome is usually poor with 60 % of patients dying of disease.

5.9.14.4 Metastasising Pleomorphic Adenoma

Definition Metastasising pleomorphic adenoma (MPA) is a histologically benign PA, which recurs locally and gives distant metastases.

Epidemiology MPA is rare, and the largest series [265] published in the last decade where the literature was reviewed between 1953 and 2005 revealed 42 patients with an average age of 33 years. Bone was the most common site for metastases (45 %), followed by the head and neck (43 %) and lung (36 %). Other cases published more recently are still regarded as *fascinating enigma* or *dramatic case report* [266–268].

Etiology and pathogenesis The development of metastasis seems to require a background of multiple loco-regional recurrences following incomplete resection of the primary tumor. Therefore, the postulated pathogenesis is vascular implantation or invasion eventually leading to metastases, but in many cases of MPA, it was not possible histologically to demonstrate actual vascular permeation [267, 269]. However, some authors [270, 271] have speculated that MPA are unrecognised (low-grade) carcinomas with a potential to kill the patient.

Clinical aspects There is usually a clinical history of multiple recurrences of PA with additional development of a tumor mass in the local lymph nodes widely or distant sites such as the bone, lung and kidney. Unusual locations include the sphenoid bone [272].

Macroscopy Well-circumscribed nodule/s similar to primary PA.

Microscopy The typical features (epithelial ducts mixed with chondromyxoid tissue) of PA are present. The tumor remains histologically ‘benign’ in the primary site, local recurrences and metastatic deposits [269, 273].

Treatment and prognosis Local recurrences and metastatic deposits are treated surgically. The prognosis depends on the sites and number of metastases and their development within the first 10 years of primary surgery [265].

5.9.15 Sebaceous Carcinoma

Definition Sebaceous carcinoma is a malignant tumor composed predominantly of sebaceous cells with varying degrees of pleomorphism and invasiveness.

Epidemiology It is rare with <50 cases reported so far [103, 274–277]. The majority occur in the parotid gland, followed by oral cavity, submandibular and sublingual gland. There is no sex predilection, and age distribution reported in literature is after the third decade [274, 275], but one group reported two cases in children [278].

Etiology and pathogenesis Sebaceous carcinoma can arise from PA [277] and sebaceous lymphadenoma [104].

Clinical aspects Patients can either present with a painful mass and facial nerve paralysis or with a painless parotid swelling.

Macroscopy Sebaceous carcinomas are frequently well or partially circumscribed tumors ranging from 6 to 95 mm in greatest dimension.

Microscopy The tumor cells are arranged in nests or sheets and show characteristic abundant clear and vacuolated cytoplasm. Nuclei show varying degree of pleomorphism that is more pronounced than the nuclear features of sebaceous adenomas. Areas of cellular necrosis and fibrosis are commonly found. In some cases, perineural invasion has been noted, but vascular invasion seems particularly uncommon. There is a background of lymphoid tissue with follicles mixed with foreign body giant cells with histiocytes [103].

Differential diagnosis Mostly with sebaceous adenoma and clear cells tumors.

Treatment and prognosis Surgery usually followed by radiotherapy in tumors of advanced stage. Metastasis is rare, but local recurrences are documented. The survival rate is approximately 60 %, but prognosis is better for oral sebaceous carcinomas [276].

5.9.15.1 Sebaceous Lymphadenocarcinoma

Definition Sebaceous lymphadenocarcinoma is the malignant counterpart of sebaceous lymphadenoma.

Epidemiology An extremely rare tumor of the salivary glands with <10 cases reported in literature [274, 275, 279–282]. It occurs in adult patients and parotid is the preferred site.

Etiology and pathogenesis The rare case reported indicates an origin from sebaceous lymphadenoma.

Clinical aspects Patients had histories of a tumor mass of varying duration up to 20 years. In one patient there was a history of cervical lymph node metastasis and involvement of the skin left cheek and neck [279].

Macroscopy Partly encapsulated, cystic and solid tumor up to 60 mm in maximum diameter.

Microscopy Malignant sebaceous cells are organised in sheets showing evidence of squamous and ductal differentiation. Identification of the underlying sebaceous lymphadenoma or pleomorphic carcinoma requires extensive sampling. Some cases show a background lymphoid infiltrate with collections of histiocytes and a foreign body giant cells.

Differential diagnosis Clear cell tumors and tumors showing sebaceous differentiation.

Treatment and prognosis Surgical excision often followed by radiotherapy. Given the limited number of cases reported in literature, the long-term prognosis is not well established yet.

5.9.16 Primary Squamous Cell Carcinoma

Definition Squamous cell carcinoma (SCC) arising in the salivary glands.

Epidemiology An extremely rare tumor of the salivary glands. It occurs in adults and the parotid gland, and its main excretory duct is the preferred site.

Etiology and pathogenesis Not known.

Clinical aspects A mass lesion of variable duration.

Macroscopy Usually invasive mass.

Microscopy As elsewhere in the head and neck region, the appearance is that of an invasive carcinoma of variable differentiation and keratinization.

Differential diagnosis The vast majority of squamous carcinomas in the salivary glands represent metastases from the skin or upper aerodigestive tract.

Treatment and prognosis Surgical excision often followed by radiotherapy.

5.9.17 Lymphoepithelial Carcinoma

Definition A malignant tumor composed of undifferentiated epithelial cells mixed with lymphocytes. It is histologically similar to undifferentiated nasopharyngeal carcinoma.

Epidemiology In the Western countries, it is an extremely rare tumor accounting for <0.5 % of all salivary gland malignancies. In contrast in southern China and in Eskimos (Inuit), the reported incidence is exceptionally high representing 80 % of the cases reported in literature. The median age is 40 years (range 10–86), and it is slightly commoner in females [48]. Familial clusters have been identified amongst patients from Greenland [283]. The parotid is involved in 80 % of cases where it can arise in an intra-glandular lymph node [284], with the rest occurring in the submandibular glands [285].

Etiology and pathogenesis Infection with Epstein-Barr virus (EBV) is a major factor. Other contributing associations are with certain human leucocyte antigen (HLA) types [286].

Clinical aspects Patients complain of a parotid mass, but in 40 % of the cases, the clinical first presentation is a metastasis in a cervical lymph node [287].

Macroscopy An invasive mass or a lymph node metastasis from the neck.

Microscopy There is a marked histological similarity to undifferentiated nasopharyngeal carcinoma, which has also been linked to EBV. Microscopic examination shows syncytial groups of large epithelial cells with vesicular nuclei and prominent nucleoli, intimately mixed with lymphocytes and plasma cells, sometimes with germinal centre formation. Mitotic figures are often numerous. At times, the epithelium is difficult to identify, but it can be highlighted by cytokeratins. Epstein-Barr virus encoded small RNA (EBER) staining of nuclei is diagnostic in this situation.

Differential diagnosis The most important differential diagnosis is a metastasis from a nasopharyngeal primary, which can present as a parotid mass [288] or possibly very poorly differentiated squamous carcinoma of usual type originating in the skin or upper aerodigestive tract. Fluorodeoxyglucose positron emission tomography-computed tomography (FDG PET-CT) has been widely recommended as sensitive at detecting both primary malignancy and metastatic spread [289].

For differential diagnosis with lymphoepithelial sialadenitis, lymphadenoma and lymphoma (see Sects. 5.10.1, 5.8.8 and 5.10.3).

Treatment and prognosis Radiotherapy has been the mainstay of treatment of nasopharyngeal carcinoma due to its radiosensitivity. Other therapy modalities include superficial parotidectomy with neck dissection. The outcome is surprisingly good for such an aggressive-looking carcinoma, and the 5-year survival rate is 60 % [287]. One report estimated 2-, 5- and 10-year survival at 91 %, 66 % and 29 %, respectively, for lymphoepithelial carcinoma of the salivary glands [290]. Further research is needed to determine the effect of different treatment modalities on survival.

5.9.18 Small Cell Carcinoma

Definition Small cell carcinoma (SmCC) is a malignant epithelial tumor composed of cells with scanty cytoplasm, round nuclei with fine chromatin and inconspicuous nucleoli similar to pulmonary SCC.

Epidemiology Primary salivary gland SmCC is very rare, accounting for <1 % of all salivary gland tumors [291]. They are seen more often in men, with a mean age of 56 years (range 5–86). Parotid gland is the most involved site in the <100 cases reported in literature [291–293].

Etiology and pathogenesis There is a known association with smoking. The pathogenesis is unknown, but SmCC is unlikely to be a single entity, as electron microscopy reveals that some neoplasms show neuroendocrine differentiation, whilst others have squamous and ductal features not apparent histologically [294, 295], and occasionally both patterns are evident in the same tumors. Unlike Merkel cell carcinomas of the skin, CK20+ SmCCs show no association with virus [295a]. Some neoplasms called SmCC may in fact be primary primitive neuroectodermal tumors and *NUT* gene rearrangement carcinomas.

Clinical aspects Patients note a rapidly growing salivary gland swelling often accompanied with lymph node enlargement.

Macroscopy The surgical specimen shows a poorly circumscribed mass often with areas of necrosis.

Microscopy The microscopic appearance may be similar to SmCC of the lung or Merkel cell carcinoma of the skin. Both comprise solid sheets, nests and cords of closely packed cells; the difference is in the cell size, small and dark cells in the former and slightly larger and with pale chromatin in the latter. Immunohistochemistry shows positive staining for chromogranin, synaptophysin, neuron-specific enolase and

CAM5.2, often with paranuclear dots in both types. However, immunohistochemistry for CK 20 seems to identify two subtypes of SmCC: CK20– lung cell type and CK20+ Merkel cell-type carcinoma [292, 293, 296].

Differential diagnosis The most important differential diagnosis is with metastases from lung SmCC and Merkel cell carcinoma of the skin, and these must be excluded before a primary SmCC can be said to be of salivary origin. Clinicopathological information is usually helpful. The solid variant of AdCC may be confused with SmCC (see Sect. 5.9.4). Lymphomas and primary primitive neuroectodermal tumors of the salivary glands may be somewhat similar morphologically and can be excluded immunohistochemically.

Treatment and prognosis Surgery with adjuvant chemotherapy is the treatment of choice. The prognosis is poor, but a study by Nagao et al. [291, 292] showed that CK20+ SmCC of the salivary glands have a better prognosis than CK20– cases [292].

5.9.19 Well and Moderately Differentiated Neuroendocrine Carcinoma ('Typical' and 'Atypical' Carcinoid)

Definition A malignant tumor showing neuroendocrine differentiation, corresponding to similar neoplasms at other sites, such as the lung. These carcinomas do not exhibit the cytomorphological features of SmCC (see Sect. 5.9.18).

Epidemiology Both well- and moderately differentiated neuroendocrine carcinomas (NEC) are very rare, with only occasional cases described. Patients reported have been adults of either sex. All reported cases have been in the major glands [296a, 296b].

Etiology and pathogenesis No known factors.

Clinical aspects Salivary gland swelling is the first clinical presentation; occasionally patients experienced pain.

Macroscopy Generally, a fairly well-circumscribed tumor but with an infiltrative pattern.

Microscopy The tumors are arranged in nests, cords and trabeculae infiltrating surrounding tissue; focal ductal differentiation was described in one case and occasional rosette-like structures in another [296a]. The cells are spindle to polygonal in shape, and the nuclei may be vesicular or display stippled chromatin; nucleoli can be prominent. In moderately differentiated NECs, mitotic figures are relatively easy to find with counts up to 8/10 HPFs. Moderately differentiated NECs may also display variable amounts of necrosis and show lymphovascular or perineural infiltration. NECs stain for neuroendocrine markers, such as synaptophysin and chromogranin, although the latter may be patchy, as well as cytokeratins; however, staining for cytokeratin 20 has not been demonstrated, unlike in SmCC (see Sect. 5.9.18). No figures are available for Ki-67 counts, but in personal experience of a single case of moderately differentiated NEC by one of the authors (RHWS), the index was 15 %.

Differential diagnosis The main differential diagnosis of a primary salivary NEC is from metastatic NEC, and this must be done by the clinical and imaging exclusion of a primary elsewhere. It can be distinguished from other primary salivary neoplasms by the almost unique immunohistochemical profile of NEC, which is shared only with SmCC, itself composed of small dark cells.

Treatment and prognosis Treatment is surgical resection. The few cases described have survived several years, in some cases even with distant metastases. However, it is reasonable to speculate that moderately differentiated NEC could cause death by extensive disseminated disease.

5.9.20 Desmoplastic Small Round Cell Tumor

Definition A malignant tumor composed of cells with polyphenotypic differentiation.

Epidemiology Exceptionally rare but possibly underdiagnosed. A recent literature review has shown six cases of extra-abdominal desmoplastic small round cell tumor reported so far [297]. Patients are young, but in one case the patient was 41 years old.

Etiology and pathogenesis Desmoplastic small round cell tumors harbour the *EWS* gene translocation that can be detected by fluorescence in situ hybridisation (FISH). The *EWS-WT1* gene fusion is also demonstrated by RT-PCR.

Clinical aspects Salivary gland rapid swelling is the first clinical presentation [297, 298].

Macroscopy Poorly circumscribed tumor with infiltrative pattern.

Microscopy The tumor is arranged in nests embedded in desmoplastic stroma. The cells have moderate amount of cytoplasm and hyperchomatic nuclei. They stain positively for cytokeratins, desmin, WT-1, EMA, NSE and CD56.

Differential diagnosis SmCC, solid variant of AdCC and other poorly differentiated carcinomas of salivary glands are the main differential diagnosis. The young age of the patient, the polyphenotypic immunoprofile and the characteristic *EWS* gene translocation are diagnostic of desmoplastic small round cell tumor.

Treatment and prognosis The affected salivary gland is surgically resected, but the prognosis is poor.

5.9.21 Nuclear Protein in Testis Rearrangement Carcinoma

Definition Nuclear protein in testis (NUT) rearrangement carcinoma is defined by abnormalities of the *NUT* gene on chromosome 15q14.

Epidemiology It has a wide age range (3–78 years), and although found throughout the body, it is well described in the head and neck region and occasionally in the salivary glands.

Microscopy The appearance is that of sheets and islands of undifferentiated malignant cells but with occasional well-differentiated squamous islands; p63 staining is seen beyond these islands, suggesting a squamous lineage. Areas of necrosis are frequent and the mitotic rate high.

Treatment and prognosis Too few cases have been reported to comment, but behaviour at other sites is generally aggressive and the development of metastases a particularly poor sign.

5.9.22 Higher-Grade Change in Carcinomas

Definition High-grade (HG) transformation (originally called 'dedifferentiation') is defined as the histologic progression of a low-grade malignant neoplasm to a high-grade one, within which the original line of differentiation is lost.

Epidemiology Rare in salivary glands. The phenomenon was first reported in mesenchymal tumors, such as dedifferentiated chondrosarcoma [299] and liposarcoma [300]. The phenomenon of HG transformation has subsequently been recognised in a variety of salivary gland carcinomas, including AciCC [114], AdCC [301], PLGA [155], EMC [163] and MASC [122].

Etiology and pathogenesis Although HG transformation of salivary gland carcinomas is always associated with tumor progression, characterised by development of a subpopulation of tumor cells within a low-grade malignancy that fails to develop along the expected lineage of differentiation, little is known about molecular genetic events that regulate this. Alteration of the p53 pathway has been recognised in AdCCs [302] and EMC [163], but no alterations of the *TP53* gene were detected so far in previous studies of HG-transformed AciCCs [114, 303] and MASC [122].

Clinical aspects Patients complain of tumor swelling with rapid increase in size.

Macroscopy Partly unencapsulated nodule with necrotic and haemorrhagic areas.

Microscopy Tumors with HG transformation are composed of conventional carcinomas juxtaposed with areas of HG morphology. The conventional/low-grade component has the features of AciCC [114], AdCC [301], PLGA [155], EMC [163] and MASC [122]. The HG component is either poorly differentiated adenocarcinoma or undifferentiated carcinoma, usually arranged in solid nests, sometimes in cribriform pattern of cells with large vesicular nuclei, prominent nucleoli and abundant cytoplasm. Frequent mitoses and extensive necrosis are evident. The Ki-67 labelling index is consistently higher in the HG component.

Treatment and prognosis Salivary gland carcinomas with HG transformation are aggressive tumors characterised by rapid progression accompanied by a higher local recurrence rate and propensity for cervical lymph node metastasis. Generally, the tumors have a very poor prognosis and therefore should be managed as high-grade carcinomas. Wide local excision, cervical lymph node dissection and aggressive radiotherapy are the treatments of choice.

5.9.23 Metastatic Malignancies

Definition Salivary gland localisation of a tumor originating from another site.

Epidemiology Metastases to the major glands and the intraparotid lymph nodes constitute approximately 10 % of all salivary carcinomas [304]; the exact figure varies from study to study depending on local factors such as different incidences of particular cancers. For example, Bergersen et al. [305] in Australia reported that metastases constituted 72 % of all malignancies, resulting from the high incidence of skin cancer. In an AFIP series and literature review in 1991 of 785 parotid metastases [304], 64 % were found to have originated from the head and neck region (including the skin), 11 % from distant sites and 25 % from an unknown primary. Of the

Table 5.6 Metastases to the parotid gland, adapted from Gnepp [304]

Location of primary	Number of tumors
Skin of head and neck	422 (53.8 %)
Upper aerodigestive tract (mouth, nose, sinuses, pharynx)	63
Eye (conjunctiva, lacrimal gland)	6
Thyroid	5
Head, not otherwise specified	4
Central nervous system	4
Submandibular salivary gland	1
Lung	28
Kidney	23
Breast	19
Colorectal	7
Prostate	4
Skin, distant	3
Stomach	2
Uterus	1
Pancreas	1
Total, distant sites	88 (11.2 %)
Skin, not otherwise specified	108
Unknown primary site	84

distant sites, lung, kidney and breast accounted for more than four-fifths (Table 5.6); only four cases were from the prostate, but it is perhaps underrecognised [306]. Metastases to the submandibular glands are less common than to the parotids but are more likely to be from distant sites [307].

Clinical aspects Patients are usually older with a clinical history of cutaneous resection of a tumor. Salivary gland (mostly parotid) swelling is the typical clinical presentation.

Macroscopy Poorly circumscribed solid and cystic tumor with areas of necrosis.

Microscopy The appearance is varied depending on the nature of the primary. Sometimes the primary site cannot be identified, and the tumor may have originated from the parotid [308, 309].

Differential diagnosis Metastases in the salivary glands can resemble almost any primary tumor, so that, for example, mammary duct carcinoma is morphologically identical (but immunohistochemically different) to SDC (see Sect. 5.9.11). Similarly, renal cell carcinoma is part of the differential diagnosis of any clear cell tumor of the salivary glands, and examples of prostate carcinoma have been mistaken for AciCC [306]. Immunohistochemistry is of some value and can identify prostate and thyroid primaries and melanoma with a reasonable degree of accuracy. Unlike most primary malignant salivary tumors, renal cell carcinomas are usually negative with cytokeratin 7; in contrast, CD10 stains most kidney carcinomas but is only positive in salivary tumors with myoepithelial differentiation. However, the possibility of metastasis is still best confirmed or excluded by imaging techniques of the kidneys.

Treatment and prognosis Surgery of salivary gland and neck dissection is the elective treatment. The long-term survival is usually poor, as the salivary glands are often only affected when metastatic dissemination is widespread.

5.9.24 Hybrid Carcinoma

Definition Hybrid tumors are defined as two separate types of tumor, each of which conforms to an exactly defined category, arising at a single site.

Epidemiology They are rare, comprising <0.1 % of neoplasms of salivary tumors [310].

Etiology and pathogenesis Some authors have speculated that hybrid tumors arise from intercalated duct lesions (adenomas and hyperplasia) with differentiation down two separate pathways [28, 29].

Clinical aspects The clinical features depend largely on the aggressiveness of the component tumor entities.

Macroscopy The reported tumor size ranged between 20 and 100 mm with a mean size of 40 mm.

Microscopy The appearance will be that of the component tumors, not infrequently a combination of adenoid cystic and EMCs.

Differential diagnosis The most important differential diagnosis is with carcinoma with high-grade transformation CS which typically shows multiple differentiation and CXPA (see Sects. 5.9.19, 5.9.14.1 and 5.9.14.3).

Treatment and prognosis Hybrid carcinomas are treated surgically. The prognosis depends on the aggressiveness of the individual components [310].

5.9.25 Endodermal Sinus Tumor

Definition Endodermal sinus tumor (EST) (yolk sac tumor) is an aggressive malignant germ cell-derived neoplasms characterised by the presence of Schiller-Duval bodies admixed with papillae, tubules, microcysts and sheets of primitive cells within myxoid extracellular background.

Epidemiology and clinical presentation ESTs are very aggressive malignant tumors with short survival time. ESTs

were documented in both adults and children. Within the head and neck area, these tumors occur mostly in sinonasal tract and in the nasopharynx. There are only two reports of primary EST of the parotid gland. One that recurred after chemotherapy was in a 2-year-old girl [311] and the other in a 16-month-old girl, who is alive and well 2 years after chemotherapy [312].

Microscopy Histologically, the tumor often consists of reticular pattern characterised by anastomosing small glandular spaces. In other places, the tumor may be composed of microcystic, solid and papillary structures lined by irregular neoplastic cell. PAS-positive diastase-resistant intracellular and extracellular globules are present.

Immunohistochemistry The tumor cells are positive for alpha-fetoprotein (AFP) and placental alkaline phosphatase (PLAP).

Differential diagnosis In differential diagnosis immature teratoma, sialoblastoma and poorly differentiated adenocarcinoma should be considered. Schiller-Duval bodies and positive staining for alpha-fetoprotein characterise EST. Particularly immature teratomas with papillary areas may be reminiscent of EST but must be distinguished because their prognosis in children is favourable after complete resection.

Treatment and prognosis ESTs in children are very aggressive neoplasms that require adjuvant cisplatin-based chemotherapy. The serum AFP is elevated, and it returns to normal levels after surgical resection of the EST.

5.9.26 Sialoblastoma

Definition Sialoblastoma is low-grade malignant neoplasm usually present at birth or shortly thereafter, composed of epithelial basaloid and myoepithelial cells that recapitulate primitive salivary gland anlage.

Epidemiology Sialoblastoma was first reported in 1996 by Vawter and Tefft [313] who used the term embryoma. Since that time, approximately 40 tumors that fit into the definition of sialoblastoma were reported under different names, such as congenital BCA, congenital hybrid BCA/AdCC and sialoblastoma [314].

Clinical presentation Sialoblastoma arises almost exclusively in the perinatal period with only exceptionally rare cases presenting after 2 years of age [315].

Macroscopy Grossly, the tumors range up to 15 cm in greatest dimension and are well circumscribed and even partly encapsulated. In other cases, they may be locally invasive with extension to adjacent soft tissues and bone.

Microscopy Microscopically, sialoblastoma is composed of numerous solid hypercellular islands of primitive basaloid cells, some with peripheral palisading, and often with small central ducts (Fig. 5.79) [316]. The tumors have variable histological patterns, composed of variably sized nests and solid sheets of basaloid cells with focal ductal differentiation and cystic and microcystic change. The tumor cells are fairly uniform with minimal cytoplasm and round-to-oval nuclei with only slight polymorphism. Mitoses are may be numerous, but none is atypical. Neural and occasionally vascular invasion may be found (Fig. 5.80). On immunohistochemistry anti-cytokeratin and EMA antibod-

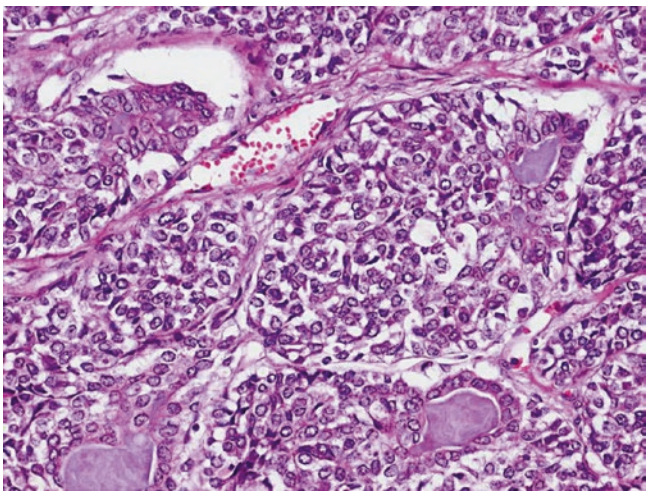

Fig. 5.79 Sialoblastoma is composed of solid hypercellular islands of primitive basaloid cells, some with peripheral palisading, and often with small central ducts

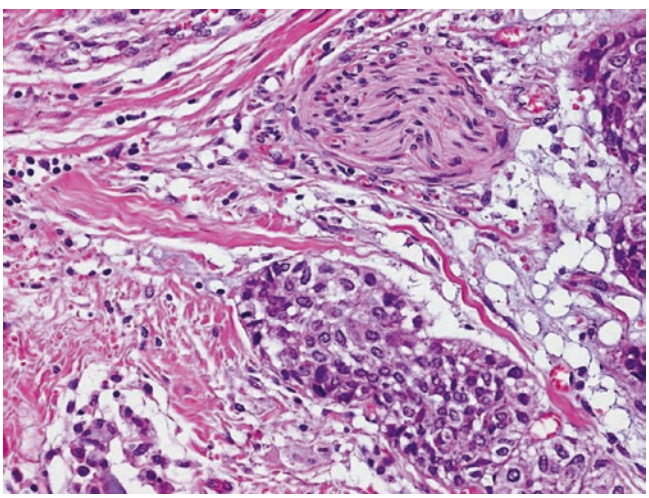

Fig. 5.80 Sialoblastoma. Neural and occasionally vascular invasion may be found

ies stain ductal elements and occasional basaloid epithelial cells in the solid nests. Luminal cells express S-100 protein and actin [316].

Differential diagnosis PA is exceedingly rare in neonatal age group and is distinguished by chondromyxoid stroma and combination of epithelial and myoepithelial cells with duct formation and metaplastic changes. BCA is also very rare in neonatal population, and it consists of uniform basaloid cells without mitoses and polymorphism. AdCC is vanishingly rare in the neonatal age group; it is characterised by invasive growth and formation of abundant extracellular matrix presenting with cribriform and pseudocystic patterns.

Treatment and prognosis Criteria for malignancy include invasion of nerves or vascular spaces, necrosis and marked cytological atypia [317]. Of 15 reported cases, 4 had recurrences and another metastases to regional lymph nodes.

5.10 Benign and Malignant Lymphoid Infiltrates

5.10.1 Lymphoepithelial Sialadenitis (Sjögren's Syndrome-Type Sialadenitis)

Definition Lymphoepithelial sialadenitis (LESA) is a destructive autoimmune lymphoid infiltrate affecting mainly salivary and lacrimal glands, in most cases correlating or progressing to sicca syndrome, clinically called Sjögren's syndrome (SS).

Epidemiology The histological correlate of the clinical disease entity SS has long been characterised by a confusing terminology, embracing terms like Mikulicz syndrome, myoepithelial sialadenitis and benign lymphoepithelial lesion, none of which was satisfactory [318]. Recently, it has been shown that the original case report by Mikulicz in 1896 has been a MALT lymphoma with multifocal manifestation in salivary glands [319]. The term myoepithelial sialadenitis also proved to be inaccurate as myoepithelial cells are not involved in the pathogenesis of the lymphoepithelial lesions/islands, previously called 'epimyoepithelial islands' [320]. In 1999 Harris et al. introduced the much more accurate term lymphoepithelial sialadenitis (LESA) [9], and this has gained general acceptance and will be used here. About 80 % of patients with LESA are female, with a mean age at presentation of 55 years.

Etiology and pathogenesis LESA is considered to be an autoimmune disease [321–323] of unknown etiology. Several viruses have been implicated [324], but they act probably only as co-factors. Recent data suggest that low vitamin D levels in patients with SS may be more associated with complications such as lymphoma and peripheral neuropathy [325].

Clinical aspects SS represents a clinical constellation of dry mouth and dry eyes, and it should not be used as a histopathological term. It is often associated with other autoimmune diseases, most frequently rheumatoid arthritis and less frequently scleroderma, lupus erythematosus, Hashimoto's thyroiditis and chronic active hepatitis. There is a major, however not total, overlap with LESA: most patients with the clinical diagnosis of Sjögren's syndrome develop the typical histological features of LESA, and most patients with LESA develop the clinical features of SS, but not all [326].

Generally, all salivary glands are involved; however, clinical manifestation with tumorous, painless and mostly bilateral swellings is most frequent and most intense in the parotid glands [321, 326]. Tumor-like lesions and, hence surgical resections, of other salivary glands due to LESA are rare.

Macroscopy Tumorous enlargement of glands with preserved lobular architecture.

Microscopy In the early stages of LESA, striated ducts are surrounded by a lymphoid infiltrate with germinal centres. B cells concentrate around the ducts and intensely infiltrate the epithelium, unlike many non-autoimmune chronic inflammatory infiltrates. Many B cells are of monocytoid or centrocyte-like type, without cellular atypia [327]. A strong plasma cell component and many T lymphocytes may be present. With advanced lymphocytic infiltration, most striated ducts transform into lymphoepithelial lesions, representing a total functional destruction of striated ducts (Fig. 5.81) [42, 318, 320, 327, 328]. These were previously inaccurately called epimyoepithelial islands [320]. As the disease progresses, the acini become atrophied and are finally totally replaced by lymphoid tissue with still preserved lobular architecture. This leads to clinical enlargement of the salivary glands and sicca syndrome. Monoclonality by PCR can be demonstrated in up to 60 % of cases with LESA [329], but this alone is obviously insufficient for a diagnosis of lymphoma [51] (Table 5.7).

The degree of infiltration in minor salivary glands is usually less intense than in parotid glands, and lymphoepithelial duct lesions are lacking. Although lymphocytic infiltration in labial glands is not specific for LESA, a semi-quantitative assessment of the amount of inflammation in a lip biopsy (so-called focus score) is advocated as part of the investigation of patients with sicca syndrome [330].

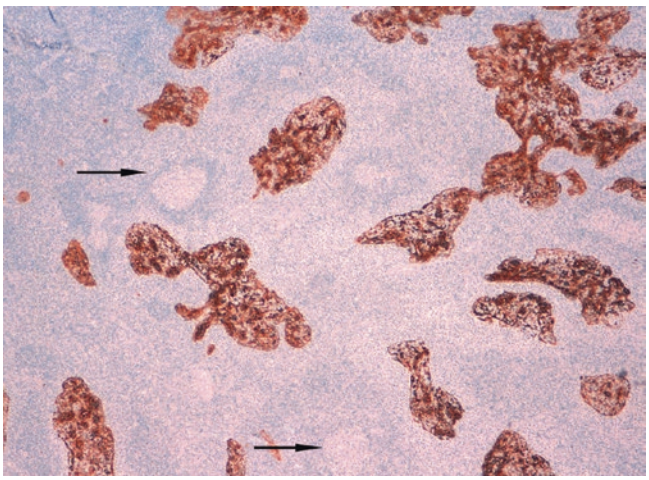

Fig. 5.81 Sjögren's syndrome-type lymphoepithelial sialadenitis of parotid gland: keratin stain identifies multiple, tightly packed lymphoepithelial lesions, embedded in reactive lymphocytic infiltration with germinal centres (*arrows*)

Table 5.7 Overview of autoimmune and neoplastic salivary lymphoid proliferations

Benign	LESA (lymphoepithelial sialadenitis), non-clonal
Borderline	*Histological or clonal evidence of neoplasia, but unlikely to disseminate*: LESA, clonal; LESA with halos of marginal zone B cells
Low-grade lymphoma	*Potential for spread to nodes and less often, systemically*: low-grade marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) – confluent proliferation of marginal zone B cells
	Low-grade marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) with plasmacytic differentiation

Adapted from Quintana et al. [329]

Differential diagnosis Cystic lymphoepithelial lesion of AIDS histologically is very similar to lymphoepithelial sialadenitis but is characterised by hyperplastic and bizarre-shaped secondary follicles and intense multifocal cystic dilatation of lymphoepithelial duct lesions [15]. Sporadic lymphoepithelial cysts of the parotid gland are a solitary cystic process without the clinical and serological characteristic of SS. The distinction of LESA from associated marginal zone B-cell lymphoma is crucial (see Sect. 5.10).

Treatment and prognosis There is still a lack of treatment for Sjögren's syndrome patients. Different types of stimulation of saliva production and of substitution of saliva are recommended. There is usually a slow deterioration of the sicca syndrome. The major complication is transformation into lymphoma (see Sect. 5.10.2).

5.10.2 Sjögren Syndrome-Associated Marginal Zone Lymphoma

Definition Marginal zone lymphoma (MALT) developing in a background of LESA/Sjögren's syndrome.

Epidemiology Overall, extranodal and nodal lymphomas represented 16 % of all malignant tumors of the major salivary glands at the AFIP [48, 331]. About half of lymphomas manifesting in salivary glands represent marginal zone B-cell lymphoma (of MALT type), which is the preferred terminology of the current WHO classification of lymphomas [332]. The vast majority develop in long-standing LESA/syndromes.

Clinical aspects Most present clinically as gradual and painless parotid enlargement, sometimes bilateral [333].

Etiology and pathogenesis The histopathology of MALT lymphoma is intimately linked with that of LESA from which it usually develops – the risk of lymphoma in LESA has been estimated at approximately 4–7 % [329]. MALT lymphoma begins as an antigen-driven lymphoid proliferation in long-standing LESA with acquisition of secondary genetic changes and slow transformation to MALT lymphoma, which can pose significant diagnostic problems in early transformational stages [327, 334].

Microscopy A restricted proliferation of marginal zone B cells to form a narrow, so-called halo around the lymphoepithelial lesions still is a physiological feature of LESA. The prerequisite to diagnose an associated MALT lymphoma is broad and focally coalescing halos of monocytoid or centrocyte-like B lymphocytes. Fully developed lymphomas are characterised by a confluent monomorphous expansion of B lymphocytes, usually involving colonisation of secondary follicles (Fig. 5.82) [10, 327, 335]. Immunohistological evidence of light chain restriction within the tumorous lymphomatous expansion is more helpful as a diagnostic criterion for manifest lymphoma as is monoclonality in PCR, which is often also positive in reactive LESA. Transformation into blastic/high-grade B-cell lymphoma is rare [333].

Prognosis and treatment Sjögren's syndrome-associated MALT lymphoma restricted to the salivary glands is an indolent disease that is often curable with local treatment [48, 331]. General lymphadenopathy and bone marrow involvement are unusual in these MALT lymphomas. Prognosis remains favourable even in the presence of other extranodal manifestation, including the bone marrow. Rituximab (anti-CD20) therapy, with or without additional chemotherapy, has been shown to be effective.

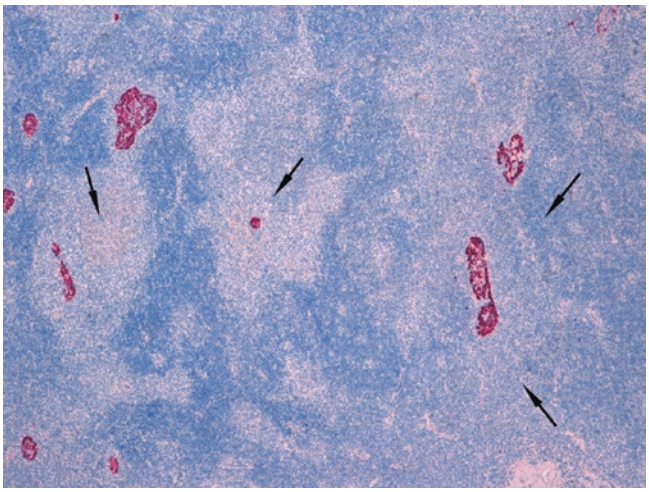

Fig. 5.82 Sjögren's syndrome-associated marginal zone (MALT) lymphoma: the keratin-stained, Sjögren's syndrome-associated lymphoepithelial lesions are widely separated by expanding lymphoma areas (bright Hemalaun counterstain: confluent right and focal around lesions on the left, *arrows*); the *dark blue* areas represent remnants of pre-existent reactive lymphoepithelial sialadenitis

Radiotherapy may aggravate the existing sicca syndrome and should be avoided.

5.10.3 Other Types of Malignant Lymphoma

Non-MALT-type lymphomas in salivary glands – especially in the parotid glands – in the majority of cases represent nodal lymphomas involving intraparotid lymph nodes. The most frequent lymphomas are follicular lymphoma and diffuse large B-cell lymphoma. They are classified according to nodal lymphomas [10, 336–338].

5.11 Other Tumors

A variety of soft tissue and other non-salivary neoplasms may rarely present as tumors of the salivary glands. These include solitary fibrous tumor, granular cell tumor, follicular dendritic cell sarcoma, inflammatory pseudotumor (inflammatory myofibroblastic tumor), primary malignant melanoma, primitive neuroectodermal tumor (PNET) and teratoma.

5.12 Unclassified Tumors

The 2005 WHO classification defined this group as benign or malignant tumors that cannot be placed in any of the categories [67]. This designation may be unavoidable if only a small quantity of tissue is available for study.

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Nasopharynx and Oropharynx

6

Alessandro Franchi, Annarita Palomba,
and Samir K. El-Mofty

Contents

A. Franchi (✉)
Section of Anatomic Pathology, Department of Surgery and Translational Medicine, University of Florence, L.go Brambilla 3, Florence 50134, Italy
e-mail: franchi@unifi.it

A. Palomba, MD
Section of Histopathology, Azienda Ospedaliera Universitaria Careggi, L.go Brambilla 3, Florence 50134, Italy
e-mail: annarita.palomba@aouc.unifi.it

S.K. El-Mofty, DMD, PhD
Division of Anatomic and Molecular Pathology, Washington University School of Medicine, Campus Box 8118, 660 S. Euclid Avenue, St. Louis, MO 63110, USA
e-mail: elmofty@path.wustl.edu

6.1 Introduction

6.1.1 Embryology of the Nasopharynx and Oropharynx

The pharynx derives from the foregut, which forms during the fourth week of development. Initially, the primitive pharynx is separated from the stomodeum by the buccopharyngeal membrane, which will break at the third week of development. At this stage, a series of five pharyngeal arches (branchial arches) appear on the ventrolateral aspect of the head and neck region, which are separated by a series of pharyngeal grooves. The inner aspect of the primordial pharynx presents a series of pharyngeal pouches, each corresponding to a branchial cleft on the external surface. The external surface of each arch is covered by the ectoderm, while the internal surface is covered by the endoderm. The inner core

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consists of mesenchyme, with an artery, a nerve, a cartilaginous and a muscular component.

The structures of the upper pharynx are mainly derived from the third arch. The second pharyngeal pouch forms most of the wall of the pharynx and gives rise to the tonsils, which derive from solid epithelial cores arising in the lateral walls of each pouch and growing into the surrounding mesenchymal tissue. These primordial epithelial evaginations branch and subsequently canalise [1]. These branches ultimately become the tonsillar crypts. These are invaded by lymphocytes and lymphoid precursors deriving from the bone marrow, approximately at the 16th week postconception [1]. At birth, vestigial tonsils are visible, hidden between the tonsillar pillars, but the immune stimulation that begins shortly after birth determines the development of secondary follicles with germinal centres and causes the rapid growth of the tonsil [1]. The base of the tongue develops from the hypobranchial eminence at the levels of the second, third and fourth pharyngeal arches.

6.1.2 Anatomy of the Nasopharynx and Oropharynx

The nasopharynx, the uppermost portion of the pharynx, is located just beneath the skull base, with the floor of the sphenoid sinus and the floor of the sella turcica to form the roof. The posterior wall is formed by the clivus, atlas and axis and contains the nasopharyngeal tonsil. Anteriorly, the nasopharyngeal cavity communicates with the posterior nasal cavities through the choanae. The lateral wall is related to the parapharyngeal space and the pterygoid plates and presents the ostium of the Eustachian tube, the torus tubarius and the lateral pharyngeal recess (fossa of Rosenmüller). Small aggregates of lymphoid tissue are present near the ostia of the Eustachian tubes to form the so-called tubal tonsil. The soft palate represents the floor of the nasopharynx and separates it from the oropharynx.

The oropharynx is the mid-portion of the pharynx, which is delimited superiorly by the plane of the superior surface of the soft palate and extends inferiorly to the horizontal plane corresponding to the tip of the epiglottis. Anteriorly, it continues with the oral cavity. The lateral walls are delimited by the anterior and posterior tonsillar pillars and contain the tonsillar fossa where the palatine tonsil is located. The inferior portion of the oropharynx comprises the base of the tongue, which contains abundant lymphoid tissue. The lymphoid tissue of the palatine tonsils, the pharyngeal tonsils and the lingual tonsil form the Waldeyer's ring.

6.1.3 Histology of the Nasopharynx and Oropharynx

The epithelial lining of the nasopharynx is mainly of the ciliated pseudostratified columnar type, variably intermixed with areas of squamous and transitional-type epithelium. The submucosa contains seromucinous glands related to minor salivary glands and aggregates of lymphoid tissue. The lymphoid component may be intermixed with the epithelium, to create the so-called lymphoepithelium. In the nasopharyngeal tonsils, the lymphoid tissue is organised to form germinal centres.

The oropharyngeal mucosa is lined by a non-keratinizing stratified squamous epithelium. The submucosa contains seromucinous glands as well as small aggregates of lymphocytes. In the palatine tonsils, the squamous epithelium forms 10–30 deep crypts that invaginate into the underlying lymphoid tissue. Here the epithelial cells show a more basaloid appearance and merge with the lymphoid cells to form the so-called lymphoepithelium. Given the irregular branching shape of the crypts, an island of squamous epithelium are commonly found within the lymphoid tissue of the tonsil and should not be misinterpreted as invasive carcinoma. The tonsils are separated from the deeper soft tissues of the parapharyngeal space by a dense fibrous capsule.

6.2 Congenital and Tumor-Like Lesions of the Nasopharynx

6.2.1 Rathke's Cleft Cyst

Definition Rathke's cleft cysts is a benign lesion, which arises from remnants of the Rathke's pouch, the embryonic structure that gives rise to the anterior lobe, pars intermedia and pars tuberalis of the pituitary gland. The Rathke's cleft is a space between the neuro- and the adenohypophysis, which normally obliterates with development, but remnants may be found in 11–33 % of unselected autopsies.

Epidemiology Symptomatic Rathke's cleft cysts represent approximately 6–10 % of sellar and suprasellar lesions in neurosurgical series [2]. The lesion is more common in female subjects (M:F = 2:1), and the age range is between 40 and 60 years [3].

Clinical aspects In most cases, they are asymptomatic and may be an incidental finding in cranial imaging. If the lesion grows enough to compress adjacent structures, it elicits symptoms including headache, visual impairment and pituitary endocrine dysfunctions [2].

Macroscopy The average size of the lesion is 15 mm [3]. The capsule has a variable thickness, and the content is mucinous, caseous, gelatinous or haemorrhagic.

Histology The epithelial lining consists of a single layer of cuboidal or columnar respiratory epithelium with interspersed goblet cells [2]. Squamous metaplasia of the epithelium is found in approximately one fourth of cases [2]. The

wall of the cyst may also present granulomatous inflammation with deposition of cholesterol crystals. A concurrent pituitary adenoma may be rarely found [2].

Differential diagnosis Rathke's cleft cyst can be distinguished from Tornwaldt's cyst based on its site, which is more cranial than the latter.

Treatment and prognosis Surgery with a trans-sphenoidal approach is the recommended first-line therapy for symptomatic cysts [2]. However, the choice of the type and extent of surgery remains controversial. Although radical resection guarantees a low recurrence rate, it leads to more complications, including cerebrospinal fluid leaks and pituitary dysfunctions. Less aggressive approaches such as evacuation of the cyst content and partial cyst obliteration are also effective in improving patient's clinical signs and symptoms with low complications [2].

A wide range of recurrence rate has been reported (0–33 %), mainly due to the type of surgical approach, extent of resection, definitions of recurrence and duration of follow-up [2]. Recurrence may occur even 10 years after the first treatment, and therefore follow-up with MRI is recommended for at least a decade after operation [2].

6.2.2 Branchial Cleft Cysts

Definition Nasopharyngeal branchial cleft cysts (synonyms: lymphoepithelial cysts, branchiogenic cysts) are thought to originate from remnants of the second pharyngeal arch and are located in the lateral nasopharynx and parapharyngeal space.

Epidemiology Branchial cleft cysts rarely localise in the nasopharynx. They are usually clinically silent and may become evident in late childhood or early adulthood as consequence of inflammation.

Clinical aspects Most nasopharyngeal branchial cleft cysts are small and asymptomatic, whereas some cause respiratory difficulties, nasal obstruction, postnasal drip, occipital headache or Eustachian tube dysfunction [4].

Histology The cysts are lined by stratified squamous epithelium or respiratory-type epithelium with underlying aggregates of lymphoid tissue (Fig. 6.1).

Differential diagnosis The differential diagnosis with other nasopharyngeal cysts is based on the anatomic location and histopathological features. Tornwaldt's cysts are located in the midline, whereas a branchial cleft cyst lies more laterally, close to the Eustachian tube orifice. The epithelial lining of the branchial cleft cyst is stratified squamous or columnar ciliated epithelium surrounded by abundant lymphoid tissue, often with germinal centres, whereas the lymphoid infiltrate surrounding a Tornwaldt's cyst is usually absent or mild.

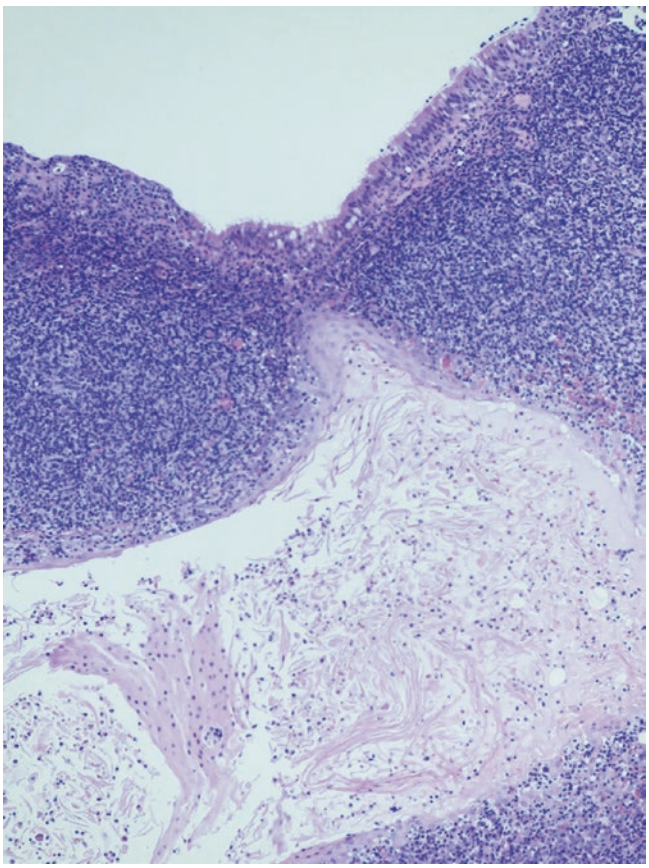

Fig. 6.1 Branchial cleft cyst. The lesion is located beneath the nasopharyngeal surface epithelium and shows a squamous epithelial lining. The cyst contains keratin and inflammatory cells

Treatment and prognosis Complete surgical excision and marsupialisation are the main treatment for nasopharyngeal branchial cleft cyst [4].

6.2.3 Tornwaldt's Cyst

Definition Tornwaldt's cyst (TC) is a benign lesion, which develops in the posterior median wall of the nasopharynx as dilatation of a persistent pharyngeal bursa. This is a persistent communication between the roof of the nasopharynx and the notochord, and its obstruction results in the cyst formation.

Epidemiology TCs are rare and have an estimated occurrence of 3 % in adult population. The peak age at onset is 15–30 years, with no reported sex predilection.

Clinical aspects They are more often an incidental finding in cranial imaging, but they may become symptomatic, with

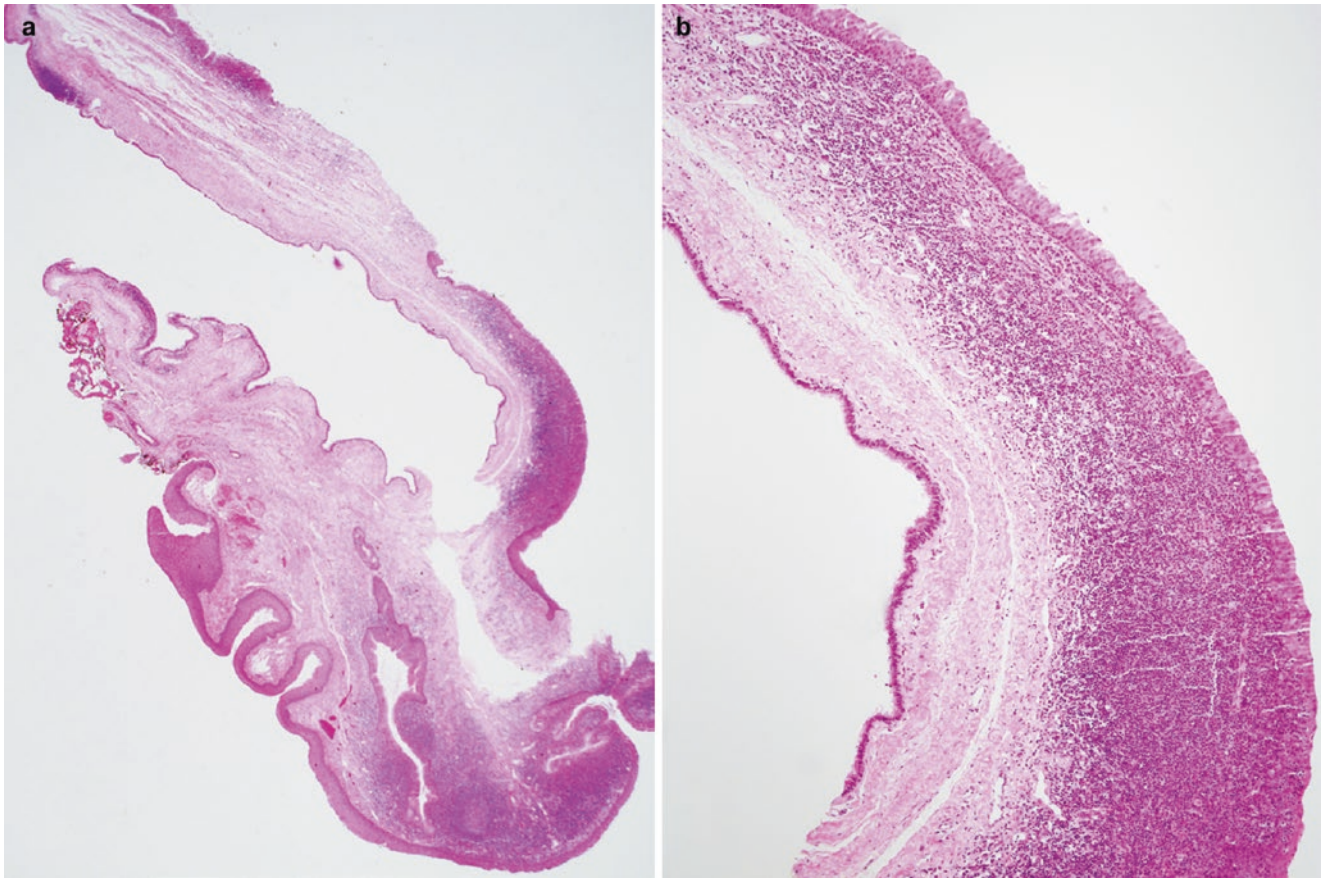

Fig. 6.2 Tornwaldt's cyst in the nasopharyngeal mucosa (**a**). The cyst is lined by columnar epithelium and separated from the surrounding mucosa by a fibrous wall (**b**)

nasal discharge, headache, seizures and dizziness/vertigo [5]. Adenoidectomy has been implicated as a possible etiologic factor, because surgery and subsequent inflammation may lead to obstruction of the diverticulum orifice and cyst formation.

Histology Tornwaldt's cysts are lined by tall, columnar, ciliated respiratory epithelium, but inflammation and infection may induce squamous metaplasia and fibrosis of the walls (Fig. 6.2).

Differential diagnosis Tornwaldt's cyst can be distinguished from other congenital nasopharyngeal cysts, mainly based on the anatomic location. While Tornwaldt's cyst is located in the posterior pharynx, branchial cleft cysts lie more laterally and Rathke's cleft cyst in the area of the sella turcica. Central nervous system herniation may also be considered in the differential diagnosis.

Treatment and prognosis Treatment consists of surgical removal or marsupialisation. Incomplete excision or simple aspiration may result in recurrence.

6.2.4 Hairy Polyp

Definition Hairy polyp (synonyms: *dermoid*, *teratoid tumor*) is a congenital lesion, which originates more commonly in the lateral wall of the nasopharynx, in the superior surface of the soft palate and in the oropharynx, while the middle ear and the mastoid cavity are sites of origin. There is no definitive agreement on its classification, since it has been considered a choristoma, a teratoma or a dermoid.

Epidemiology Hairy polyps are found in the naso- and oropharynx of neonates or young infants, with a 6:1 predilection for female infants. Occasional cases have been reported in adolescents and young adults.

Clinical aspects Hairy polyps cause respiratory distress or feeding problems. Other symptoms include hearing loss, otorrhoea, vomiting, earache, bleeding, snoring and recurrent ear infections [6]. Simultaneous congenital abnormalities may be associated [7–9].

Histology The polyp is composed of superficial keratinizing squamous epithelium associated with adnexa (hair,

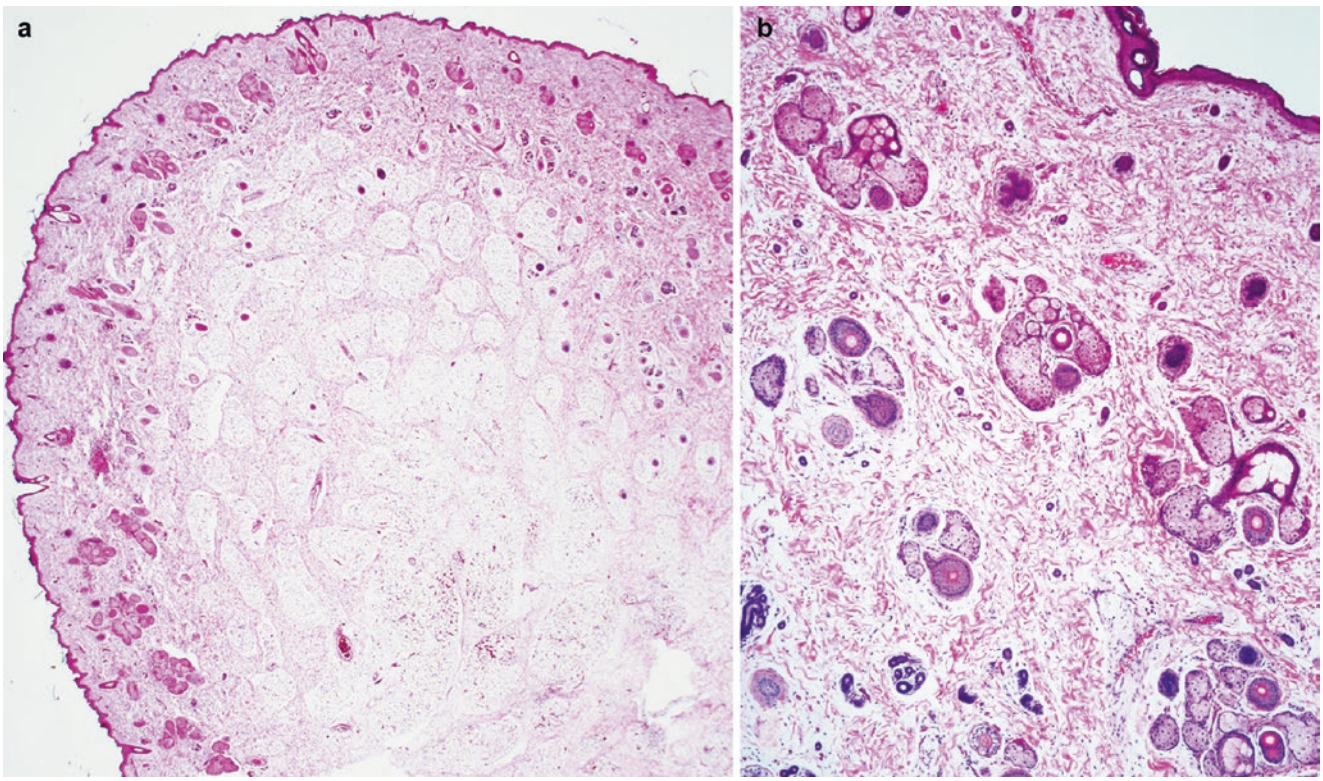

Fig. 6.3 Hairy polyp. The lesion consists of superficial keratinizing squamous epithelium and numerous adnexal structures set within a fibrovascular oedematous core (**a**, **b**)

sebaceous glands and sweat glands) and a mesenchymal core comprising blood vessels, mature fibroadipose tissue, cartilage, smooth muscle and striated muscle fibres (Fig. 6.3). Lymphoid tissue and bone formation have also been recorded [6].

Differential diagnosis Hairy polyp can be distinguished from teratomas for the presence of endodermal derivatives. Hamartomas are ruled out by the presence of single germ layer (usually mesodermal) components and dermoid cysts by presence of keratin material [6].

Treatment and prognosis Treatment is simple surgical resection, which results in complete and uneventful recovery.

6.2.5 Encephalocele/Heterotopic Brain Tissue

Definition Nasopharyngeal encephalocele is an uncommon congenital malformation consisting of herniations of brain tissue through a skull defect. Heterotopic brain tissue is a lesion composed of neuroglial tissue located within the nasopharynx. It is commonly accepted that heterotopic brain tissue is a variant of encephalocele in which the connection to the central nervous system has been lost.

Epidemiology Nasopharyngeal encephalocele is rare, representing approximately 10 % in a series of 133 anterior encephaloceles. Nasopharyngeal heterotopic brain tissue is also extremely unusual [10, 11]. Both lesions occur in newborn and infants.

Clinical aspects The most frequent presentation is with airway obstruction; cerebrospinal fluid rhinorrhoea may be the presenting symptom in nasopharyngeal encephalocele.

Histology Histologically, nasopharyngeal encephalocele and heterotopic brain tissue are undistinguishable and consist of a mixture of glial elements of various types and neuronal cells. The intervening stroma shows various degrees of inflammation and neoangiogenesis.

Immunohistochemistry Positivity for S-100 protein and GFAP may be helpful to recognise the glial elements in lesions dominated by fibrosis and with scant cellularity. Synaptophysin highlights the neural cells, when present.

Differential diagnosis Teratomas can be excluded based on the absence of other tissue types and also on clinical presentation. The distinction between nasopharyngeal encephalocele and heterotopic brain tissue is clinical, and it is based on the identification of a communication with the cranial cavity.

Treatment and prognosis Surgery is the treatment of choice for repair of the encephalocele and correction of the deformity [11]. The management of heterotopic brain tissue consists of complete surgical resection. Attention must be paid to the integrity of vital structures and to distinguish these lesions from encephalocele, because this latter lesion may have relationship to intracranial structures [10].

6.2.6 Respiratory Epithelial Adenomatoid Hamartoma

Definition Respiratory epithelial adenomatoid hamartoma is a benign lesion characterised by a glandular proliferation in continuity with the surface epithelium.

Epidemiology It typically involves the nasal cavities and paranasal sinuses (see Chap. 3), but rare cases have been described in the nasopharynx as well [12, 13]. It mainly occurs in adult patients, with a male predominance.

Clinical aspects The most common presenting symptom is unilateral nasal obstruction [13].

Histology It consists of a glandular proliferation in direct connection with the surface epithelium. Glands are lined by ciliated respiratory epithelium, with interspersed mucin-producing cells, and are surrounded by hyaline stroma.

Differential diagnosis The differential diagnosis includes Schneiderian papilloma and adenocarcinomas of various types.

Treatment and prognosis Complete surgical removal of the hamartoma is the treatment of choice [13].

6.2.7 Glandular Retention Cysts

Definition Nasopharyngeal cysts can originate from any anatomical component of the mucosa, including the surface epithelium, salivary-type glands and lymphoid tissue, and can be classified in congenital and acquired forms. Nasopharyngeal acquired cysts include retention cysts from the seromucinous glands or adenoid crypts and oncocytic cysts.

Epidemiology Nasopharyngeal acquired cysts are rather common, as they can be encountered in up to 6% of the individuals [14], but only rarely they become large enough to be clinically evident and to mimic a nasopharyngeal tumor. Among nasopharyngeal cysts, the oncocytic one has a lateral preferential topography, whereas the other acquired cysts can be indifferently median or lateral [15].

Clinical aspects Nasopharyngeal cysts are usually asymptomatic and can be fortuitously discovered on rhinoscopy or imaging. When they become clinically evident, symptoms include hearing loss, fullness in the ear, nasal obstruction, sore throat, dysphagia, odynophagia, dysphonia and tinnitus [16].

Histology Retention cysts are lined by a single layer of cuboidal epithelium, which may undergo squamous metaplasia. Retention cysts may rupture, determining perilesional fibrosis and inflammation, eventually resulting in a mucin-filled pseudocyst without epithelial lining, which can be designated as a mucocele. When the epithelial lining is mainly of the oncocytic type, the lesion can be designated as *oncocytic cyst* or *nasopharyngeal oncocytoma*.

Differential diagnosis The differential diagnosis includes congenital cysts, such as branchial arch cysts, Tornwaldt's cysts and Rathke pouch cysts.

Treatment and prognosis Different treatment options can be considered for cystic lesions of the nasopharynx. Complete surgical removal of the cyst should be performed when possible. Other options include aspiration or incision and drainage of the cyst content, or marsupialisation, but in these cases, recurrence is common [16].

6.2.8 Melanotic Oncocytic Metaplasia

Definition Nasopharyngeal melanotic oncocytic metaplasia is a rare benign lesion characterised by the coexistence of glandular oncocytic metaplasia and deposition of melanin pigment. Its importance resides in the distinction from mucosal malignant melanoma.

Epidemiology The largest series reported so far included 12 patients, all of Asian origin, with a predominance of males (11:1), aged 56–80 years (mean, 68 years) [17].

Clinical aspects It appears as a small, pigmented, brown to black mucosal lesion, localised in most cases near the Eustachian tube opening. Multiple lesions may be present. Associated symptoms included otitis media, tinnitus, hoarseness, rhinorrhoea, epistaxis, discomfort of the throat and haemoptysis [17].

Histology The lesion consists of diffuse oncocytic metaplasia of the nasopharyngeal seromucinous glands associated with cytoplasmic deposition of melanin pigment (Fig. 6.4). Pigmentation can also be detected in the surface epithelium, even in absence of oncocytic metaplasia. There is no atypia or mitotic activity. Fontana–Masson staining is positive and

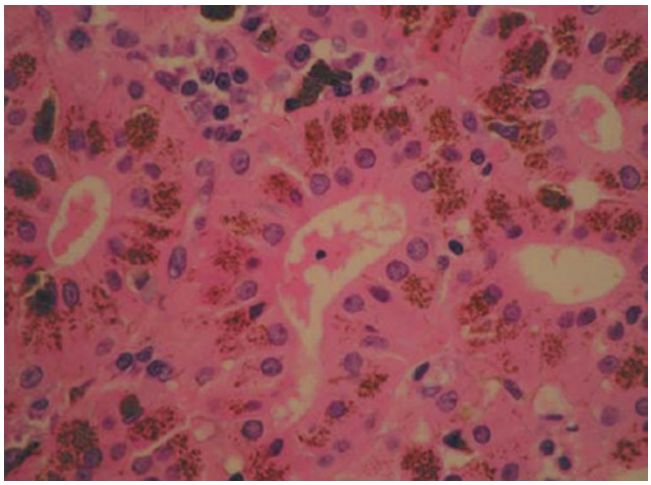

Fig. 6.4 Melanotic oncocytic metaplasia. The epithelium of the glands shows oncocytic metaplasia with melanin pigmentation in the cytoplasm (Reprinted from Sasaki et al. [17], with permission)

highlights several dendritic melanocytes between the metaplastic and the surface epithelial cells.

Immunohistochemistry Dendritic cells are positive for S-100 protein and negative for HMB45 [17].

Differential diagnosis Clinically, melanotic oncocytic metaplasia may be misdiagnosed as carcinoma or melanoma, but histologically the differential diagnosis is straightforward.

Treatment and prognosis There has been no recurrence or progression of the lesion reported after excisional biopsy [17].

6.3 Benign and Locally Aggressive Tumors of the Nasopharynx

6.3.1 Schneiderian Papilloma

Definition Schneiderian papillomas (synonymous inverted papilloma) are benign but locally aggressive epithelial tumors, which mainly involve the sinonasal tract (see Chap. 2), but may occasionally arise in the nasopharynx [18, 19]. Three distinct subtypes can be recognised: exophytic, inverted or endophytic and cylindrical cell or oncocytic papilloma.

Epidemiology These lesions affect predominantly male subjects, with a median age of 60 years [18].

Clinical aspects Nasopharyngeal Schneiderian papillomas more often represent an incidental finding or may occasionally cause nasal airway obstruction [18].

Histology Histologically they are identical to the sinonasal counterparts (see Chap. 3).

Differential diagnosis The differential diagnosis includes benign lesions like respiratory epithelial adenomatoid hamartoma and malignant neoplasms, such as squamous cell carcinoma variants for the exophytic and inverted forms, and adenocarcinomas for the oncocytic papilloma.

Treatment and prognosis Surgery is the treatment of choice. As the sinonasal counterparts, they are prone to recur, with the potential of malignant transformation. One patient affected by nasopharyngeal Schneiderian papilloma developed a separate nasopharyngeal squamous cell carcinoma and died for disease [18].

6.3.2 Ectopic Pituitary Adenoma

Definition Ectopic pituitary adenoma (EPA) is defined in the WHO classification as a benign pituitary gland neoplasm occurring separate from, and without involvement of, the sella turcica [20]. The occurrence of ectopic pituitary adenomas can be explained considering the embryology of the adenohypophysis, which originates from Rathke's cleft pouch and migrates to the sellar region, via a pharyngeo-cranial path. Embryologic remnants along this path of migration may give rise to ectopic pituitary adenomas.

Epidemiology EPA is a rare tumor, and cases exclusively involving the nasopharynx are exceptional [21]. In a series of 32 patients affected by sphenoid sinus EPA, 9 showed involvement of the nasopharynx [21]. Most patients are adult, with a mean age of 50 years. There is no significant gender predilection.

Clinical aspects Nasal obstruction and respiratory difficulty are the most common reported presenting symptoms. The majority of nasopharyngeal EPA region are hormone non-active. Among active EPA, there is a predominance of ACTH-secreting tumors in the first decade, while prolactinomas are more frequently detected in the second to fourth decades [22].

Histology EPAs involving the nasopharynx appear as polypoid lesions, covered by normal respiratory epithelium. The tumor shows a solid, organoid nested architecture and is composed of small polygonal to plasmacytoid cells with a low nuclear-to-cytoplasmic ratio [21]. Gland-like spaces and perivascular pseudo-rosettes may be seen. Calcifications may also be present. Mitotic figures are usually infrequent and never atypical. Necrosis is absent.

Immunohistochemistry Tumor cells are positive for pancytokeratins. Notably, cytokeratin 5/6 and 7 are negative [21]. Neuroendocrine markers, such as neuron-specific enolase, chromogranin, CD56 and synaptophysin, are positive in most cases, while S-100 immunostaining is not detected. CD99 immunoreactivity is present in a minority of cases [21]. Variable immunoreactivity is observed with antibodies against pituitary hormones [21].

Differential diagnosis EPA is often misdiagnosed and the differential diagnosis is broad, especially for those tumors that are not limited to the nasopharynx, but involve also adjacent structures [21]. In these cases, EPA should be distinguished from neuroendocrine tumors, such as olfactory neuroblastoma, paraganglioma, neuroendocrine carcinoma as well as other epithelial malignancies, such as sinonasal undifferentiated carcinoma (SNUC) and nasopharyngeal carcinoma. Positivity for epithelial markers rules out olfactory neuroblastoma and paraganglioma. Neuroendocrine carcinoma, SNUC and nasopharyngeal carcinoma present histological features of high-grade malignancies, including marked atypia, necrosis and brisk mitotic activity, which are not present in EPA. In addition, nasopharyngeal carcinoma is positive for cytokeratin 5/6 and for EBV, while EPA is negative.

Treatment and prognosis Surgical removal of the lesion is the treatment of choice, although large or incompletely excised lesions may be treated with postoperative radiotherapy to achieve local control.

6.3.3 Salivary Gland Anlage Tumor

Definition Salivary gland anlage tumor (SGAT; synonym *congenital pleomorphic adenoma*) is a rare nasopharyngeal tumor of salivary gland origin [23, 24]. Although current terminology indicates that SGAT is a neoplasm, others consider it as a hamartoma.

Epidemiology SGAT is a very rare lesion, and to date approximately 30 cases have been reported in the literature [23–32]. Most lesions are recognised post-partum or within the first 2 months, and there is a strong male predominance [27]. It is often attached by a small pedicle to either the posterior septum or posterior nasopharyngeal wall.

Clinical aspects Most patients present with respiratory distress or difficulty due to nasal airway obstruction, but difficulty feeding and bleeding have also been reported [27].

Histology SGATs are multinodular and usually solid tumors. The microscopic pattern is characteristically biphasic with epithelial and mesenchymal components. There are tubular and cord-like structures seemingly derived from the surface nasopharyngeal epithelium, occasionally containing complex intraluminal papillations, which blend with stromal–mesenchymal nodules (Fig. 6.5).

The epithelial proliferation can be extensive with solid squamous areas with focal keratinization, keratinized nests, cyst and pearls (Fig. 6.5). Calcification within the cysts occurs. The surrounding stroma may be loose and myxoid with numerous inflammatory cells, but may also show some fibrosis. Other SGATs consist predominantly of densely packed sheet and nodules of small fusiform spindle cells with occasional regular mitoses. Rare keratinized duct and cystic structures are seen in these areas. Haemorrhage and focal necrosis can be present [25].

Immunohistochemistry SGAT stains positive with salivary gland amylase in all the components. The spindle cells are immunoreactive with antibodies to vimentin, cytokeratins, EMA, calponin and smooth muscle actin, but negative for S-100 and GFAP (Fig. 6.6). The epithelial structures stain for cytokeratins and EMA (Fig. 6.6).

Genetics Vranic and co-workers studied by fluorescent in situ hybridisation (FISH) one example of SGAT [28], and they found no abnormalities in 1p and 12p regions, in contrast with teratocarcinoma. Based on these findings, they concluded that SGAT might be a hamartomatous, developmental disorder rather than true neoplastic lesion.

Differential diagnosis The histopathological features of SGAT are unique among the various tumors, which may occur at this site in infants. The biphasic character of the proliferation with epithelial and mesenchymal components may resemble synovial sarcoma, which is located in the head and neck region in approximately 10 % of cases. However synovial sarcoma has a distinctive fasciculated fibrosarcoma-like pattern, which is not present in salivary anlage tumor. Teratomas may also occur in the nasopharynx in infants, but they are composed of a mixture of immature and mature tissues, including neuroepithelium, cartilage and enteric or respiratory type epithelia. In SGAT, the presence of mitotic activity and focal areas of necrosis should not be interpreted as evidence of malignancy.

Treatment and prognosis Excellent results have been obtained with simple excision. No recurrences have been reported thus far, but since SGAT histologically resembles pleomorphic adenoma, periodic follow-up is recommended to evaluate for recurrence [27].

6.3.4 Teratoma

Definition Mature teratomas are benign lesions that consist of mature tissues of ecto-, meso- and endodermal derivation in a more or less organised fashion. The term epignathus is

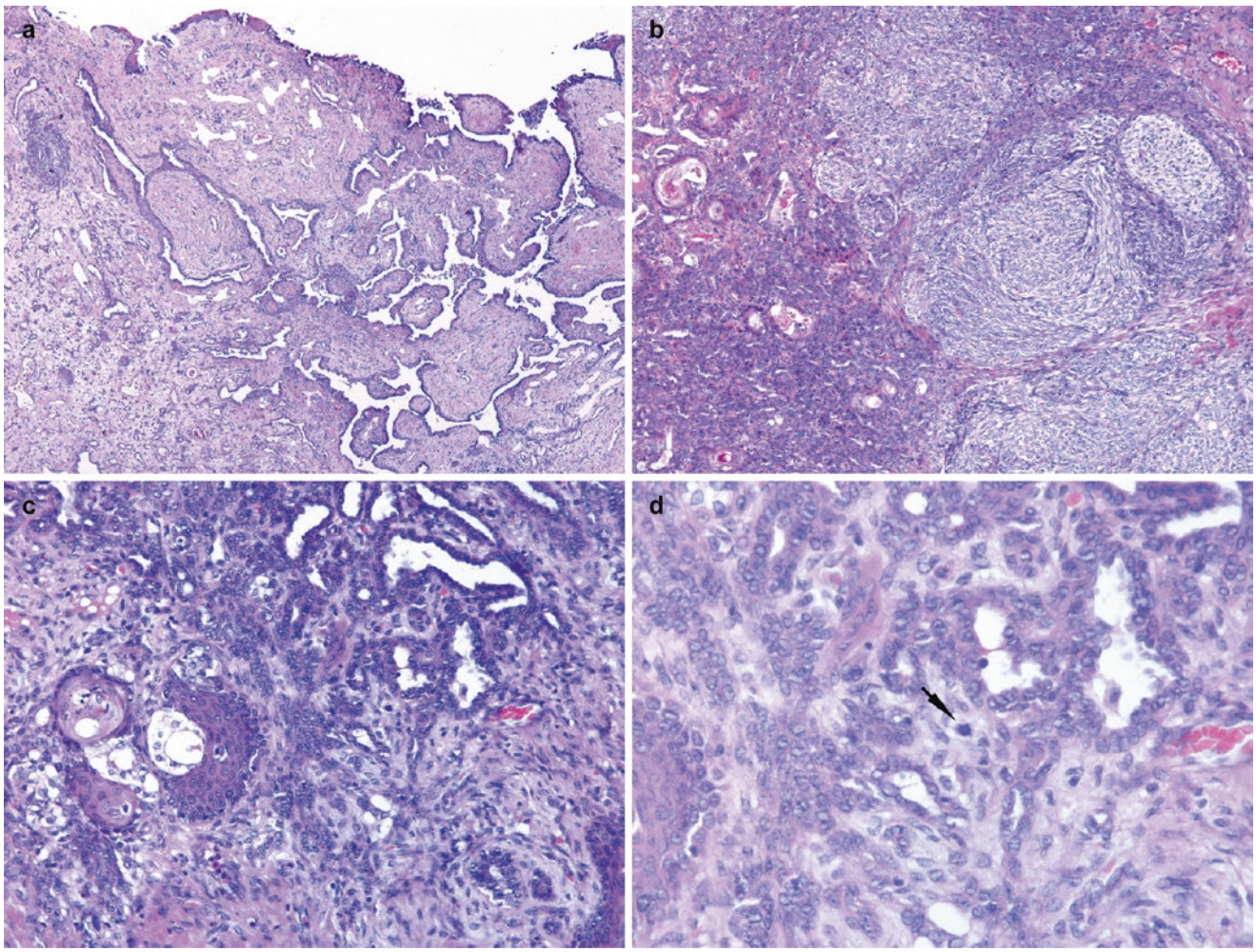

Fig. 6.5 Salivary gland anlage tumor. The surface of the lesion has a papillary architecture and blends with the surface epithelium of the nasopharynx (**a**). The microscopic pattern is characteristically biphasic with epithelial nests and spindle cell components (**b**). The epithelial component consists of solid squamous nests and tubules (**c**). There is no cytologic atypia, while mitotic figures are frequently observed (**d**)

used when fetal organs, including formation of limbs, can be identified. A variant of epignathus is fetus in fetu, which may be considered as an incomplete twinning of monozygotic twins at a primitive stage, and shows vertebral development, internal organs and limb formation.

Epidemiology Teratomas are most commonly observed in the paraxial and midline locations, and the involvement of the head and neck region is the second most common location for teratomas in early infancy, after the sacrococcygeal region. The nasopharynx is an exceptionally rare location, accounting only for 2 % of all teratomas [33, 34].

Clinical aspects Teratomas typically present in the neonatal period with airway obstruction. Congenital malformations, particularly cleft palate, may be associated.

Histology Nasopharyngeal teratomas are usually pedunculated lesions, sometimes showing a cystic component. Typically, the tissue components identified are epithelia of various types (squamous keratinizing and non-keratinizing, columnar ciliated respiratory or gastrointestinal type, cutaneous adnexa, salivary gland, liver, pancreas), mesenchymal derived tissues (bone, cartilage, fat, smooth and striated muscle) and neural elements. Immature teratomas contain tissues of varying degrees of differentiation and maturation.

Differential diagnosis The differential diagnosis mainly includes hairy polyp, dermoid cysts and encephalocele.

Treatment and prognosis Optimal treatment is complete surgical excision, which is usually curative, with a low rate of recurrence. Immature teratomas in infants have an excellent prognosis, quite in contrast to adult patients with immature teratomas. To the best of our knowledge, malignant transformation of a nasopharyngeal teratoma has not been reported.

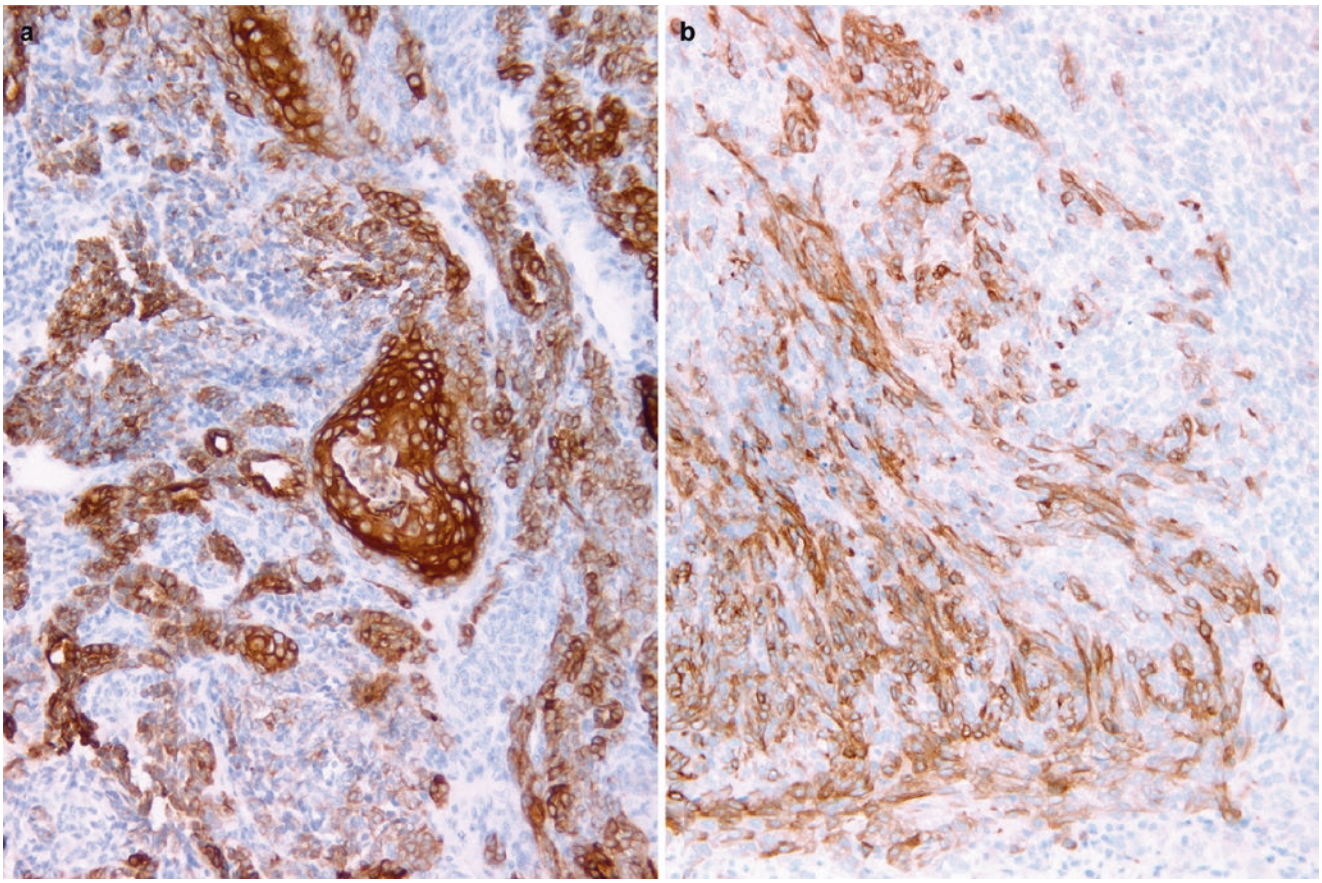

Fig. 6.6 Salivary gland anlage tumor. Immunohistochemical staining for cytokeratin AE1/AE3 mainly marks the epithelial elements of the lesion (**a**), whereas calponin highlights the myoepithelial spindle cell component (**b**)

6.3.5 Craniopharyngioma

Introduction Craniopharyngioma is a rare benign, locally invasive intracranial tumor, which occasionally may have an extracranial localisation. They may arise along the path of the Rathke's pouch ascent into the sella (the so-called craniopharyngeal duct), which extends from the oropharynx to the third ventricle. Extracranial craniopharyngiomas may therefore localise in the nasopharynx, sella turcica or sphenoid sinus [35–38]. Craniopharyngioma involving the skull bones is discussed in Chap. 4.

Epidemiology Nasopharyngeal craniopharyngiomas are extremely rare tumors occurring during the first two decades of life.

Clinical aspects The most common presenting symptoms include headache, nausea, vomiting, visual disturbances, polydipsia and polyuria, nasal obstruction and epistaxis [37, 38].

Macroscopy The tumor shows solid and cystic areas containing brown fluid.

Histology It consists of cords and islands of basaloid cells with foci of squamous differentiation and keratin pearls. Areas of necrosis, foci of calcifications and deposits of cholesterol crystals may also be present.

Treatment and prognosis The main treatment is surgical removal of the lesion. In large lesion, which cannot be removed completely without important morbidity, postoperative radiotherapy, including interstitial radiation, has improved patients' survival [37].

6.3.6 Nasopharyngeal Angiofibroma

Definition Nasopharyngeal angiofibroma (NA; synonym *juvenile nasopharyngeal angiofibroma*) is a rare, benign, but locally aggressive mesenchymal tumor, which predominantly occurs in adolescent males. It is thought to originate from the superior border of the sphenopalatine foramen at the junction of the pterygoid process and the sphenoid process of the palatine bone. The lesion grows slowly in the nasopharynx, nasal cavity and laterally into the pterygomaxillary space,

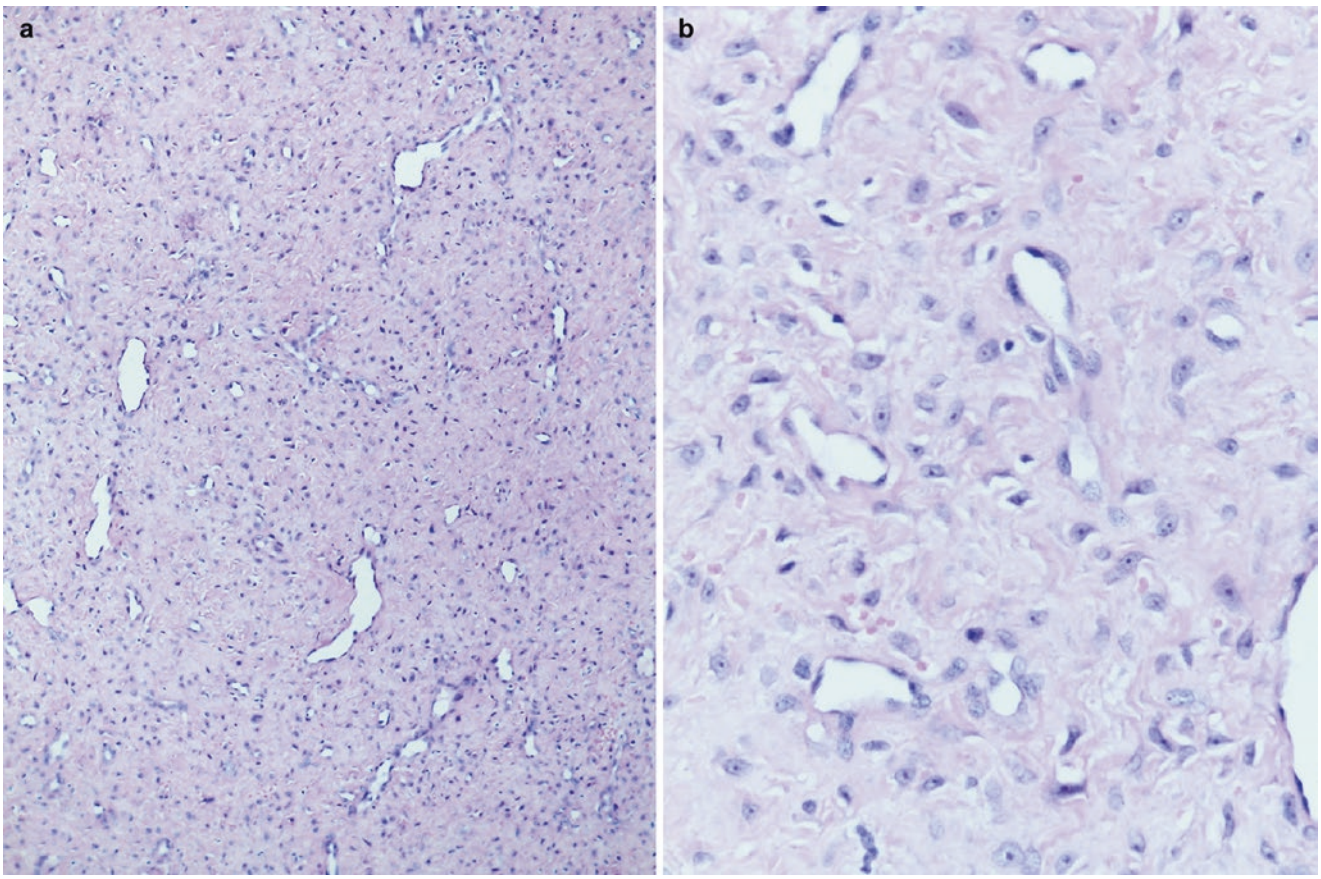

Fig. 6.7 Nasopharyngeal angiofibroma. The neoplasm is rich in blood vessels and consists of a proliferation of fibroblast-like cells set in a densely collagen stroma (**a**). The neoplastic fibroblast-like cells show no significant atypia (**b**)

and with time, it may erode the cranial bones and extend in the orbit and middle cranial fossa.

Epidemiology NA is a rare tumor with an estimated incidence of 0.05–1 % of all head and neck tumors. It shows a clear predilection for adolescent male subjects, ranging in age between 10 and 25 years.

Clinical data Presenting symptoms include nasal obstruction, epistaxis, respiratory distress, headaches or sometimes visual disturbances. The diagnosis of NA is based on clinical examination and imaging studies including CT, MRI and angiography for confirmation [39]. Pretreatment biopsy should not be performed, due to the high risk of extensive bleeding. Several staging systems have been proposed, based on the extension of the tumor and the involvement of adjacent structures, which may serve as a guide for management [40].

Macroscopy The tumor has a polypoid or multinodular appearance, and the cut surface may vary in colour according to the preoperative embolisation treatment from pink-red to grey-white.

Histology NA presents as a richly vascular lesion, with a proliferation of fibroblast-like cells set in a densely collagen stroma (Fig. 6.7). The blood vessels are of variable size and they are mainly thin walled, but vessels with a muscular wall are also present. The endothelial lining is single layered and cells have a flattened appearance. The stromal component consists mainly of dense collagen fibres, but myxoid areas can also be present. The cellular component consists of spindle and stellate cells without nuclear atypia (Fig. 6.7). Multinucleated stromal elements can be sometimes present. Tumors removed after embolisation show areas of necrosis and presence of foreign material in the blood vessels. Patients treated with testosterone receptor blocker (flutamide) show tumor shrinkage [41], with a reduction of the cellular and vascular component, and increased stromal collagen.

Immunohistochemistry The stromal cells show no evidence of epithelial, myoid, endothelial marker expression [42], and ultrastructurally they appear as fibroblast-like cells [43]. Endothelial cells express glucose transporter 1 (GLUT-1) and CD105, and this seems to correlate with clinical parameters [44, 45].

Immunohistochemical studies of steroid receptor expression have given variable results, mostly showing positivity for androgen receptors and oestrogen receptor beta and negativity for oestrogen receptor alpha [46–48].

Differential diagnosis The differential diagnosis includes other spindle cell mesenchymal neoplasms, which are however exceedingly rare in this anatomic location, such as solitary fibrous tumor or nerve sheath tumors, and sinonasal hemangiopericytoma, which can be separated based on the morphology of the proliferating cells, which are more oval and uniform, and the expression of markers of myoid differentiation, which are negative in nasopharyngeal angiofibroma. The distinction from other vascular tumors, such as capillary lobular hemangioma, may be difficult in small biopsy samples, but the typical clinico-radiologic presentation of NA and its immunohistochemical positivity for beta-catenin can help in the differential diagnosis. Antrochoanal polyp lacks the vascular component and has a certain degree of inflammatory background.

Genetics Genetic data on NA are sparse and controversial. An association between NA and familial adenomatous polyposis had been initially suggested [49, 50], but this has not been confirmed in other series [51]. In a recent study employing high-resolution comparative genomic hybridisation (HR-CGH), the endothelial and stromal cells were analysed separately, after laser microdissection [52]. The majority of chromosomal alterations were common by both components, thus suggesting a common mechanism of functional regulation. In this study *AURKB, FGF18* and *SUPT16H* genes were identified as potential molecular markers in NA [52]. Different conclusions were reached in another study, in which activating *CTNNB1* gene mutations were present in 75 % of NA examined, and this was accompanied by immunohistochemical localisation of β-catenin only to the nuclei of stromal cells but not to those of endothelial cells, indicating that the stromal cells, rather than endothelial cells, are the neoplastic cells of nasopharyngeal angiofibroma [53]. Finally, angiofibroma does not appear to be associated with a viral etiology, since both HHV-8 and EBV were not detected in one study [54].

Treatment and prognosis The choice of the appropriate treatment is based on tumor extension [40]. Surgical resection is currently accepted as the treatment of choice, and transnasal endoscopic approach is the main modality employed in early-stage disease [55]. In cases with intracranial extension, endoscopic resection can be considered as approach to treatment [56]. A multimodality approach, with surgical resection followed by treatment with radiosurgery, has also been proposed for tumors with significant skull base involvement and intracranial extension. Malignant transformation of nasopharyngeal angiofibroma in fibrosarcoma is a rare occurrence, which has been mainly related to radiation therapy [57].

6.3.7 Meningioma

Definition Meningioma is a well-characterised tumor of the central nervous system, which is defined as a benign tumor of meningothelial cells. It may occasionally present as a sinonasal mass (see Chap. 3), while primary involvement of the nasopharynx is exceedingly rare.

Epidemiology In a series of 146 extracranial meningiomas, 5 involved the nasopharynx only [58]. One example presenting as a primary tonsillar ectopic meningioma has also been reported [59]. These tumors occur over a wide age range, with a mean age of 45 years, and they show no significant gender predilection.

Clinical data Symptoms are non-specific and include nasal obstruction and visual changes.

Histology The majority of tumors are of the meningothelial type, being composed of lobules of spindle and ovoid cells with indistinct borders.

Differential diagnosis In the nasopharynx, the main differential diagnosis is with juvenile angiofibroma and is supported by the immunohistochemical positivity for EMA in meningioma.

Treatment and prognosis Patients have been treated surgically, sometimes with adjuvant radiation therapy, and the prognosis is good [58].

6.4 Malignant Tumors of the Nasopharynx

6.4.1 Nasopharyngeal Carcinoma

Definition The 2005 WHO classification of nasopharyngeal carcinomas (NPCs) includes squamous cell carcinoma, non-keratinizing carcinoma (differentiated or undifferentiated) and basaloid squamous cell carcinoma. This section deals with non-keratinizing carcinoma; for squamous cell carcinoma and variants, the reader is referred to Chap. 1. These carcinomas represent 75–95 % of all nasopharyngeal malignancies in low-risk populations and nearly all nasopharyngeal malignancies in high-risk populations [60]. In a single-centre retrospective review of NPC in a UK-based population, 88 % were non-keratinizing and 12 % were keratinizing carcinomas [61].

Epidemiology The incidence of NPC shows remarkable geographic variations. In most countries, NPC is a rare tumor, with an annual incidence below 1 per 100,000 inhabitants per year, whereas in provinces of southern China, the

incidence is around 25 per 100,000 inhabitants per year [60]. This high risk is maintained in immigrant populations. A high incidence is also observed in the Inuit populations of the Arctic region, while intermediate rates are observed in several populations of Southeast Asia, such as Thais, Vietnamese, Malays and Filipinos, and in Arabs of North Africa [60]. In Europe, the estimated number of new cases per year is 1626, with an overall rate of 3.27 per million [62].

Several factors have been implicated in the development of NPC. Epidemiological studies indicate a link between the populations at increased risk in childhood intake of locally consumed preserved foods, particularly salted fish and other foods containing nitrosamines. Genetic factors, including the presence of susceptibility loci in the HLA regions, polymorphisms of genes involved in the activation and detoxification of chemical carcinogens and in the repair of DNA, as well as in cell cycling, have also been implicated [63, 64]. The role of Epstein–Barr virus in the pathogenesis of NPC is discussed below. A subset of NPC, mainly of the non-keratinizing subtype, are associated with oncogenic HPV [65–67]. In such cases, however, an extension of an oropharyngeal HPV-related carcinoma must be ruled out [68].

Other risk factors for developing NPC include cigarette smoking and occupational exposures to wood dust and to formaldehyde [63, 64].

NPC involves more frequently male subjects, with a M:F ratio of 2–3:1. Age distribution varies across different populations: in low-risk populations, the incidence rises with age, whereas in high-risk populations of southern China, the incidence increases with age until it peaks between the fifth and sixth decades and declines at older ages. A minor peak in incidence is observed among adolescents and young adults in low- to moderate-risk populations. Familial clustering has also been observed in the population of southern China [69].

Etiology and pathogenesis Current consensus indicates that the development of NPC in endemic regions involves the interaction of environmental and genetic factors, in conjunction with EBV infection (see also section "Epstein-Barr virus: carcinogenesis and detection"). Exposure to environmental carcinogens, which may be present, for example, in salted fish and preserved food commonly used in endemic areas, together with infection may determine chronic inflammation and increase DNA damage in nasopharyngeal mucosa. This is of greater importance in individuals with cancer-prone genotypes, who may bear higher susceptibility to EBV infection for their HLA haplotypes and to DNA damage for polymorphisms of genes involved in the activation and detoxification of chemical carcinogens and in the repair of DNA. This may lead to the development of multiple epithelial precursor lesions with various clonal genetic changes. EBV, derived from infected B lymphocytes, reactivates and transfers in the epithelial cells, maintaining persistent latent infection only in the cells with specific genetic alterations, such as *p16* inactivation and/or *cyclin D1* amplification (see Genetics section). In such EBV-infected cells, the viral proteins' latent membrane proteins 1 and 2 (LMP-1 and LMP-2) drive clonal expansion and transformation, eventually resulting in the onset of invasive carcinoma. Inflammatory cytokines produced by the tumor stroma including TNF-a, TGF-b, IL-6 and IL-8 further contribute to the growth of NPC cells infected by EBV.

Clinical aspects The most common presenting sign is a palpable neck mass, due to metastatic lymph node localisation. Since NPCs often arise in the region of the fossa of Rosenmüller, obstruction of the Eustachian tube opening may cause unilateral deafness or tinnitus. Other common presenting symptoms include epistaxis and nasal obstruction as well as signs of involvement of cranial nerves in more advanced cases (Fig. 6.8).

Histology Non-keratinizing nasopharyngeal carcinoma can be further subclassified into undifferentiated and differentiated subtypes, although this distinction is of no clinical or prognostic significance [70]. The *undifferentiated* subtype is more common and consists of a proliferation of large cells with indistinct borders, growing in cohesive nests (syncytial pattern) (Fig. 6.9). They show a large round to oval vesicular nucleus with prominent nucleolus and scant amphophilic or eosinophilic cytoplasm (Fig. 6.9). Neoplastic cells may also occasionally have a spindle shape and be organised in fascicles (Fig. 6.10). Small foci of primitive squamous differentiation can seldom be present. The *differentiated* subtype shows a more organised cellular stratification reminiscent of transitional cell carcinoma of the urinary bladder (Fig. 6.11). Neoplastic cells tend to be smaller than those of the undifferentiated subtype, show more distinct cell borders and have more chromatin-rich nuclei that do not usually show prominent nucleoli. Areas of necrosis can be present.

The neoplastic epithelial cells are usually associated with an inflammatory infiltrate of variable density, consisting of lymphocytes, plasma cells and less frequently eosinophils. In most cases, this infiltrate is abundant and separates single epithelial cells or small groups of epithelial cells, thus determining the so-called lymphoepithelioma or lymphoepithelial carcinoma appearance. Other minor features, which can be sometimes identified, include the presence of amyloid globules, epithelioid granulomas and extracellular oedema and accumulation of extracellular [71] or intracellular mucins.

Immunohistochemistry The immunoprofile of non-keratinizing NPC is characterised by strong and diffuse staining for pan-cytokeratins (Fig. 6.12), high-molecular-weight cytokeratins and cytokeratin 5/6, while staining for low-molecular-weight cytokeratins and EMA is more limited. Cytokeratins 7 and 20 are negative. Nuclear

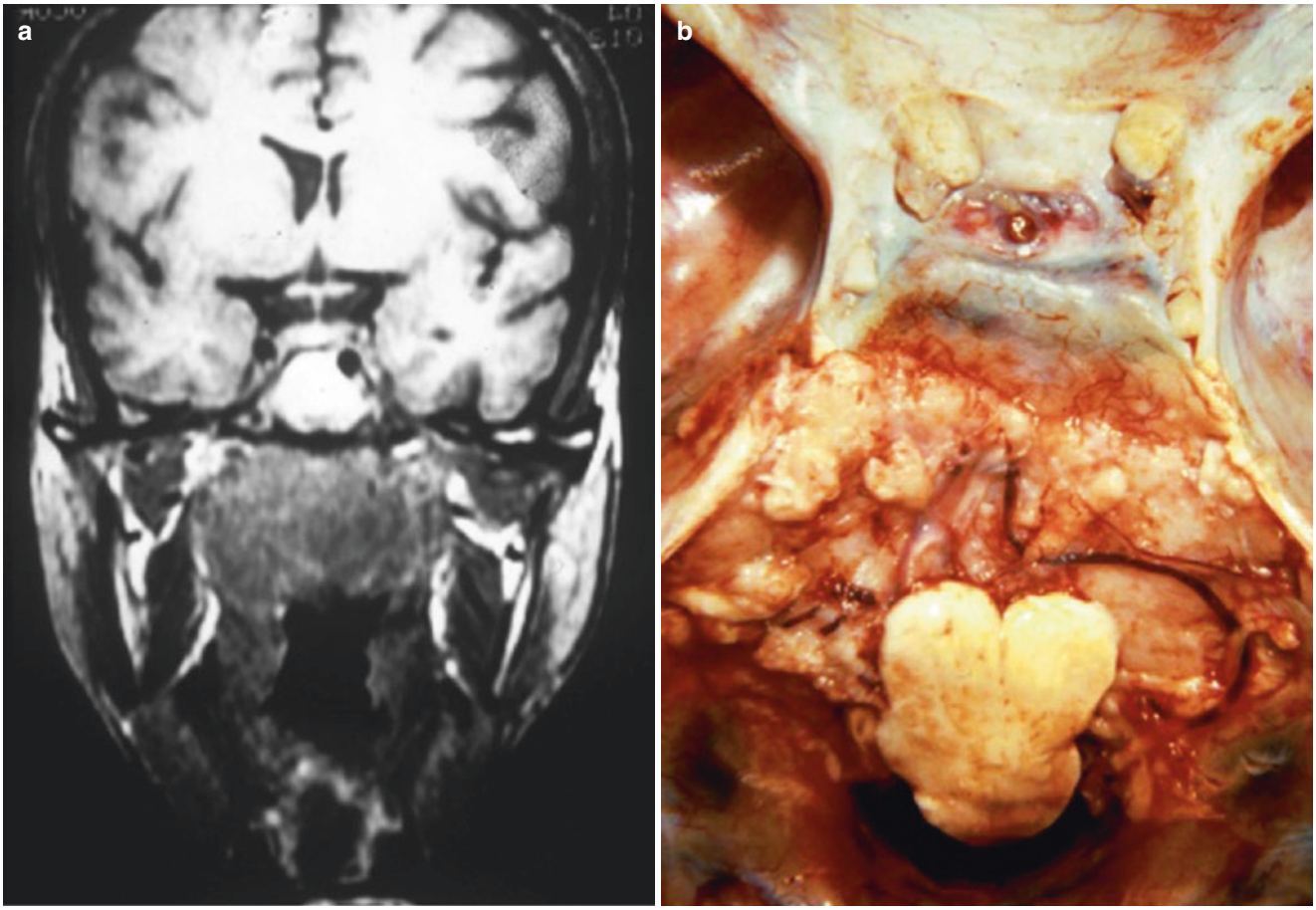

Fig. 6.8 (**a**) Advanced-stage nasopharyngeal carcinoma. MRI showing invasion of the skull base and parapharyngeal space. (**b**) Grossly, the tumor infiltrates the skull base, the pons and the cranial nerves (Courtesy of Professor J. Traserra, Barcelona, Spain)

immunoreactivity for p63 is strong and diffuse. The accompanying infiltrate is positive for B and T lymphoid markers as well as for S-100 protein. A subset of NPCs may show positivity for syndecan-1 (CD138) in epithelial neoplastic cells [72]. P16 immunostaining is negative, with the exception of the rare cases of non-keratinizing NPCs which are related to HPV [65].

Epstein–Barr virus: carcinogenesis and detection EBV is a ubiquitous γ herpes virus with a double-stranded linear DNA genome of about 172 kb. It becomes circularised to form viral episomes shortly after infection. The virus enters the cells through CD21 membrane receptor which is mainly expressed on B lymphocytes but is also present on human pharyngeal cells [73] and other epithelial cells [74]. EBV is a B-lymphotropic virus with growth-transforming properties. It has strong tropism for human lymphocytes and the epithelium of the upper respiratory tract. It infects approximately 90 % of the population worldwide. As is typical of herpes viruses, it can either undergo a cycle of lytic infection during which it replicates and the majority of the viral genes are expressed or it can go into a state of latency in the infected cells during which the virus does not replicate [75]. Primary infection in early childhood is usually asymptomatic and results in lifelong virus persistence in a latent state. Delayed infection occurring during adolescence is often lytic leading to infectious mononucleosis which is also followed by persistent asymptomatic latency in which the virus can persist for life in the host cells. During this latency phase, no viral proteins are expressed and the virus is therefore shielded from the immune system. In this type of latency which is termed latency type 0 or true latency [76], viral DNA can be identified in the infected cells by ISH and PCR.

EBV-induced malignant transformation of host cells occurs during latency and is associated with transcriptional activation of a set of viral genes. The products of transcription vary in different tumor types, corresponding to specific latency phases. For example, in the case of EBV-related post-transplantation lymphoproliferative disease (PTLD), arising in immunosuppressed transplant patients, EBV expresses the full spectrum of latent genes (latency type III); these include two small early nuclear RNAs (EBER-1 and EBER-2), six nuclear antigens (EBNAs) and three latent membrane proteins (LMPs) [77]. Burkitt's lymphoma cells display latency type I form characterised by expression of a single viral protein EBNA-1 [78, 79].

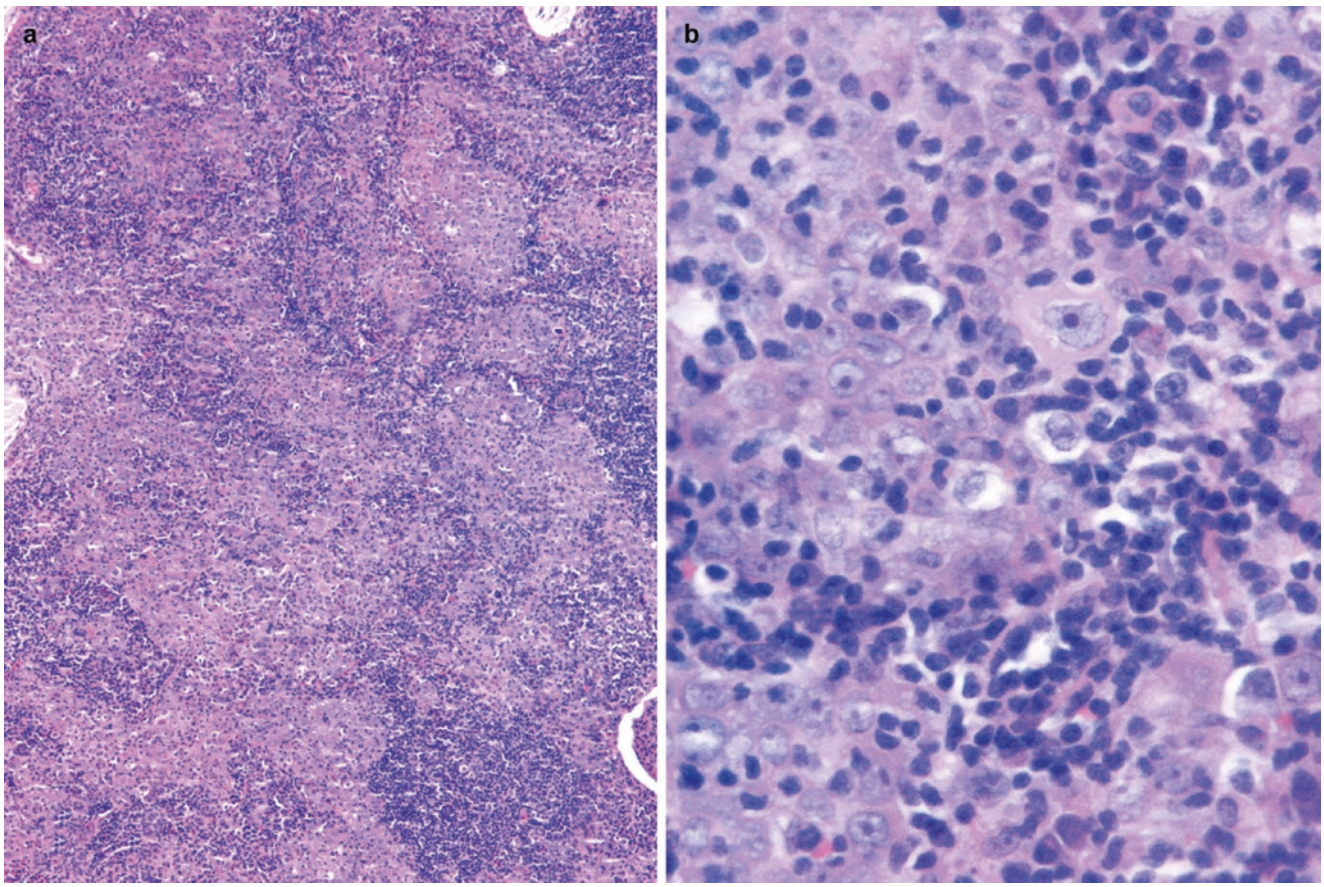

Fig. 6.9 Non-keratinizing nasopharyngeal carcinoma, undifferentiated subtype. The tumor is formed by a proliferation of cohesive nests of tumor cells, set in a lymphoid background (**a**). At higher power, neoplastic cells have indistinct borders, eosinophilic cytoplasm and large vesicular nucleus with prominent nucleolus (**b**)

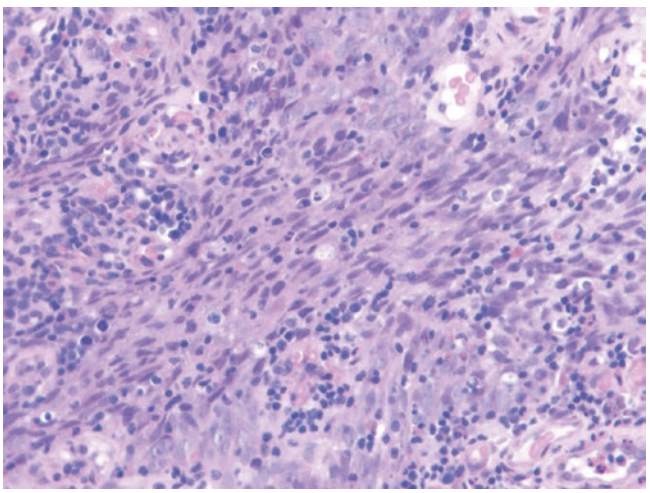

Fig. 6.10 Non-keratinizing nasopharyngeal carcinoma, undifferentiated subtype. In some instances, neoplastic cells show a spindle shape

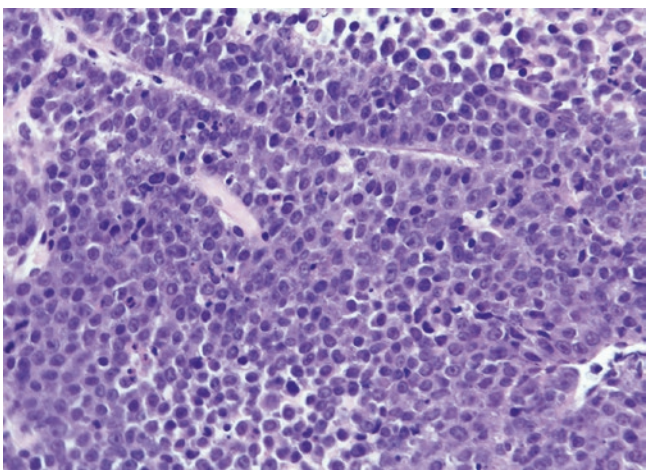

Fig. 6.11 Non-keratinizing nasopharyngeal carcinoma, differentiated subtype. Tumor cells show well-defined borders, and tendency towards nuclear palisading can be appreciated at the periphery of the tumor nests

In the case of nasopharyngeal carcinoma, EBV displays a latency type II. The tumor cells express limited set of the viral latency transcripts including EBNA-1, LMP-1, LMP-2, EBER-1 and EBER-2 [80]. These gene products contribute to both mitogenic and antiapoptotic functions of EBV, leading to host cell immortalisation and transformation [81].

EBNA-1 is a DNA-binding nuclear phosphoprotein which is required for the replication and maintenance of the episomal EBV genome. EBNA-1 also acts as a transcriptional

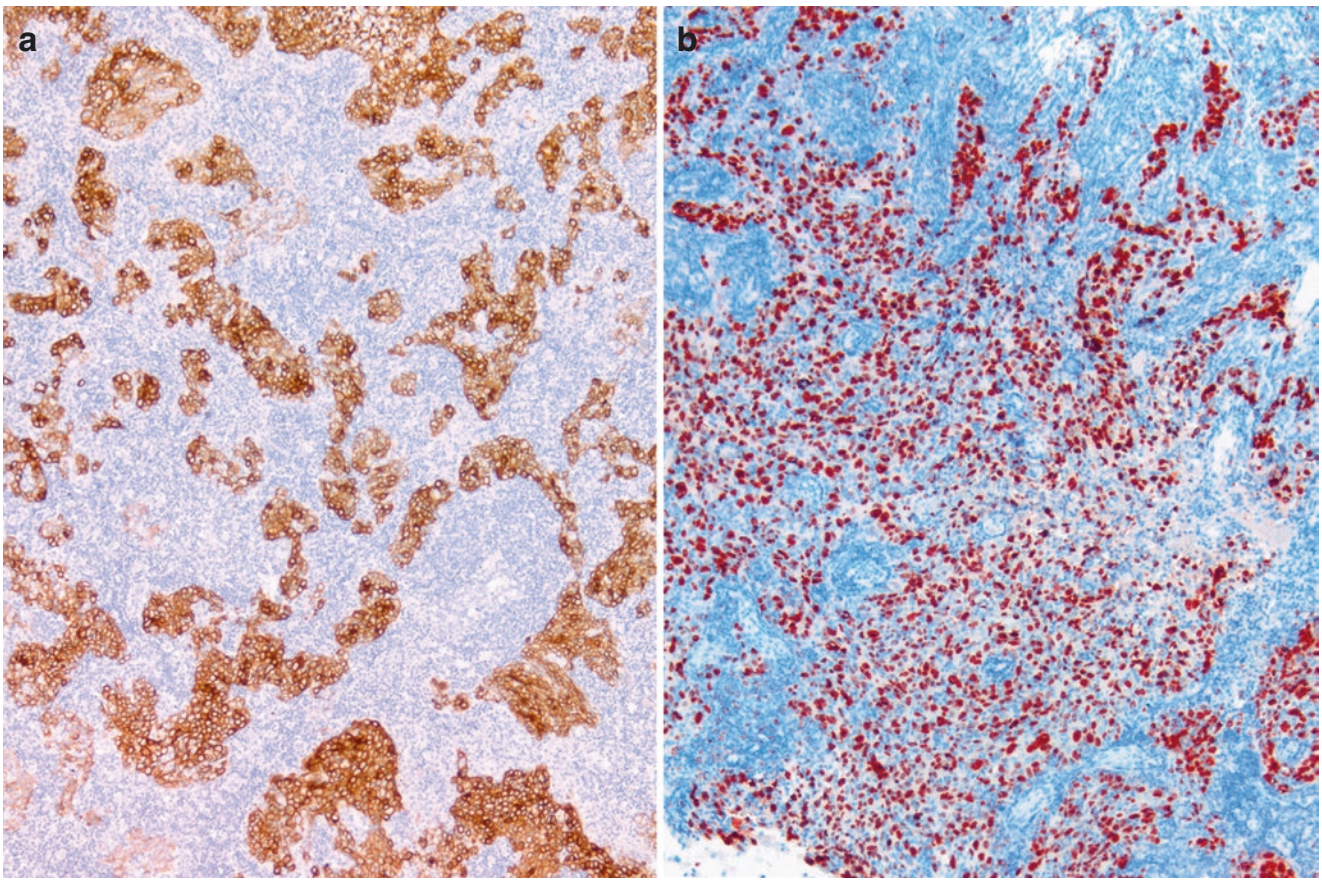

Fig. 6.12 Ancillary techniques for the diagnosis of nasopharyngeal non-keratinizing carcinoma. The tumor is diffusely positive with pancytokeratin antibodies (**a**). In situ hybridisation for EBV (EBER) results in nuclear positivity in virtually all neoplastic cells (**b**)

activator and has been shown to upregulate LMP-1 [82]. It has also been suggested that EBNA-1 can inhibit apoptosis by suppressing p53 level, due to its ability to bind p53-regulatory protein USP7 [83].

The latent membrane proteins LMP-1 and LMP-2 are also implicated in the transforming ability of EBV. LMP-1 mode of action is to stimulate several signal transduction pathways [84]. The MAP-kinase and phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt) are activated. The latter has important pro-survival action on the cells. LMP-1 also activates NFKB pathway which can upregulate the expression of antiapoptotic genes at the transcriptional level. LMP-2, like LMP-1, can activate the PI3K/Akt pathway and may promote the expression of genes involved in cell cycle regulation [85].

EBER-1 and EBER-2 are two small non-coding RNAs, 167 and 172 nucleotides, respectively. The expression of EBERs is abundant and is restricted to the cell nucleus where they are present at approximately 10^7 copies per cell [86]. The role of EBERs in the process of transformation has not been completely elucidated. They do not code for any proteins but may nevertheless regulate apoptosis by their ability to bind protein ligands [81]. In addition EBER expression has been reported to stimulate the production of autocrines such as IL-9, IL-10 and IGF-1 [87, 88].

EBER in situ hybridisation (EBER-ISH) on formalin-fixed paraffin-embedded sections is widely used and is the standard method to detect and localise EBV in the diagnosis of EBV-related neoplastic disease (Fig. 6.12). EBER-ISH shows identical pattern of positivity as EBV DNA-ISH in nasopharyngeal carcinoma samples, with multiple discrete dots within the cell nuclei in every cell [80].

Studies have shown the presence of LMP-2 mRNA transcripts with high prevalence in nasopharyngeal carcinoma cases [89]. LMP-2 protein expression is detected by immunohistochemistry in almost 50 % of nasopharyngeal carcinoma cases [90]. Identification of LMP-1 in nasopharyngeal carcinoma by immunohistochemistry is variable (20–65 %). The use of more sensitive techniques like RT-PCR increases the detection to >90 % [91].

Genetics Loss of heterozygosity (LOH) studies have identified allelic deletions at chromosomes 3p and 9p as the most common genetic alterations in NPC [92], and the *p16* gene appears to be inactivated in the majority of tumors. *RASSF1A* (Ras-association domain family 1A) tumor suppressor gene

is likely to be the inactivated target on chromosome 3p [93]. Notably, both gene products are important cell cycle regulators.

Data obtained from comparative genomic hybridisation (CGH) analysis have also indicated deletion at 3p and gains at 12p as important early events [94]. In addition, *CCND1*/cyclin D1 and lymphotoxin-beta receptor (*LTBR*) appear to be important NPC-associated oncogenes, which are localised in amplified chromosomal regions [95, 96]. Activation of *PIK3CA* at 3q26.1 is another common oncogenic alteration in NPC [97]. Finally, several important oncogenes, including *BCL-2, C-MET, C-MYC, EGFR* and *RAS*, are often upregulated, at least in part through modulation by EBV latent genes [98].

Differential diagnosis The main differential diagnosis of non-keratinizing NPC is with lymphomas of various types and is greatly facilitated by the use of an immunohistochemical panel including lymphoid markers, cytokeratins and p63. Other undifferentiated malignancies that can be entered in the differential diagnosis are malignant melanoma, which is positive for S-100 protein and melanocytic markers, and sinonasal undifferentiated carcinoma, which is negative for EBV and shows only limited expression of p63 [99].

Treatment and prognosis Patients with early-stage NPC are treated with radiotherapy with a high cure rate [100]. However, over 60 % of NPC patients present with locoregionally intermediate to advanced UICC stages IIB–IV disease [61], and they are preferentially treated with combined chemoradiotherapy [101]. The outcome for patients with advanced disease is significantly poorer, with 5-year survival rates ranging between 50 and 80 % for stages I–II and between 30 and 60 % for stages III and IV. Stage is therefore the most important prognostic factor [102]. Other independent adverse prognostic factors include older age at diagnosis and keratinizing histology [61].

Patients affected by NPC cured by radiation therapy may develop second tumors of the nasopharynx and of the sinonasal tract, including squamous cell carcinomas and neuroendocrine carcinomas [103–105]. The second tumor develops after a mean interval of 10 years and shows major morphological and molecular differences from the primary, including the absence of EBV infection.

6.4.2 Nasopharyngeal Adenocarcinomas

Adenocarcinomas of the nasopharynx are extremely uncommon tumors and can be separated into adenocarcinomas arising from the surface epithelium or from the mucoserous salivary glands. The salivary gland-type carcinomas are more common than those arising from the surface epithelium [106].

6.4.2.1 Salivary Gland-Type Adenocarcinomas of the Nasopharynx

Definition Salivary gland-type nasopharyngeal adenocarcinomas (SGAN) are histologically identical to the major and minor salivary gland counterparts. The most common subtypes are adenoid cystic carcinoma and mucoepidermoid carcinoma. Rare examples of polymorphous low-grade adenocarcinoma have also been described.

Epidemiology SGAN is a rare malignancy that represents from 0.5 to 1.5 % of all nasopharyngeal carcinomas [106, 107]. Adenoid cystic carcinoma of the nasopharynx is more common in Japan than in the western world.

Clinical aspects The median age at presentation is 45 years, and there is no gender predilection [108]. The most common presenting symptoms include epistaxis, nasal obstruction, tinnitus and headache [108]. Cranial nerve symptoms occur in 40 % of the patients, the most common being the trigeminal nerve [109]. Staging according to TNM AJCC 2002 criteria showed an approximately equal distribution of patients in early (I–II) and advanced (III–IV) stage categories [108].

Microscopy For a detailed description of the histological features of salivary gland tumors, see Chap. 5. *Adenoid cystic carcinomas* are classified in tubular, cribriform and solid subtypes. In the nasopharynx, most adenoid cystic carcinomas are high-grade tumors [108]. Examples of adenoid cystic carcinoma of the nasopharynx arising from a pre-existing pleomorphic adenoma have also been reported [110].

Mucoepidermoid carcinoma shows nesting architecture with multiple well-circumscribed squamous nests containing clear and mucin-producing cells, and microcystic areas are frequently seen [111]. In one of the cases reported by Kuo and Tsang [106], several psammoma bodies were detected within the cystic spaces and in the solid tumor nests. The majority of nasopharyngeal mucoepidermoid carcinomas are low-grade tumors [108].

The *polymorphous low-grade adenocarcinoma* has a wide diversity of histological patterns including solid areas, papillary growth, ductal differentiation, cystic spaces and an infiltrative growth pattern with perineural invasion. The main bulk of the carcinoma is found in the submucosa and the surface epithelium is often intact.

Immunohistochemistry The immunohistochemical profile overlaps that of the oral salivary gland counterparts (see Chap. 5 for detailed discussion). No useful immunohistochemical marker was found by Kuo and Tsang to distinguish SGAN from nasopharyngeal undifferentiated carcinoma [106].

Genetics The search for the presence of EBV infection in nasopharyngeal adenocarcinomas has produced controversial

results. Kuo and Tsang detected presence of EBV by EBER-ISH in 60 % of their cases and by PCR of the *LMP-1* gene in 67 % [106]. Conversely, EBER in situ hybridisation analysis was negative in two cases of mucoepidermoid carcinoma studied by Kusafuka et al. [111].

Differential diagnosis In the nasopharynx, adenoid cystic carcinoma with predominantly solid architecture should be distinguished from poorly differentiated squamous cell carcinoma and from non-keratinizing undifferentiated carcinoma. High-grade mucoepidermoid carcinoma may mimic the appearance of conventional squamous cell carcinoma. Polymorphous low-grade adenocarcinoma should be differentiated from nasopharyngeal papillary adenocarcinoma [112].

Treatment and prognosis Treatment of SGAN includes surgery and radiotherapy. Chemotherapy has been employed as palliative treatment. Indications for definitive radiotherapy are poorly differentiated histotype, early stage, incomplete surgical resection, perineural invasion and deep muscle invasion [108].

In the series reported by Pineda-Daboin et al., the prognosis for patients with SGAN was poor, since most patients had died of disease or were living with disease after a median survival of 3 years. Patients with low-grade tumors (polymorphous low-grade adenocarcinoma, hyalinizing clear cell carcinoma and acinic cell carcinoma) had a good prognosis [109].

In the series reported by Liu and co-workers, the 5-year disease-free survival and overall survival rates were 41.1 % and 57.1 %, respectively. Lymph node metastases occurred in approximately 30 % of patients and distant metastases in 25 % [108]. Presence of cranial nerve invasion, residual tumor and distant metastases were independent factors affecting overall survival, while no significant difference was observed among different histological subtypes [108].

6.4.2.2 Papillary Adenocarcinoma of the Nasopharynx

Introduction Nasopharyngeal papillary adenocarcinoma (NPA) is a slow-growing, low-grade surface tumor characterised by a papillary and glandular architecture [113].

Epidemiology NPA is an extremely uncommon tumor, without known risk factors [106, 113]. The age range is between 11 and 64 years, and there is no significant gender predilection [113]. No definitive risk factors have been associated to the development of NPA [109].

Clinical aspects NPA involves more often the roof and the posterolateral walls of the nasopharynx [113]. The most common presenting symptoms are nasal obstruction and otitis media [113].

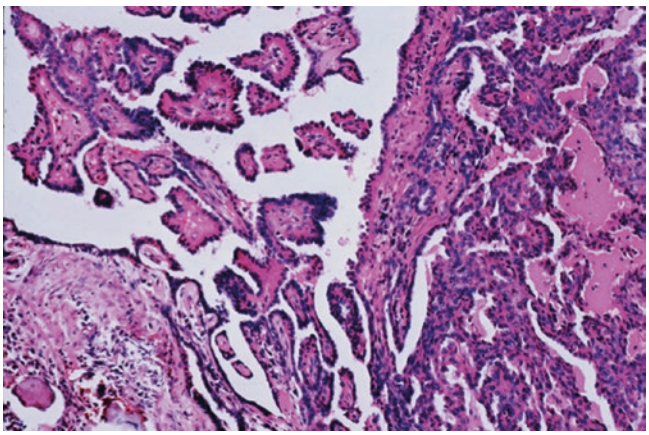

Fig. 6.13 Papillary adenocarcinoma of the nasopharynx. Infiltrating papillary tumor occurring in continuity with nasopharyngeal surface epithelium. Reprinted with permission from Shanmugaratnam K et al. [249]

Histology NPAs are unencapsulated, exophytic, cauliflower-like tumors with an occasional gritty consistency due to numerous psammoma bodies. Complex papillations arise from the surface epithelium. The papillae and crowded back-to-back glands are lined by uniform, bland, tall, columnar cells (Fig. 6.13) with intermixed mucin-containing, PAS-positive goblet cells. Some areas show overlapping vesicular nuclei with granular cytoplasm resembling thyroid carcinomas. These tumors have also been designated thyroid-like low-grade NPA [114]. Stromal calcifications and psammoma bodies can be found. Vascular, lymphatic or neural invasion is uncommon.

Immunohistochemistry NPA is positive for epithelial markers cytokeratins and EMA [109, 113, 114], while S-100 protein is negative or only focally positive [109]. TTF-1 is positive in the thyroid-like lesions, but not in conventional NPA, whereas thyroglobulin is negative in both variants [109, 114].

Genetics In situ hybridisation for EBV is negative in NPA [114].

Differential diagnosis NPA of the nasopharynx should be distinguished from the polymorphous low-grade carcinoma of salivary gland origin because they do not metastasise. In addition, the thyroid-like variant should be distinguished from metastatic thyroid carcinoma. Immunoperoxidase studies are helpful in distinguishing the different nasopharyngeal adenocarcinomas with papillary architecture. Indeed, polymorphous low-grade papillary adenocarcinoma shows diffuse expression of S-100, whereas papillary NPA of surface origin is negative or only focally positive [109, 113]. Thyroid-like low-grade NPA can be distinguished from metastatic thyroid carcinoma based on its negativity for thyroglobulin [109, 114].

Treatment and prognosis NPA can be treated with complete simple surgical excision and the results are excellent. In the series reported by Wenig et al., all patients were alive with no evidence of disease after a median follow-up of 6 years after treatment [113]. In the series reported by Pineda-Daboin et al. [109], all of the patients were alive and free of recurrent or metastatic disease after a median follow-up of 9 years.

6.4.3 Chordoma

Definition Chordoma is a locally aggressive malignant tumor that derives from remnants of the notochord. This entity is discussed more extensively in Chap. 4.

Epidemiology About a third of chordomas takes origin from the base of the skull, with possible extension in the nasopharynx, while only exceptional cases may arise in the nasopharynx and paranasal sinuses [115]. Patients are more often adults, but it may occur at any age.

Clinical aspects Symptoms include disturbances of vision, headache and nasal obstruction [116].

Histology Chordoma is characterised by a lobular architecture and consists of a proliferation of polygonal and ovoid cells, with eosinophilic or vacuolated cytoplasm, embedded in a mucoid matrix.

Immunohistochemistry Chordoma cells are positive for epithelial markers, S-100 protein, vimentin and the notochordal marker brachyury [117].

Differential diagnosis Chordoma should be distinguished from benign notochordal remnants, which have rarely been reported in the nasopharynx [118].

Treatment and prognosis Treatment consists of debulking surgery and irradiation, but recurrences are frequent [115].

6.4.4 Sarcomas

For a detailed discussion of sarcomas of the head and neck region, the reader is referred to Chap. 12.

Epidemiology In general, sarcomas of the nasopharynx are rare tumors. However, in the paediatric age group, their relative incidence is more relevant. For example, in areas with high risk for the development of nasopharyngeal carcinoma such as southern China, the carcinoma-to-sarcoma ratio is 4:1, while in adults it is 443:1 [119]. Sarcomas are the most common malignancies of the head and neck in children, and approximately 15 % arise in the nasopharynx [120].

Histology The most common histological type is rhabdomyosarcoma, but several entities have been reported as single-case reports [121–125].

Treatment and prognosis Treatment depends on the histological type and grading and on the involvement of adjacent structures. Surgical tumor resection can be followed by radiation therapy and chemotherapy.

6.4.5 Malignant Melanoma

Introduction Primary malignant melanoma of the nasopharynx is rare, representing approximately 5 % of head and neck mucosal melanoma [126]. The nasopharynx, however, is often involved by advanced stage melanomas arising in the sinonasal tract, which are more frequent. Primary melanomas of the oropharynx are exceedingly rare (3 % of head and neck mucosal melanomas), whereas this site may be involved by metastases from cutaneous melanoma [126, 127].

Epidemiology The mean age at presentation is around 65 years, and there is no significant gender predilection.

Clinical aspects The main presenting symptoms for nasopharyngeal melanoma are epistaxis, difficulty in breathing and obstructive symptoms [128]. Patients with nasopharyngeal tumors have a mean duration of symptoms of approximately 4 months, which is significantly shorter than those with sinonasal tumors [128].

Macroscopy The tumor may vary in colour from pink to brown or black, according to the presence of pigment. Most lesions have a nodular or polypoid appearance.

Histology The histological appearance of neoplastic cells is quite variable, being more often "undifferentiated", with round to oval shape, but spindle or epithelioid cytology can also be observed. The surface epithelium is frequently ulcerated, but its involvement with pagetoid spread of neoplastic cells can be documented. The presence of melanin pigment is variable. An infiltrate of melanophages and lymphocytes is often detected. Mitotic activity is brisk.

Immunohistochemistry As melanomas of other sites, they are positive for S-100 protein and for various melanoma markers, including tyrosinase HMB-45, melan A and microphthalmia transcription factor (MITF) [128]. Neural markers and CD99 are expressed in a minority of cases. EMA, cytokeratins, actins and desmin are negative [128].

Differential diagnosis The differential diagnosis is wide and depending on the morphology of neoplastic cells includes undifferentiated and poorly differentiated carcinomas, lymphomas and sarcomas of various types. Considering the variable immunophenotype of melanoma, the use of a panel of markers is recommended. Cytokeratins, S-100 protein, HMB45, CD45, actins and myogenin can be included. Metastatic cutaneous melanoma must also be excluded on clinical basis.

Treatment and prognosis The standard therapy for melanoma continues to be surgical resection, possibly associated with adjuvant radiation [129]. Patients with unresectable tumors may be treated with definitive radiotherapy [130]. The overall prognosis remains poor, due to the difficulty in eradicating the primary tumor. The incidence of nodal metastases is 20 % for oropharyngeal and 6.3 % for nasopharyngeal melanomas [126]. Nasopharyngeal and paranasal melanomas have a worst prognosis in comparison with melanomas of the oral and nasal cavities [126, 129].

6.4.6 Metastatic Tumors of the Nasopharynx

The occurrence of metastatic carcinoma to the nasopharynx is exceedingly rare, and only few cases have been reported in the literature. Nasopharyngeal metastases tend also to involve the nose and paranasal sinuses. The primary sites have been the lung, the breast, the kidney and the thyroid [131–135]. The possibility of a metastatic lesion should be considered in case of a glandular tumor of the nasopharynx, even in absence of a known primary. The differential diagnosis with nasopharyngeal primary adenocarcinomas is discussed in the respective paragraphs. Metastases to the nasopharynx in patients with cutaneous malignant melanoma have also been reported [136, 137].

In case of solitary metastasis from carcinomas, radiation therapy treatment may be beneficial for survival [133].

6.5 Tonsillitis

Definition The lymphoid tissues of the Waldeyer's ring are located at the gateway of the respiratory and alimentary tracts, and thus they play a key role in the antimicrobial defence of the body by initiating immune responses against inhaled and ingested pathogens. During life, oropharyngeal tonsils undergo morphological alterations, with an increase in size due to the increase of lymphoid tissue related to the antigen challenge. This increase in size of the palatine tonsil, or tonsillar hypertrophy, can be graded according to Brodsky's scheme [138] in five grades, which are based on the reduction of the airflow: 0, intravelic tonsils; I, slightly hypertrophied tonsils, 25 % reducing the air flow; II, medial amygdalian hypertrophy, 25–50 % reducing the airflow; III, increased amygdalian hypertrophy, 50–75 % reducing the airflow; and IV, huge amygdalian hypertrophy, achieving more than 75 % airflow reduction.

In general, surgical specimens of tonsillitis are rarely encountered, as routine histological examination of tonsillectomy specimens is considered unnecessary, except for patients with certain risk factors [139]. Gross examination is still recommended, and clinical suspicion and specimen asymmetry should be used as criteria to determine when thorough histological examination is necessary [140].

Epidemiology Bacterial and viral tonsillitis are among the most frequent paediatric infections, and there is no gender predilection. Children with acute streptococcal tonsillitis are significantly older than children with viral tonsillitis.

Etiology and pathogenesis The most often involved bacterial agents are group A beta-haemolytic streptococci, *Haemophilus influenzae*, *Streptococcus pyogenes* and *Staphylococcus aureus*. The most commonly detected viruses in chronic adenotonsillitis are adenovirus, enterovirus, rhinovirus, bocavirus, metapneumovirus and human respiratory syncytial virus [141]. EBV infection is common in the general population, but only a small subset of young infants and children presents with infectious mononucleosis. The virus is transmitted by saliva, and lytic infection involves crypt epithelial cells and B lymphocytes of the Waldeyer's ring, resulting in viral reproduction and high levels of salivary shedding. Latently infected memory B lymphocytes circulate systemically and serve as reservoir for lifelong viral persistence [142]. EBV infection has been implicated in the development of post-transplant lymphoproliferative disorder (PTLD).

Human immunodeficiency virus (HIV) infects the lymphoid tissue of the Waldeyer's ring determining miscellaneous pathological changes, including enlargement of the adenoids and/or tonsils, which is often accompanied by large ulcers and extensive necrosis [143].

Clinical aspects Patients present with fever, odynophagia, sometimes associated with otalgia, and dysphagia. Signs include tonsillar enlargement, rubor and exudates and anterior cervical lymphadenopathy [144]. Sore throat and malaise or fatigue are the most common presenting symptoms of infectious mononucleosis, while the classic triad of signs includes tonsillitis/pharyngitis, cervical lymphadenopathy and fever. The diagnosis in typical cases is made clinically and/or serologically.

Histology In acute tonsillitis specimens, there is more often lymphoid hyperplasia, with enlarged germinal centres containing tingible body macrophages, surrounded by mantle lymphocytes, and interfollicular expansion. In acute bacterial tonsillitis, an infiltrate of neutrophilic granulocytes may

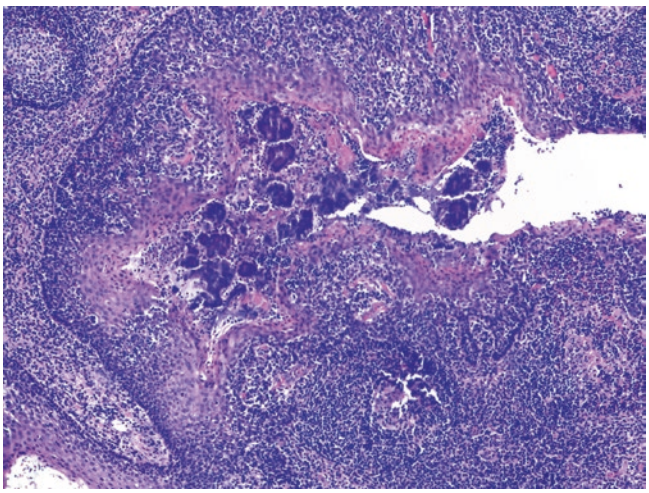

Fig. 6.14 Actinomyces colonies within tonsillar crypts are a common finding in tonsillectomy specimen

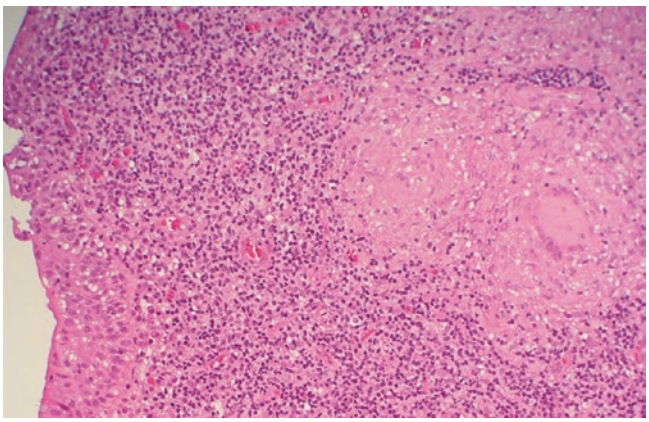

Fig. 6.15 Non-caseating tonsillar granuloma, composed of epithelioid histiocytes and Langhans' type giant cells (Courtesy of Prof. A. Cardesa, Barcelona, Spain)

be present in the tonsil mucosa and in the crypts (crypt abscesses). In chronic tonsillitis, fibrosis may be present, together with atrophic lymphoid tissue. The presence of *Actinomyces* colonies (sulphur granules) in the tonsillar crypts in absence of tissue reaction does not necessarily indicate an active infection (Fig. 6.14) [145]. Primary tonsillar tuberculosis is rare and is characterised by the presence of caseating granulomas, with acid-fast-positive bacilli visible after Ziehl–Neelsen staining. Other causes of granulomatous adenotonsillitis include fungal infections, toxoplasmosis and systemic disorders like Crohn's disease and sarcoidosis. However, in a significant number of cases of adenotonsillitis with non-caseating epithelioid granulomas, a specific etiology cannot be recognised (Fig. 6.15) [146]. It has been hypothesised that these granulomatous infiltrates may represent an exaggerated immune response to repeated episodes of adenotonsillitis [146].

The histological features of EBV tonsillitis are worrisome and can be easily mistaken for malignancy. They are characterised by an expansive polymorphous infiltrate, which alters, but does not obliterate, the normal tissue architecture. The interfollicular infiltrate consists of aggregates of immunoblasts, in a background of small- and intermediate-sized lymphoid cells, plasma cells, histiocytes and admixed high endothelial venules (Fig. 6.16). Mitotic activity is moderate to brisk. Reed–Sternberg-like cells and mononuclear Hodgkin-like cells are often detected and tend to be more numerous near foci of geographic necrosis. An important diagnostic feature is that tonsillar architecture is retained, with preservation of crypts and presence of reactive follicles.

HIV-associated lymphoid hyperplasia of the adenoids and tonsils is characterised by prominent irregular germinal centres with folliculolysis. Occasionally, multinucleated giant cells can be observed (Fig. 6.17). These are similar to giant cells present in other tissues infected by HIV and present either as isolated elements or in small clusters, mainly localised beneath the epithelium of the tonsillar crypts. The nature of these cells is yet to be elucidated, but they are currently considered as activated macrophages that are infected with HIV present in the oropharyngeal secretions [147].

Immunohistochemistry and differential diagnosis The most important differential diagnosis is between viral tonsillitis and lymphomas. Indeed, viral tonsillitis may be associated with important tonsillar enlargement that may be biopsied to rule out lymphoma. However, virus-induced histological changes may result in worrisome morphologies that closely resemble both Hodgkin and non-Hodgkin lymphomas. In particular, infectious mononucleosis involving the palatine tonsil may be difficult to separate from Hodgkin and non-Hodgkin lymphomas.

Diffuse large B-cell lymphoma characteristically shows a diffuse pattern of tonsillar involvement, usually resulting in total effacement of the architecture. On the other hand, infectious mononucleosis is characterised by the presence of a polymorphous infiltrate, which, besides the aggregates of atypical immunoblasts, includes small lymphoid cells, plasma cells, histiocytes and high endothelial venules [148]. Moreover, significant areas of preserved tonsillar architecture can be identified. Immunohistochemical and molecular markers can help in the differential diagnosis, because the combination of EBV-encoded RNA+ and CD30+ large activated cells with nongerminal centre phenotype (MUM1/IRF4+, CD10-,BCL-6-), in a polymorphous background with inverted CD4:CD8, is uncommon in diffuse large B-cell lymphoma [148].

The differential diagnosis between the rare EBV+ diffuse large B-cell lymphoma, which mainly occurs in immunocompromised subjects, and acute EBV infection can be challenging, also for the presence of a polymorphic infiltrate, which mimics an infectious process. In these cases, the demonstration of polyclonal κ and λ light chain expression by EBV+ immunoblasts may help in the differential diagnosis [148].

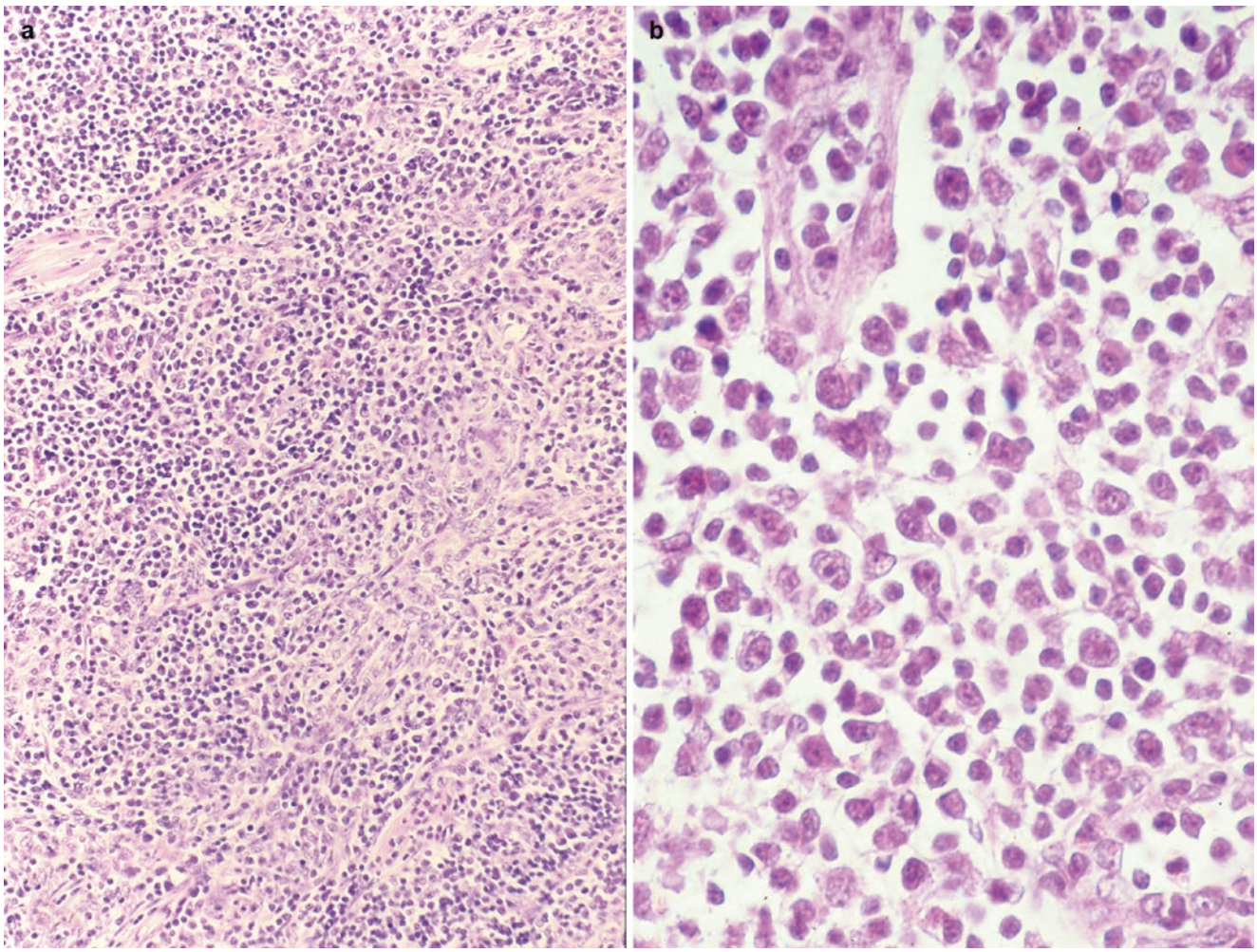

Fig. 6.16 EBV tonsillitis is characterised by an interfollicular infiltrate consisting of small- and intermediate-sized lymphoid cells, plasma cells, histiocytes, with admixed high endothelial venules, (**a**) and large immunoblasts (**b**)

The presence of Reed–Sternberg-like cells in infectious mononucleosis is a well-known source of diagnostic confusion with Hodgkin lymphoma. Moreover, both are characterised by a similar polymorphous background infiltrate, although eosinophils are rare in infectious mononucleosis and numerous in Hodgkin lymphoma. A further source of confusion is the presence of expression of CD30 and EBV and the lack of expression of CD45 in both Reed–Sternberg cells of Hodgkin lymphoma and the Reed–Sternberg-like cells of infectious mononucleosis. However, the Reed–Sternberg-like cells of infectious mononucleosis lack the expression of CD15 and express other markers like OCT-2 and BOB-1 that are not expressed by Reed–Sternberg cells [148].

Herpes simplex virus (HSV) infection of the nasopharynx can be associated with a dense CD4+, CD56+ T-cell infiltrate that may simulate a nasal NK/T-cell lymphoma. This possibility can be ruled out based on the presence of HSV, the lack of angioinvasion and angiodestruction, the absence of EBV and the polyclonal T-cell nature of the infiltrate [149].

Treatment and prognosis For bacterial tonsillitis, treatment of choice is penicillin administration for 10 days. Prevention of acute rheumatic fever is the principal goal of treatment. Acute bacterial infections may complicate with intratonsillar [150] and peritonsillar abscesses (quinsy). Tonsillitis of viral origin is usually treated with supportive care, avoiding the use of antibiotics [151]. Complications include the development of peritonsillar abscesses.

6.6 Benign Tumors of the Oropharynx

6.6.1 Squamous Papilloma

Introduction Squamous papilloma is a common benign squamous exophytic lesion thought to be related to HPV infection. For a detailed discussion of squamous papilloma, see Chap. 1.

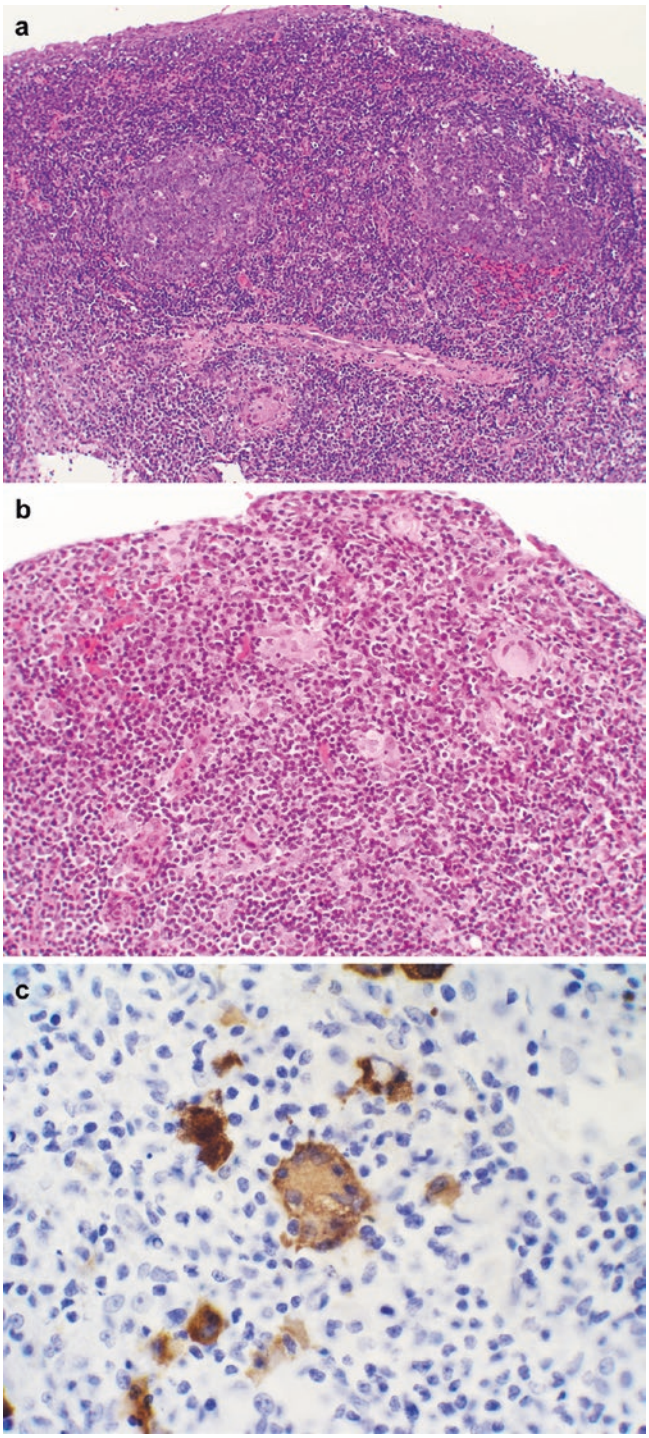

Fig. 6.17 HIV-associated lymphoid hyperplasia of the tonsils is characterised by prominent irregular germinal centres (**a**) and occasional presence of giant cells that tend to be localised close to the lymphoepithelia (**b**). These cells stain positive for the HIV-associated p24 protein (**c**) (Courtesy of Prof. A. Cardesa, Barcelona, Spain)

Epidemiology Squamous papilloma is the most common benign oropharyngeal tumor. It occurs at any age, but it is more commonly diagnosed in adult subjects, with no significant gender predilection.

Clinical aspects Although any site in the oropharynx can be involved, it most commonly originates from the soft palate and uvula. It is usually asymptomatic and presents as an exophytic lesion. Rarely, larger lesions (>1 cm) arising in the uvula or lesions with a long stalk may cause dysphagia.

Histology The lesion has a benign appearance and consists of a branching proliferation of stratified non-keratinizing or keratinizing squamous epithelium, covering a fibrovascular core [152]. Presence of HPV 6 and 11 has been demonstrated by in situ hybridisation [153].

Differential diagnosis The differential diagnosis of squamous papilloma includes acuminate condyloma and verrucous and papillary squamous cell carcinoma (see Chap. 1).

Treatment and prognosis Conservative surgical excision is curative.

6.6.2 Tonsillar Lymphangiomatous Polyp

Definition Tonsillar lymphangiomatous polyp (synonyms: lymphangioma, papillary lymphoid polyp) is a benign lesions of the palatine tonsil, which consists of varying proportions of fibrous, adipose and lymphoid mature tissues. In rare instances, the lesion may arise from the adenoids. Their nature is debated, with some authors considering them as hamartomatous proliferations and others as benign neoplasms.

Epidemiology It represents approximately 2 % of all tonsillar neoplasms and occurs over a wide age range, with a median of 26 years [154].

Clinical aspects Most patients present with a tonsillar mass, causing dysphagia and sore throat [154]. Bilateral involvement of the palatine tonsils has been reported [155].

Macroscopy The lesions range in greatest dimension from 0.5 to 4 cm and appear as polypoid masses with a smooth white, tan or yellow cut surface [154].

Histology It consists of a proliferation of dilated lymphatic vessels within varying amounts of fibrous connective tissue, sometimes accompanied by mature adipose tissue (Fig. 6.18). A lymphatic component is present within the stroma, the vessel lumina, or more frequently in association with the surface epithelium, which is either squamous or respiratory (Fig. 6.18). The endothelial lining is flat and shows no atypia.

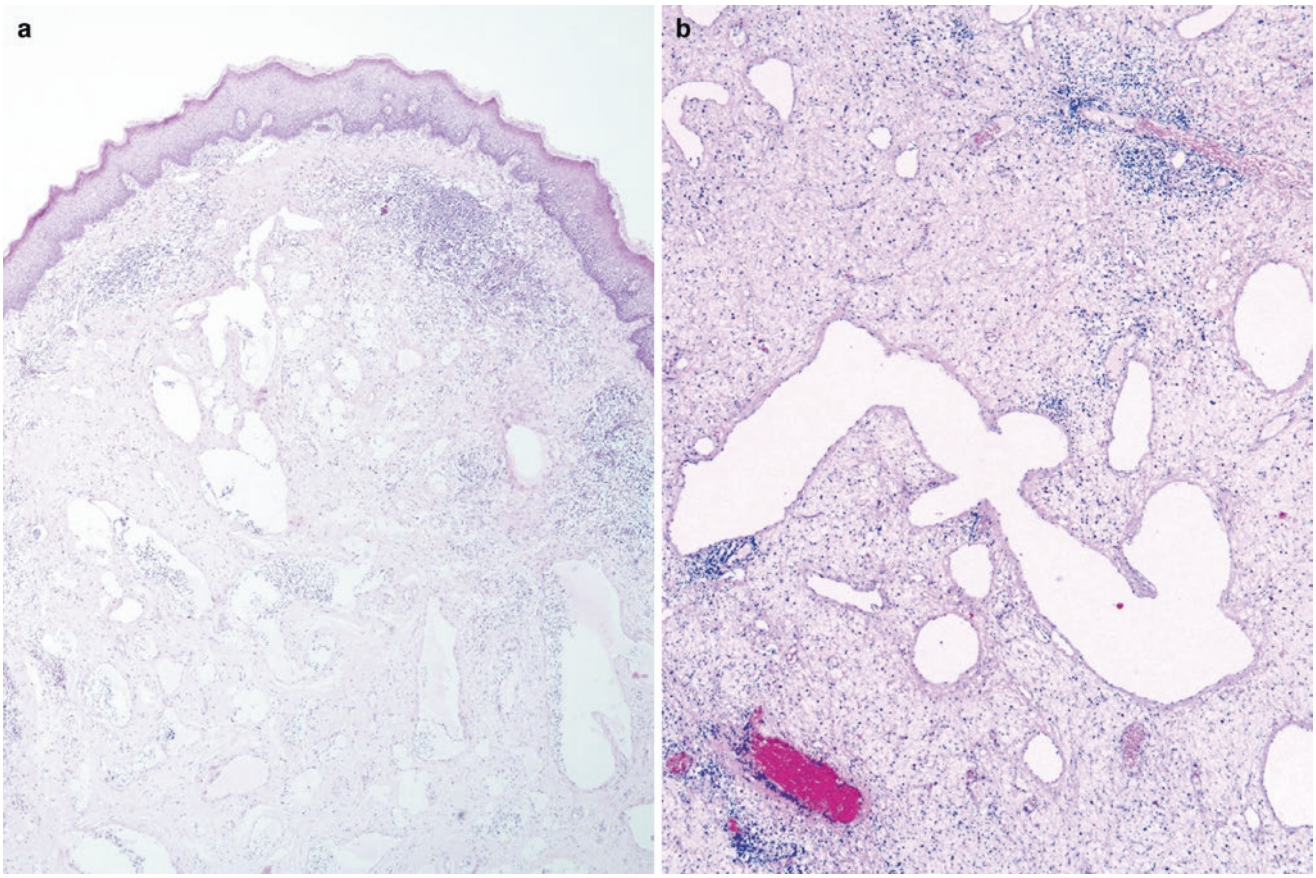

Fig. 6.18 Lymphangiomatous polyp consists of a fibrovascular stalk with several thin-walled lymphatic vessels lined by flattened endothelial cells and a squamous epithelial covering (**a**, **b**). Small lymphocytes are present within dilated lymphatics, scattered within the stalk, or associated with surface epithelium (**a**)

Immunohistochemistry The endothelial lining stains for factor VIII, CD31 and CD34, while lymphocytes are positive for CD45, CD3 and CD20.

Differential diagnosis The differential diagnosis includes juvenile angiofibroma, fibroepithelial polyps and squamous papilloma. Juvenile angiofibroma has a characteristic clinical setting, as it occurs in the nasopharynx of adolescent males, and often shows a locally aggressive growth. In addition, this tumor differs histologically from lymphangiomatous polyp because its stroma is more cellular and contains staghorn-like thin-walled vascular channels, whereas the lymphangiomatous polyp has a paucicellular fibrous background often with several lymphocytes. Fibroepithelial polyp and squamous papilloma are easily separated because they lack the lymphatic vascular component and the lymphocytes.

Treatment and prognosis Lymphangiomatous polyps are cured by simple surgical excision, with no evidence of recurrence at follow-up.

6.7 Malignant Epithelial Tumors of the Oropharynx

6.7.1 Squamous Cell Carcinoma and Variants

Definition Oropharyngeal squamous cell carcinoma represents approximately 15–20 % of all head and neck squamous cell carcinomas, but its incidence is rapidly increasing. The anatomic subsites of this region include the palatine and lingual tonsils, where the majority of these tumors originate, the soft palate and uvula, the anterior and posterior tonsillar pillars and the lateral and posterior walls. Some authors include also tumors of the retromolar trigone, because they often involve the adjacent sites of the oropharynx.

Epidemiology The incidence of oropharyngeal carcinoma has increased in recent years worldwide, and this has been more apparent in economically developed countries, among both men and women and in younger patients (<60 years) [156]. This figure is largely explained by the increasing incidence of HPV-positive cancers, whereas the incidence

of HPV-negative cancers has declined. The important role of HPV is also more evident in economically developed versus developing countries [156]. In Europe, there are significant geographical variations in the incidence of oropharyngeal carcinoma, and the highest rates occur in eastern countries [62].

As for other head and neck sites, heavy tobacco and alcohol consumption are the most important risk factors for HPV-negative oropharyngeal squamous cell carcinoma. Tobacco and alcohol abuse is less prevalent in patients affected by HPV-related carcinoma, although its potential synergistic role with HPV remains to be determined [157]. Patients affected by HPV-related carcinoma are more frequently white male subjects, of higher socio-economic status, and have sexual behaviours characterised by increased lifetime number of oral and genital sexual partners [158].

Etiology and pathogenesis

Molecular mechanisms involved in HPV-driven malignant transformation

HPVs are small non-enveloped double-stranded DNA viruses with a circular genome of approximately 8000 base pairs. The genome of the papillomavirus virion is enclosed within a capsid which is composed of two proteins; a major L1 and a minor L2 protein. Seventy-two pentamers of L1 and 72 copies of L2 are assembled to produce the icosahedral structure of the capsid. The genome is composed of early genes *E1, E3, E4, E5, E6* and *E7* and late genes *L1* and *L2*. A non-coding region, of about 800 bp which is situated between *E6* and *L1* open reading frames (ORFs), is called long control region (LCR). The LCR contains promoter sequences that are critical for regulation of viral transcription and replication. In the OP, infection typically starts when the virus gains entry into the basal cells of the crypts of the palatine and lingual tonsils. Evidence shows that some cell surface HPV receptors may be involved in this process, including integrins and the syndecan-1 heparan sulphate proteoglycan [158, 159].

In tumor cells, the virus is found in episomal (extrachromosomal) form, integrated in the host DNA or a combination of both forms. Viral integration typically occurs at the *E2* region, downstream of the early genes *E6* and *E7* oncogenes. Disruption of *E2* and consequently its regulatory protein leads to viral genes overexpression [160]. It is believed that transcripts from integrated virus DNA are more stable than those derived from episomal forms, giving the affected cells a greater growth advantage. Viral integration is thought to develop only after an initial phase of episomal replication leading to high viral load [161].

Malignant transformation of HPV-infected cells occurs as a result of interactions between viral oncogene products, particularly E6 and E7, with cell cycle regulatory proteins. E6 binds the tumor suppressor protein p53 and, with the help of a cellular ubiquitin-protein, leads to its total degradation. A major role for E7 protein is mediated through its binding to, and functional inactivation of, pRB, which in turn leads to release of E2F transcription factors and continued cell cycle progression [162, 163]. The consequence of overexpression of HPV oncoproteins and the interference with these cellular pathways are genomic instability and continued accumulation of mutations in cellular genes ultimately leading to malignant transformation [161, 164].

One of the molecular manifestations of inactivation of pRB by E7 is a paradoxical overexpression of cellular CDKN2A (p16), a cell cycle protein involved in tumor suppression by pRb. p16 inhibits hyperphosphorylation of pRb, thus preventing its dissociation from E2F. Overexpression of p16 in actively replicating cells results from feedback control, secondary to pRb deregulation [165]. p16 overexpression is considered as a molecular signature of HPV-related squamous cell carcinoma (SCC). It was initially identified as a surrogate marker for HPV-related SCC of the cervix [166] and was later shown, for the first time, as a marker in head and neck HPV-related SCC [167].

Molecular techniques clinically used for identification of HPV-related squamous cell carcinomas in the head and neck

Unlike in the cervix, where almost all SCCs are HPV related, in the head and neck, only a fraction of carcinomas are associated with HPV infection. It has been overwhelmingly proven that a majority of these carcinomas are more responsive to treatment and have a better clinical outcome with improved disease-free and overall survival. Clinical trials on the efficacy of using less toxic treatment modalities in management of HPV-related SCC of the head and neck are in progress. It is thus of importance to identify the best methodologies to distinguish between HPV-related and unrelated carcinomas. Methods used in this regard can be divided into the following broad categories:

1. Direct identification of viral DNA and proteins
2. Direct detection of transcriptional activation of viral oncogenes *E6/E7*
3. Indirect identification of transcriptionally active virus using the surrogate marker p16

The most widely used techniques used for identification of HPV in tumor specimens for clinical purposes are in situ hybridisation for HPV DNA (ISH) and p16 immunohistochemistry.

In Situ Hybridisation (ISH) for HPV DNA In situ hybridisation techniques, with fluorophore (FISH) or chromogenic (CISH) probes, allow for direct visualisation of targeted nucleotides in FFPE tissue sections. Probes could be either

cocktails for identification of multiple HR-HPV types or specific for one or two HPV types (e.g. type 16 or 16/18). Two commercially available chromogenic probes that are commonly used for clinical applications are Dako and Ventana [168]. The hybridisation results are examined microscopically. Positivity is indicated by the presence of a nuclear precipitate which could be punctuate (dot-like) or diffuse: the punctuate signal is suggestive of an integrated virus, while diffuse signal is for episomal form (Fig. 6.19). While ISH techniques are highly specific, their sensitivity varies considerably in different studies. A range of 21–83 % has been reported [168, 169]. It is estimated that ten copies of virus per cell is needed in order for ISH to detect HPV. Signal enhancement techniques such as tyramide signal amplification have drastically increased the sensitivity of ISH, which have been shown to be increased by 10- to 100-fold, to the extent that one to two copies of DNA per cell can be detected [170–173]. While the sensitivity of ISH is less than PCR, it is more specific for clinically relevant infections.

Direct Detection of Transcriptional Activation of Viral Oncogenes E6/E7 As stated above, expression of the early viral oncogenes E6/E7 is a prerequisite for the malignant transformation of the infected cells. It is generally accepted that detection of the transcriptional activation of these genes is the most reliable method to identify biologically relevant association between HPV, as a causative agent, and the malignant cells. The mere detection of HPV DNA in tumor cells, in the absence of evidence of its transcriptional activation, may indicate that the virus is likely a bystander (passenger) rather than a causative agent (driver). E6/E7 expression is now considered the "gold standard" for identification of clinically relevant HPV infection in tumor specimens. Reverse transcriptase polymerase chain reaction (RT-PCR) and real-time quantitative PCR (RT-qPCR) have been used for detection and quantitation of E6/E7 mRNA [174, 175]. However, these techniques are thought to be reliable only when used on fresh frozen tissue [175]. More recently, an in situ hybridisation method was developed for detection of transcriptionally active HR-HPV in head and neck squamous cell carcinomas [176, 177].

ISH for E6/E7 mRNA is a slide-based chromogenic assay that has been recently developed under the name RNAscope (Advanced Cell Diagnostics, Inc., Hayward, CA) [176, 177]. Formalin-fixed, paraffin-embedded tissue sections are hybridised with target probes to HR-HPV E6/E7 mRNA. The probes are used for either one specific genotype such as HPV 16 or as a cocktail for 7 or 18 high-risk HPV genotypes [176, 177]. A horseradish peroxidase-based signal amplification is used with the probes followed by colour development with 3,3′ diaminobenzidine. Positive staining is identified as brown, punctuate dots present in the nucleus and/or cytoplasm (Fig. 6.19). Positive control probes for the housekeeping gene *ubiquitin C* and negative control probes for the bacterial gene *DapB* are used. Results from HPV E6/E7 mRNA ISH were found to be highly concordant with p16 immunohistochemistry and RT-qPCR [176, 177]. RNA ISH is not a quantitative assay. However, it confirms the presence of transcriptionally active virus directly in tissue sections.

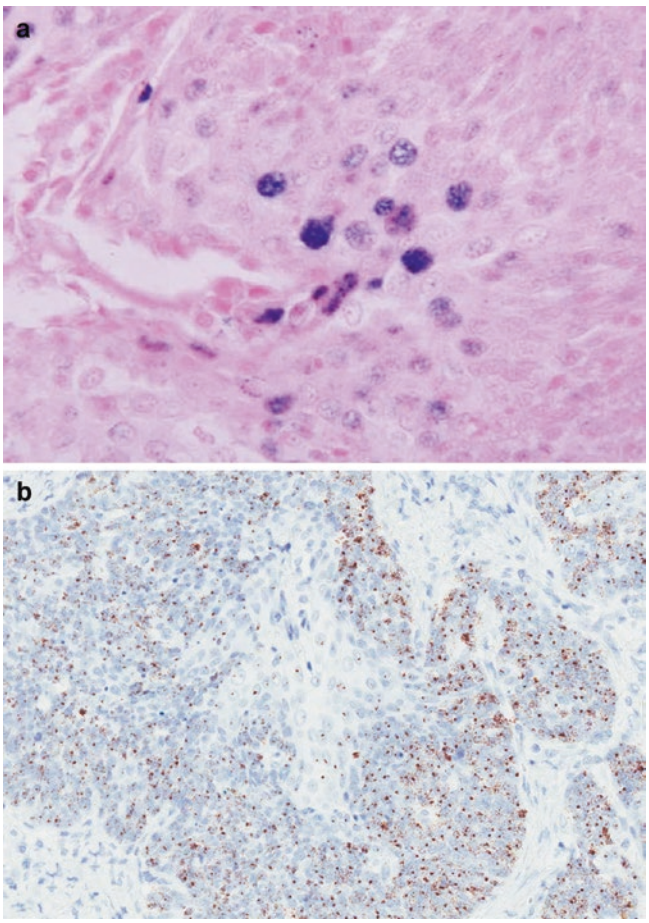

Fig. 6.19 In situ methods for HPV detection commonly used in head and neck squamous cell carcinoma. (**a**) Positive diffuse nuclear staining using HPV DNA in situ hybridisation. (**b**) Positive staining for E6/E7 mRNA in a squamous cell carcinoma of the tonsil

Indirect Identification of Transcriptionally Active Virus Using the Surrogate Marker CDKN2A (p16) Immunohistochemistry Immunohistochemistry of p16 overexpression is a widely used method for the identification of HPV-related carcinomas in head and neck clinical samples. It is considered as a robust surrogate marker for transcriptionally active and biologically significant HPV (see above). The technique is simple and inexpensive and can be used on FFPE archival material. p16 monoclonal antibodies are commercially available. The utility of this method is of par-

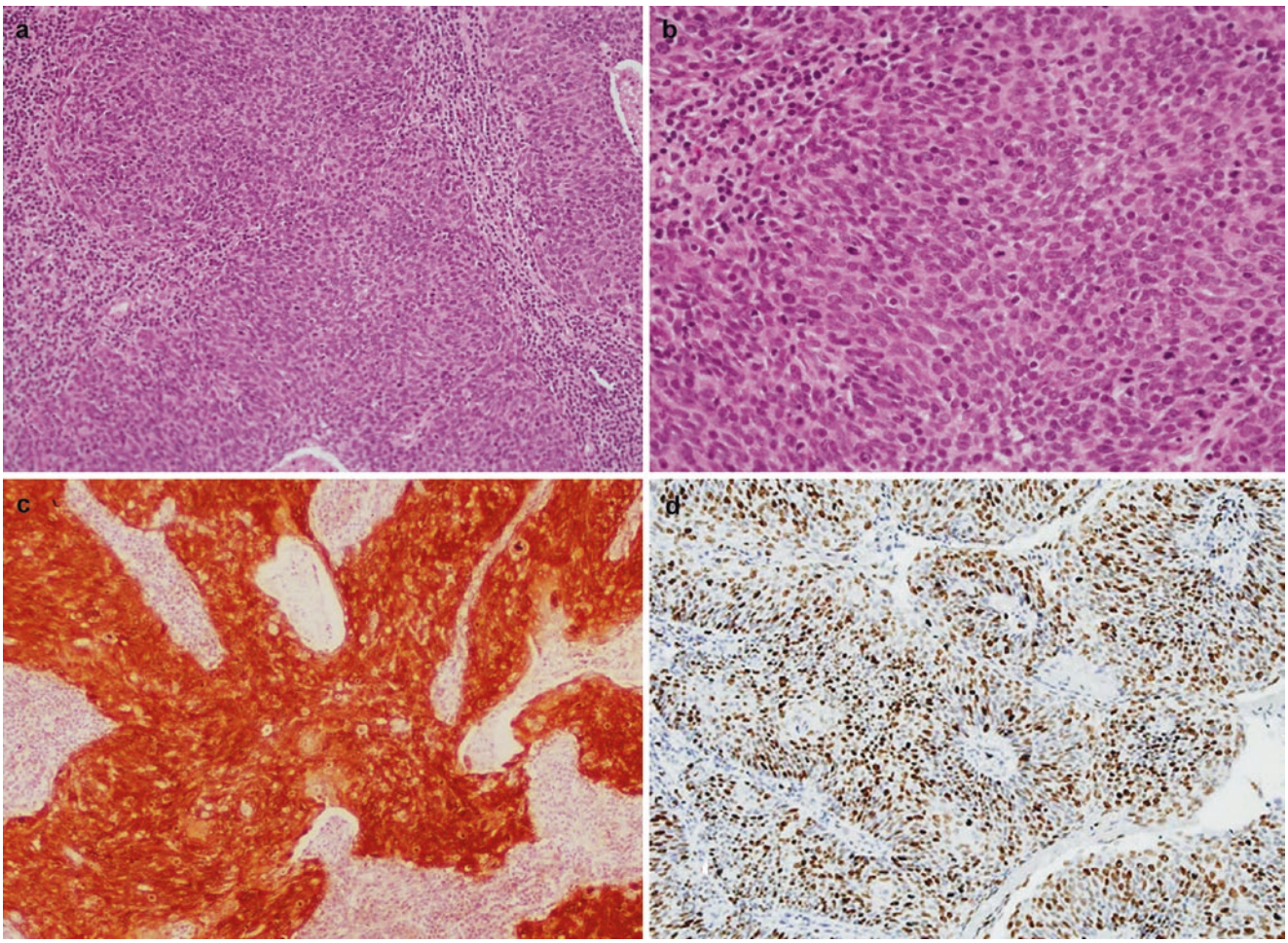

Fig. 6.20 Non-keratinizing HPV-related squamous cell carcinoma of the oropharynx. The tumor infiltrates the lymphoid tissue of the tonsil with nests and trabeculae (**a**). There is no evidence of keratinization and neoplastic cells show a high nuclear-to-cytoplasmic ratio. Mitotic activity is brisk (**b**). HPV-related squamous cell carcinomas show strong and diffuse nuclear and cytoplasmic staining for p16 (**c**) and high Ki-67 labelling index (**d**)

ticular significance in oropharyngeal squamous cell carcinoma where positivity approaches 100 % in HPV-related non-keratinizing carcinomas [167, 178]. In contrast, p16 is usually consistently inactivated in HPV-negative head and neck cancers, leading to minimal, if any, detectable protein by immunohistochemistry. However, variations in sensitivity of p16 immunohistochemistry were illustrated in some studies. Lewis et al. [179] reported that 13.9 % of p16-positive oropharyngeal squamous cell carcinomas were HPV negative when tested by ISH and PCR. Yet another study [180] showed that less than 2 % of p16-positive tumors were HPV negative. These differences may represent differences in sensitivity of HPV identification methods or may be related to true differences in tumor types. Indeed, lack of concordance between p16 overexpression and HPV positivity is not unusual in non-oropharyngeal carcinomas of the head and neck such as those of the larynx and the oral cavity [181–183].

The majority of oropharyngeal carcinomas show either strong and diffuse reactivity for p16 (Fig. 6.20) or they are totally negative. A minority of cases, however, show partial staining, which could be difficult to interpret. In a recent study, the pattern and extent of p16 partial staining were correlated to the presence of HPV, as determined by ISH and RT-PCR, in a number of oropharyngeal squamous cell carcinomas [184]. It was determined that greater than 75 % staining associated with confluent pattern of reactivity correlated with HPV positivity.

Clinical aspects Patients are typically adults, with a peak between the fifth and seventh decades, and there is a significant predominance of male subjects [62]. HPV-positive tumors are more prone to present with neck node metastases, which are often cystic, and with an unknown primary site [185, 186]. These tumors may indeed grow undetected in the crypts of the palatine and lingual tonsils for long time.

Moreover, these anatomic sites are difficult to access for clinical examination, often causing a delay in the diagnosis [186]. Larger tumors may present with tonsillar asymmetry and cause dysphagia, odynophagia and otalgia. While patients with non-HPV-related carcinoma may be affected by multiple tumors, either synchronous or metachronous, related to a field effect, this is not a feature of HPV-related carcinoma.

Macroscopy Large tumors appear as ulcerated or cystic masses, while less frequently the lesion has an exophytic appearance. Tumors of the tonsil and of the base of the tongue may be small and difficult to identify. Neck lymph node metastases from HPV-related carcinomas characteristically have a cystic appearance.

Histology The histological appearance of conventional keratinizing squamous cell carcinoma does not differ from that of other head and neck sites (see Chap. 1). HPV-related squamous cell carcinoma has a distinctive morphology, characterised by a non-keratinizing pattern [167, 187, 188]. Neoplastic cells have a basaloid or spindled appearance with indistinct borders and high nuclear-to-cytoplasmic ratio (Fig. 6.20). They form sheets, nests and cords, often with central comedo-type necrosis, without intervening desmoplasia. Mitotic activity is brisk. Hybrid lesions, showing both areas of keratinizing and non-keratinizing carcinoma, can also be observed, and these tend to be less frequently associated with HPV.

Variants of squamous cell carcinoma that have been reported to occur in the oropharynx, and are often associated with HPV infection, include basaloid squamous cell carcinoma [189, 190], undifferentiated carcinoma (lymphoepithelioma) [191, 192], adenosquamous carcinoma [193] and papillary carcinoma [194–196]. Their histological features are described in detail in Chap. 1.

Immunohistochemistry The immunohistochemical profile of HPV and non-HPV-related carcinoma differs significantly.

The non-keratinizing HPV-related squamous cell carcinomas show strong and diffuse nuclear and cytoplasmic staining for p16 (Fig. 6.20), absent or low p53 expression and high Ki-67 labelling index (Fig. 6.20) in comparison with HPV-negative carcinomas. There are no differences in the expression of other markers, including p63 and cytokeratins.

Genetics HPV-positive carcinomas show a distinct molecular profile, characterised by low frequency of *TP53* mutations, reduced *p14ARF-p15INK4b* deletion and lack of *EGFR*-activating mutations, *EGFR* amplification and *cyclin D1* overexpression in comparison with HPV-negative tumors [197].

Differential diagnosis The main differential diagnosis of non-keratinizing HPV-related squamous cell carcinoma includes small cell carcinoma, adenoid cystic carcinoma and basaloid squamous cell carcinoma [198]. Small cell carcinoma is rare in the oropharynx and is associated with both human papillomavirus (HPV) infection and tobacco exposure [199]. These tumors are positive for synaptophysin and/or chromogranin, while p63 is negative [199]. Adenoid cystic carcinoma differs from non-keratinizing HPV-positive carcinoma because the neoplastic population is more uniform and shows at least focal immunoreactivity for myoepithelial markers; moreover, p63 is diffusely positive in non-keratinizing carcinoma and only focally in adenoid cystic carcinoma, while cytokeratin 7 is expressed only by adenoid cystic carcinoma. The distinction from basaloid squamous cell carcinoma is difficult, if not impossible on morphologic basis in small biopsies. They are both positive for the same cytokeratin classes and for p63. For practical purposes, basaloid squamous cell carcinoma is not associated with HPV and is negative for p16.

Treatment and prognosis Several retrospective and prospective studies have now shown that patients with HPV-associated oropharyngeal squamous cell carcinoma of the tonsil and base of the tongue have better outcomes than patients with non-HPV-related carcinomas, both in terms of overall survival and progression-free survival, even though they tend to present in more advanced stage [200–204].

The 5-year survival rate for HPV-positive oropharyngeal squamous cell carcinoma ranges between 60 % and 90 %, while that of HPV-negative tumors ranges between 30 and 60 % [157]. As shown by multivariate analysis, this prognostic advantage is intrinsic to the tumor type and only partially related to the lower tobacco exposure, younger age and fewer comorbities of the population affected. A risk stratification of patients based on tumor HPV status, history of tobacco use, tumor stage and nodal stage has been proposed [205]. Regarding treatment, HPV-related tumors have a better response to induction chemotherapy and concomitant chemoradiotherapy in comparison with HPV-negative tumors [204] and are therefore more often eligible for organ preservation treatments.

Regarding squamous cell carcinoma at other locations in the oropharynx, including the uvula, the soft palate, the faucial pillar and the lateral and posterior walls, the 5-year survival rate is around 65 % [206]. About half of the patients present with advanced stage tumors, and 30 % show nodal metastases. The survival rate is reduced in the presence of higher stage, lymph node metastases and tumor extension to the tongue base and in midline tumors that extend across the palatine arch [206]. Squamous cell carcinoma of the uvula appears to have a higher rate of nodal metastases, even in small lesions [207]. However, the prognosis does not seem to differ significantly from that of tumors arising at other oropharyngeal sites [207, 208]. This apparent discrepancy could be explained by early diagnosis and definitive surgical treatment in uvular carcinomas [207].

6.7.2 Adenocarcinomas of the Oropharynx

Definition Malignant epithelial glandular neoplasms primarily involving the oropharynx mainly belong to the group of salivary gland tumors. More recently, HPV 16-positive adenocarcinomas arising in the base of the tongue have been reported [209, 210].

Epidemiology Oropharyngeal adenocarcinomas are rare tumors, which occur in adult patients (median age in the fifth decade of life), with no significant gender predilection. A history of cigarette smoking was evidenced in more than half patients affected by minor salivary gland tumors [211]. HPV-related adenocarcinomas of the base of the tongue occurred in adult male subjects with no significant history of cigarette smoking and alcohol drinking [209, 210].

Clinical data Presenting symptoms included progressive dysphagia, odynophagia and hemoptysis. For salivary gland-type neoplasms, the most common anatomic subsite was the base of the tongue (60 %), followed by the soft palate (30 %) and tonsils (10 %); cases with bilateral localisation in the tonsils have been reported [212].

Histology The most common salivary-type adenocarcinomas of the oropharynx are mucoepidermoid carcinoma and adenoid cystic carcinoma; less frequent subtypes are adenocarcinoma NOS, malignant mixed tumor, polymorphous low-grade adenocarcinoma [211, 213, 214], cribriform adenocarcinoma [215] and hyalinizing clear cell adenocarcinoma [216–218]. For a detailed description of their histological features, see Chap. 5.

HPV-associated adenocarcinomas of the base of the tongue show variable growth patterns, including cribriform, glandular, solid and papillary areas [209, 210]. A minor component of squamous cell carcinoma was evidenced in one case [210]. Neoplastic cells are cuboidal to columnar and show amphophilic to pale eosinophilic cytoplasm. Numerous mitotic figures and areas of necrosis are present.

Finally, a few examples of intestinal-type adenocarcinoma of the tongue have been reported [219, 220]. Their histological appearance is identical to that of colonic adenocarcinomas, including the presence of a mucinous component.

Immunohistochemistry The immunoprofile of salivary-type adenocarcinomas is described in detail in Chap. 5. HPV-related adenocarcinomas are positive for cytokeratin 7 and p16, while cytokeratin 20, CDX2 and myoepithelial markers are negative [209, 210]. Intestinal-type adenocarcinoma shows cytoplasmic immunoreactivity for cytokeratin 20 and nuclear immunoreactivity for CDX-2 [219].

Genetics HPV type 16 can be detected by PCR and localised in tumor cells by in situ hybridisation in HPV-related adenocarcinomas [209, 210].

Differential diagnosis The differential diagnosis of oropharyngeal adenocarcinomas includes metastases from other sites (see below). HPV-related adenocarcinomas lack light microscopic and immunophenotypic features of a specific "salivary gland-type" carcinoma and are positive for p16 and HPV by different methods.

Treatment and prognosis Treatment includes surgery and adjuvant postoperative radiotherapy [211]. Significant data on outcome are available only for salivary-type adenocarcinomas. Overall survival at 5 and 10 years were 80 % and 53 %, respectively, in a large series. Approximately one third of patients developed local or distant recurrences [211]. In multivariate analysis, clinical T stage, anatomic subsite (base of the tongue had the worst clinical behaviour) and margin status were independent predictors for overall survival [211]. Ten-year survival was significantly worse for patients affected by adenoid cystic carcinoma [211]. No significant difference in survival rates has been observed between oropharyngeal salivary gland carcinomas and squamous cell carcinoma [221].

6.7.3 Malignant Non-epithelial Tumors of the Oropharynx

6.7.3.1 Lymphomas of the Waldeyer's Ring

This section gives a brief introduction on the general features of lymphomas of the Waldeyer's ring. For a detailed description of these entities, see Chap. 13.

Epidemiology The Waldeyer's ring is the most commonly affected site by extranodal lymphomas [222]. Overall, Waldeyer's ring lymphomas represent 5–15 % of all extranodal non-Hodgkin lymphomas and 60–70 % of all extranodal lymphomas of the head and neck region. A higher incidence has been reported in Asian populations [223]. Patients are predominantly adults, and there is a slight male predominance.

Clinical data The tonsil is the most common site of involvement (60–80 % of all Waldeyer's ring lymphomas) [224]. The most common presenting symptoms are dysphagia, respiratory obstruction or clinical manifestations related to Eustachian tube obstruction. While squamous cell carcinoma presents most frequently as an ulcerative lesion, lymphomas appear as painless masses covered by healthy looking or slightly hyperemic mucosa. Systemic symptoms are reported in less than 10–20 % of patients [225, 226].

Histology The most common histological subtype is diffuse large B-cell lymphoma, but other histotypes have been documented, including marginal zone lymphoma, mantle cell lymphoma and T-cell lymphomas, such as T-cell lymphoblastic lymphoma and NK/T-cell lymphoma [227]. Moreover, although exceptionally rare, Hodgkin's lymphoma may also primarily affect the Waldeyer's ring [228].

Differential diagnosis The main differential diagnoses include benign reactive and infectious processes (infectious mononucleosis) on one side and undifferentiated high-grade malignancies, such as carcinomas and melanoma, on the other.

Treatment and prognosis Stage, histological subtype and use of combined modality treatments are relevant prognostic factors. Preferred treatment regimens include combined chemotherapy and radiotherapy [229].

6.7.3.2 Sarcomas of the Oropharynx

The clinico-pathological features of sarcomas are discussed in detail in Chap. 12.

Epidemiology Sarcomas involving primarily the oropharynx are exceedingly rare tumors.

Histology The most common histological subtypes are rhabdomyosarcoma [230], Kaposi sarcoma [231], synovial sarcoma [232, 233], leiomyosarcoma [234, 235] and follicular dendritic cell sarcoma [236].

6.7.4 Metastatic Tumors of the Oropharynx

The oropharynx is a very unusual site for metastatic spread from other organs. In a review of approximately 2000 palatine tonsillar malignancy from the Armed Forces Institute of Pathology, less than 1 % represented metastatic lesions [237].

The most common primary site is the lung, followed by the liver and the gastrointestinal tract, the kidney, the prostate and the breast [238–240]. Among non-epithelial malignancies, metastases of cutaneous melanoma have been repeatedly reported in the tonsil [241–243].

6.8 Systemic Disease Affecting the Oropharynx

6.8.1 Tangier Disease

Definition Tangier disease is an autosomal recessive disorder of lipid metabolism, characterised by absence of plasma high-density lipoprotein (HDL) and deposition of cholesteryl esters in the reticuloendothelial system [244].

Etiology and pathogenesis The disease is caused by mutations in the *ATP-binding cassette transporter A1* (*ABCA1*) gene, which encodes the membrane transporter ABCA1 [244]. This molecule plays a key role in the first step of reverse cholesterol transport, through which the efflux of free cholesterol from peripheral cells is transferred to lipid-poor apoA-I. The deranged fat metabolism results in storage of cholesterol esters in the reticuloendothelial system and macrophages of the pharyngeal and gastrointestinal tract mucosa but also in smooth muscle cells, pericytes and Schwann cells of peripheral nerves.

Epidemiology To date there are approximately one hundred patients diagnosed with the disease. The index cases of the disease were recognised in the Tangier Island, Chesapeake Bay, Virginia.

Clinical data Clinically, most patients are asymptomatic. Children with Tangier disease have enlarged tonsils with a characteristic yellow colour.

Histology Histologically, there is an infiltrate of macrophages with foamy cytoplasm in the tonsillar tissue.

Differential diagnosis The differential diagnosis includes other lipid storage diseases, which can be separated on the basis of different clinical presentation. Focal collections of foamy macrophages may also be the result of non-specific chronic inflammation of the tonsils.

Treatment and prognosis To date there is no specific treatment for Tangier disease.

6.8.2 Amyloidosis

Definition Amyloidosis is a disease characterised by the presence of amorphous extracellular eosinophilic deposits of protein fibrils that reveal an apple-green birefringence under polarised light after staining with Congo red. It may present with localised or systemic involvement and can either be primary or secondary to infectious processes, chronic inflammatory diseases, neoplasia or myeloma.

Epidemiology In the upper respiratory tract, the most commonly affected site is the larynx. Involvement of the oropharynx and nasopharynx by localised amyloidosis is rare. Patients are adult, with a slight male predominance. Paediatric cases are extremely rare [245].

Clinical aspects Patients may be asymptomatic or may present with foreign body sensation in the throat, dysphagia and sore throat.

Histology Microscopically, there are homogeneous, amorphous, eosinophilic deposits within the tonsillar structures. This material is positive for Congo red staining and gives an apple-green birefringence at the polarising light examination. Foreign body giant cell reaction and plasma cells infiltration may be present around the amyloid deposits. Areas of bone metaplasia have been documented [246].

Treatment and prognosis The treatment for localised amyloidosis is surgical, and the prognosis is very good. However, in patients with localised amyloidosis, it is mandatory to rule out the presence of systemic involvement.

6.8.3 Sarcoidosis

Definition Sarcoidosis is a chronic granulomatous disease of unknown etiology, which may occur as systemic or isolated involvement.

Epidemiology It generally affects young patients or middle-aged adults. Involvement of the naso- and oropharynx by systemic sarcoidosis is a well documented, but very unusual in the absence of systemic disease [247, 248].

Clinical aspects Localised oropharyngeal disease presents with symptoms of chronic tonsillitis and/or with foreign body sensation. Patients may also be asymptomatic and the disease detected on routine histological examination [248].

Histology Histologically, there are multiple non-caseating granulomas in the interfollicular areas, composed of densely packed epithelioid histiocytes.

Differential diagnosis The differential diagnosis includes other tonsillar diseases which may present histologically with granulomatous inflammation. These include mycobacterial infections, toxoplasmosis, neoplasia (squamous cell carcinoma and Hodgkin's lymphoma) and Crohn's disease [146]. A specific cause for tonsillar granulomatous inflammation is not always recognisable, thus representing a non-specific finding possibly related to chronic recurrent tonsillitis.

Treatment and prognosis Corticosteroid therapy is the elective treatment of symptomatic sarcoidosis.

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Larynx and Hypopharynx

7

Nina Zidar, Nina Gale, Antonio Cardesa, and Luis Ortega

Contents

N. Zidar (✉) • N. Gale
Faculty of Medicine, Institute of Pathology,
University of Ljubljana, Korytkova 2, Ljubljana 1000, Slovenia
e-mail: nina.zidar@mf.uni-lj.si; nina.gale@mf.uni-lj.si

A. Cardesa
Department of Anatomic Pathology, Hospital Clinic,
University of Barcelona, Villarroel 170, Barcelona 8036, Spain
e-mail: acardesa@clinic.ub.es

L. Ortega, PhD
Department of Pathology, Hospital Clínico San Carlos,
C/Martín Lagos sn, Madrid 28040, Spain
e-mail: lortega.hcsc@salud.madrid.org;
luis.ortega@salud.madrid.org

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7.1 Introduction: Anatomy, Histology and Embryology of the Larynx and Hypopharynx

The larynx and hypopharynx are constituent parts of the upper aerodigestive tract, intimately linked with their connective tissue elements and different epithelia. The anatomy and histology of both organs are very complex and details are available in various standard textbooks and specialised papers [1–5]. Only essential data will be given here.

The larynx is a hollow tube, which communicates cranially with the hypopharynx. Its upper limits are the free edge of the epiglottis and the two aryepiglottic folds. The lower laryngeal part continues caudally with the trachea and its inferior limit is the lower edge of the cricoid cartilage. The anterior border consists of the lingual surface of the epiglottis, thyrohyoid membrane, thyroid cartilage and the anterior arch of the cricoid cartilage. Posteriorly, the cricoid cartilage and arytenoids' area limit the larynx.

The larynx is divided into supraglottic, glottic and subglottic regions, which have particular significance for the biological behaviour and staging of tumors. The supraglottic region extends from the tip of the epiglottis down to the superior edge of the true vocal cord and includes the epiglottis, aryepiglottic folds, arytenoids, the false vocal cords and ventricles. The glottis includes the vocal cords with anterior and posterior commissures. The subglottis extends below the true vocal cords to the lower border of the cricoid cartilage.

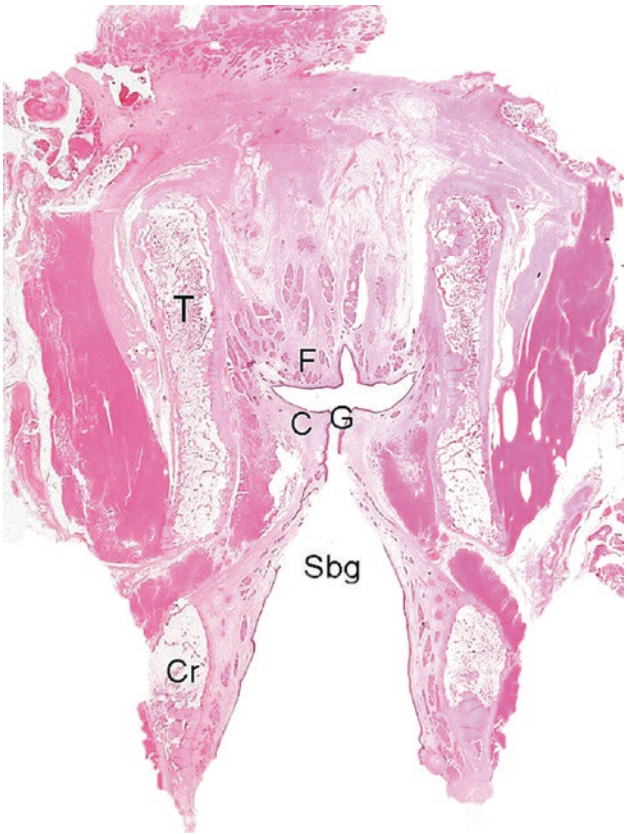
Our own experience suggests that marked variations in the distribution of different types of laryngeal epithelia related to age seem to be the rule [4]. The lingual and, variably, laryngeal side of the epiglottis and the true vocal cords are covered by a non-keratinizing stratified squamous cell epithelium; the rest of the larynx is lined by a respiratory epithelium. The seromucinous glands are abundant in all compartments of the larynx except in the vocal cords, where they are essentially missing on their free edges and are sparse in the rest of the cords.

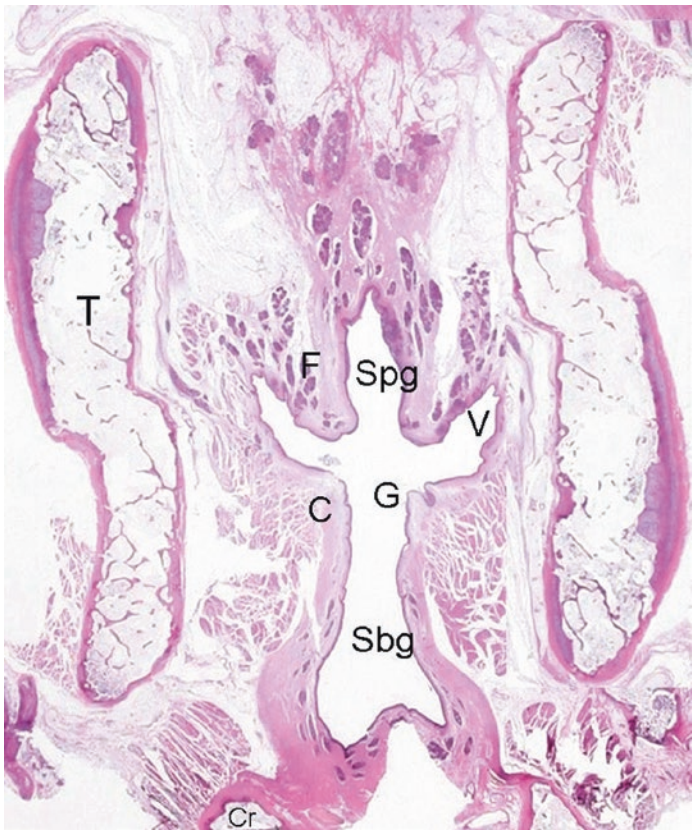
It is important to highlight some particularities in the laryngeal structure that considerably influence the spread of malignant tumors. The elastic cartilage of the epiglottis, with numerous fenestrations for vessels, nerves and glands, provides a "locus minoris resistentiae" for the progress of malignant growth from the laryngeal to the lingual side or vice versa. In the anterior commissure, where the true vocal cords meet in the anterior midline, a band of fibrous tissue (protrusions of the two vocal ligaments) with lymphatic and blood vessels is attached to the thyroid cartilage. There is no perichondrium at this point, which certainly facilitates the ingrowth of malignant tumors in the thyroid cartilage. The paraglottic space extends on each side of the glottis and is bounded laterally by the perichondrium of the thyroid cartilage and the cricothyroid membrane and posteriorly by the mucous membrane of the pyriform sinus; anterosuperiorly it extends into the preepiglottic space. It is an important route of transglottic and extralaryngeal spread of the laryngeal malignant tumors.

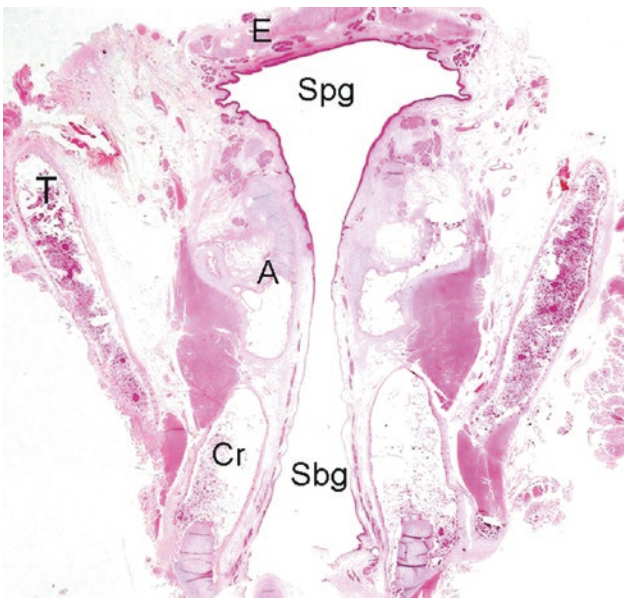
The network of capillaries is poorly developed in Reinke's space of the vocal cords and lymphatics are lacking. These specificities contribute to the development of various exudative lesions of the vocal cords and delayed metastases of glottic cancers (Figs. 7.1, 7.2, 7.3, and 7.4).

Embryologically, the supraglottic part of the larynx arises from the third and the fourth branchial arches, while the glottic and subglottic portions are derived from the sixth arch. The first appearance of the respiratory tract occurs at approximately 21 days during embryogenesis as an evagination or a vertical groove of the cephalic portion of the foregut. This evagination is the precursor of the epiglottis, the earliest portion of the larynx. Its outlines appear at the 6 mm foetal stage by 30 days. The respiratory groove begins to close and with the formation of the arytenoids, the closure becomes complete [1]. The covering epithelium of the groove appears in the 3–5 mm embryo as three lines of polyhedral embryonic cells of endodermal origin. In a 30 mm foetus, by 60–70 days, the thickness of the embryonic stratified squamous epithelium increases and the vocal cords begin to differentiate. A ciliated epithelium occurs in a 40 mm embryo on the epiglottis and laryngeal vestibule. A sharp distinction between the two epithelia appears after the embryo reaches a length of 95 mm. The larynx of a newborn is covered by a ciliated epithelium, except on the true vocal cords. In addition to this location, a stratified squamous epithelium is also present in the interarytenoid area and on the tip of the epiglottis.

The hypopharynx is the caudal part of the pharynx, with the wide part superiorly, extending from the tip of the epiglottis to the inferior level of the cricoid cartilage, where it becomes narrow and continuously proceeds to the oesophagus. The hypopharynx is divided into three compartments: left and right pyriform sinuses, postcricoidal region and posterior pharyngeal walls. The pyriform sinuses are limited medially by the aryepiglottic folds and laterally by the







Figs. 7.1, 7.2, and 7.3 Normal microscopical anatomy of the larynx. Coronal sections through the anterior third, midportion, and posterior third of the vocal cords, respectively. The thyroid (T), cricoid (Cr) and arythenoid (A) cartilages construct the cartilaginous framework of the organ. The larynx is divided into supraglottic (Spg), glottic (G) and subglottic (Sbg) compartments. The true (C) and false (F) vocal cords and ventricles are functionally important laryngeal components

thyroid cartilage. The postcricoid area is the posterior side of the cricoid cartilage. The posterior wall is situated in front of the cervical spine. The entire hypopharynx is covered by a stratified squamous cell epithelium.

Embryologically, the pharyngeal gut or pharynx extends from the buccopharyngeal membrane to the tracheobronchial diverticulum. The hypopharynx is almost entirely of endodermal origin. In the eighth through the tenth gestation

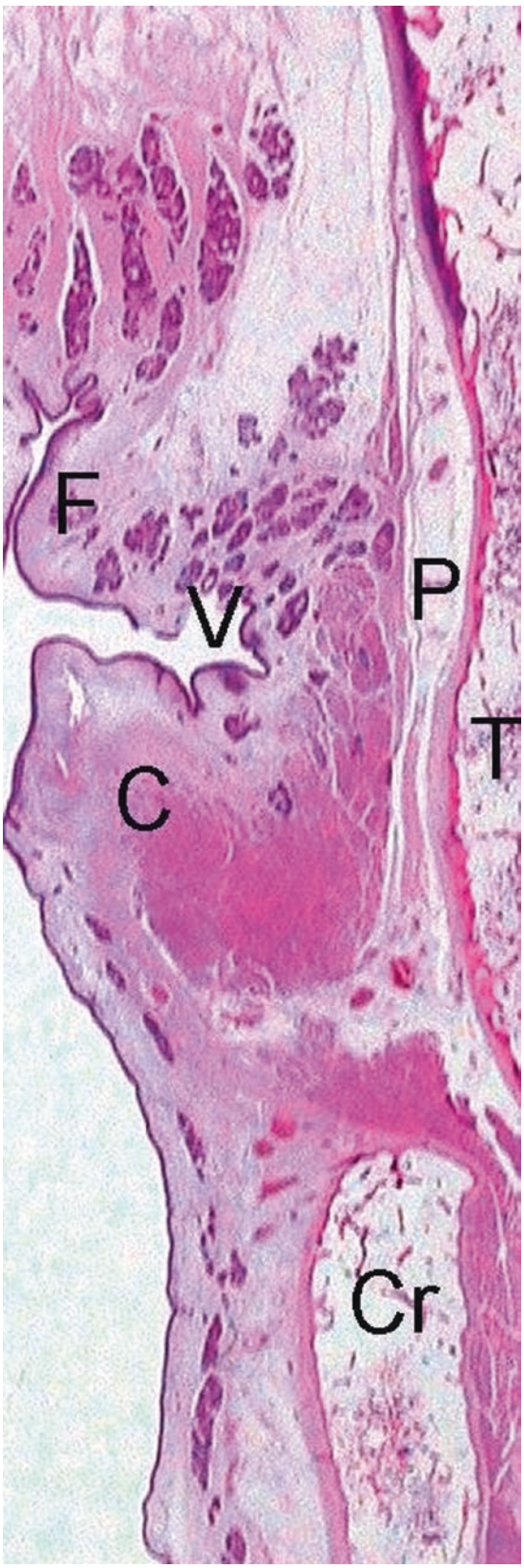


Fig. 7.4 Normal microscopical anatomy of the larynx. Coronal section. Detail of the hemilarynx with the cartilages, thyroid (T) and cricoid (Cr), with the paraglottic space (P), ventricle (V), true (C) and false (F) vocal cords

week, the pharynx, as well as the hypopharynx, is fairly small but after the tenth week of gestation, remarkable growth occurs in this region [6].

7.2 Laryngomalacia

Definition and etiology Laryngomalacia (LM) is the commonest congenital laryngeal disease and cause of stridor in newborns. Aetiologically, LM may be related to neuromuscular alteration resulting in supraglottic collapse during inspiration, consequently causing airway obstruction. It can be associated with various comorbidities, such as gastro-oesophageal reflux disease, neurologic diseases or congenital heart diseases [7, 8].

Epidemiology and clinical aspects Newborns are mainly affected. Inspiratory stridor is the leading symptom, which worsens with feeding, crying and agitation [7, 8]. LM typically presents with supra-arytenoid tissue prolapse during inspiration, omega-shaped epiglottis, retroflexed epiglottis, short aryepiglottic folds and oedematous mucosa of the posterior larynx.

Microscopy Oedematous subepithelial stroma with myxoid degeneration, lymphatic dilatation and focal lymphocytic infiltrates are the usual histological findings.

Treatment and prognosis LM is a benign disease that resolves spontaneously in 70 % of infants by 1 year of age. From 4 to 31 % of patients require surgical intervention (supraglottoplasty and tracheostomy), according to various studies, due to failure to thrive, cyanosis, cor pulmonale or apnoea [7–9].

7.3 Laryngocele and Laryngeal Cysts

A laryngocele is a rare congenital or acquired laryngeal lesion that appears as a pouching of the laryngeal ventricle and saccule and is normally filled with air [10]. Laryngeal cysts (LCs) account for approximately 5 % of benign laryngeal lesions [4]. They can be congenital or acquired, solitary or multiple; laryngeal cysts have various origins and, consequently, specific sites of appearance [11, 12]. In terms of their origin, LCs are divided into ductal and saccular cysts; ductal cysts are additionally subdivided with regard to the lining epithelium and predominant subepithelial tissue into oncocytic and tonsillar cysts.

7.3.1 Laryngocele

Definition A laryngocele (L) is defined as an excessive elongation and dilatation of the air-filled laryngeal ventricle and saccule (ventricular appendix), which communicate directly with the laryngeal lumen.

Epidemiology The location of the L determines its type: internal, external or mixed. An internal L extends in a superior-posterior direction towards the area of the false vocal cord and aryepiglottic fold. An external L expands cranially and laterally to the neck through the weak zone of the thyrohyoid membrane. It presents as a lateral neck mass that varies in size depending on variations in intralaryngeal pressure. A mixed or combined form has both internal and external components, with a swelling of the neck and endolaryngeal bulging [13, 14]. About half of Ls are of the mixed type. They are usually unilateral; only 15 % are bilateral [15]. L is most frequently observed in infants and adults between 50 and 60 years. A male predominance is evident, with a ratio of 7:1 [10, 16].

Etiology and clinical aspects The lesion occurs in persons with a congenital large saccule and weakness of the periventricular soft tissue. In adults, various conditions involving a repeated increase of intralaryngeal pressure, such as professional glass-blowers, wind instrument musicians, singers, professional speakers and patients with a chronic cough, have been reported [16]. Stenosis of the saccule neck, giving rise to valve system behaviour, may also lead to the occurrence of a laryngocele. Symptoms vary in terms of the location and size of the L. Internal and mixed Ls present with a foreign body sensation, cough, dyspnoea and hoarseness. An external L presents as a soft, reducible neck swelling, changing in size in relation to an increase or decrease in intralaryngeal pressure. The lesion may, however, also be asymptomatic. The diagnosis is established on the basis of history and physical and radiological examination, especially computed tomography (CT) and magnetic resonance imaging (MRI).

Microscopy Histologically, a cystic extension of the saccule is evident, the wall of which tends to lose its folded surface. A laryngocele is covered by respiratory epithelium; an oncocytic or cuboidal metaplasia is occasionally present. Focally, chronic mononuclear inflammatory cells are seen in the subepithelial stroma.

Treatment and prognosis Treatment of Ls depends on disease size and location. Small asymptomatic Ls are only observed. Endoscopic laser resection is the treatment of choice for small symptomatic internal lesions; larger and external ones are surgically treated by an external approach [10]. L-related complications include upper airway obstruction, infection (laryngopyocele) [14], aspiration and subsequent pneumonia [10]. This alarming clinical state demands fast diagnosis and urgent surgery. L may be an associated lesion in patients with laryngeal amyloidosis or carcinoma. Literature data report that between 4.9 % and 28.8 % of laryngeal carcinomas are associated with Ls [17].

7.3.2 Saccular Cyst

Definition A saccular cyst (SC) is a mucous-filled dilatation of the laryngeal saccule that has no communication with the laryngeal lumen [18–20].

Epidemiology, etiology and clinical aspects Most SCs are congenital in origin, some may also appear as acquired lesions caused by various inflammatory processes, traumatic events or tumors [20, 21]. SCs, which may occur at any age, are divided into anterior and lateral. The former are located between the true vocal cords and ventricular folds while a lateral SC is evident between the ventricular and aryepiglottic folds. Lateral SCs are generally larger and extend towards the false vocal cord and aryepiglottic fold (Fig. 7.5). They may rarely spread through the thyrohyoid membrane [12, 20, 22]. SCs may be asymptomatic, but the commonest symptoms are progressive cough, dysphagia, hoarseness, dyspnoea and foreign body sensation. Diagnosis is often made by laryngoscopy combined with CT scan.

Microscopy SCs are lined by a ciliated respiratory epithelium. An increased number of goblet cells may be present. Rarely, the cysts are partially or entirely lined by a metaplastic squamous or oncocytic epithelium. The subepithelial stroma, i.e. the cyst wall, usually contains focal lymphocytic infiltrates [4].

Treatment and prognosis Treatment is surgical; with current improved endoscopic techniques, most SCs can be treated endoscopically, often without the need for an external approach [18].

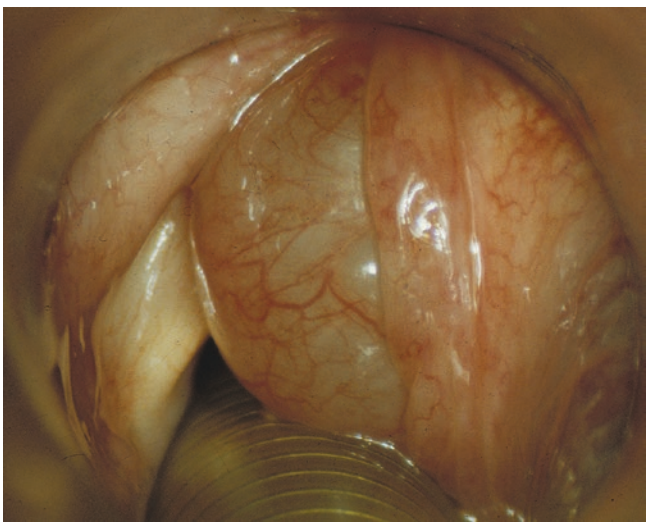


Fig. 7.5 Saccular cyst. Large cyst arises from the right saccule

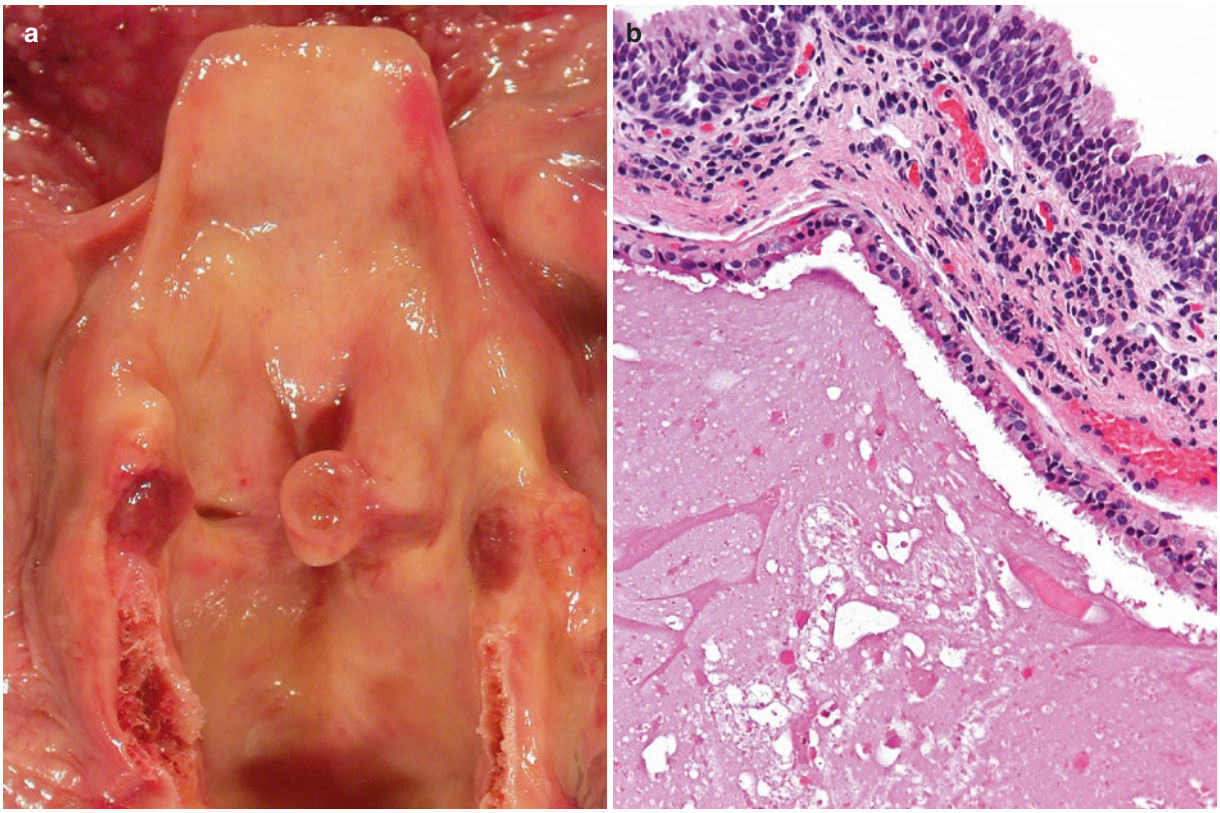


Fig. 7.6 (**a**) Ductal cyst arising from the right Morgagni sinus. Incidental finding at autopsy. (**b**) Ductal cyst of the false vocal cord. The cyst filled with mucin is lined by the ductal double layered epithelium

7.3.3 Ductal Cyst

Definition Ductal cysts (DCs) are the commonest laryngeal cysts and account for around 75 % of all laryngeal cystic lesions [23, 24]. The characteristic retention of mucus in the dilated collecting ducts of the intramucosal seromucinous glands can be found anywhere in the larynx [4].

Epidemiology and etiology DCs are found at any site of laryngeal mucosa in which glands are present but with a predilection to being close to the vocal cords, ventricles of Morgagni, ventricular folds and epiglottis. Free vocal cord margins are usually excluded because of the lack of small seromucinous glands at this location. DCs develop after occlusion of the seromucinous glandular ducts, caused mainly by chronic inflammation or any other obstructive factor, such as tumor or trauma. The origin of a so-called epidermoid cyst (EC) of the vocal cord is probably related to microtraumatic inclusion of small fragments of a squamous epithelium into the subepithelial tissue or to the remnants of the vocal cord sulcus.

Clinical aspects Symptoms and complaints of patients with LC depend on their location and size. They present most frequently with hoarseness, coughing, inspiratory stridor, foreign body sensation and respiratory disorders, but they may also be asymptomatic and identified as an incidental finding.

DCs are almost invariably less than 1 cm in diameter; only a ductal cyst of the pharyngeal side of the epiglottis may be an exception. These cysts are usually smaller than other laryngeal retention cysts, measuring 1–4 mm and not exceeding 10 mm in diameter [4].

Macroscopy Laryngoscopically, ductal cysts are seen as a sharply delineated spherical protrusion; the overlying mucosa is smooth and stretched (Fig. 7.6a). Larger cysts, mainly in newborns or in small children, can obstruct breathing.

Microscopy The microscopical picture of DCs is influenced by origin. DCs are covered by a double-layered cylindrical, cuboidal or flattened epithelium (Fig. 7.6b). As in SC, a partial or complete squamous or oncocytic metaplasia may also be seen. Microscopically, an EC is usually lined by atrophic keratinizing epithelium with intraluminal stratified basophilic keratin scales (Fig. 7.7).

Treatment The therapy of choice for DC is endoscopic removal [4].

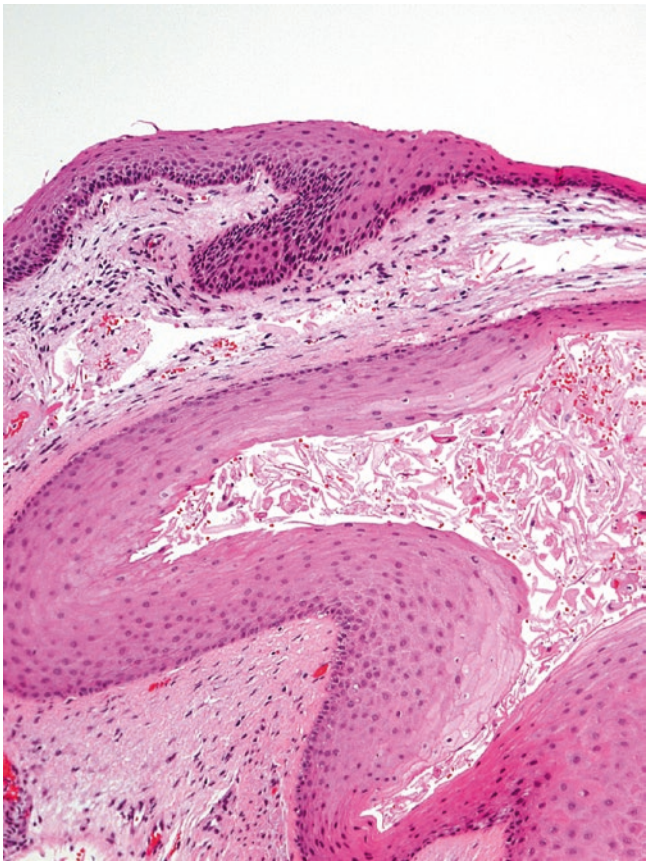


Fig. 7.7 Epidermoid cyst of the vocal cord. The cyst is lined by squamous epithelium and filled with keratin flakes

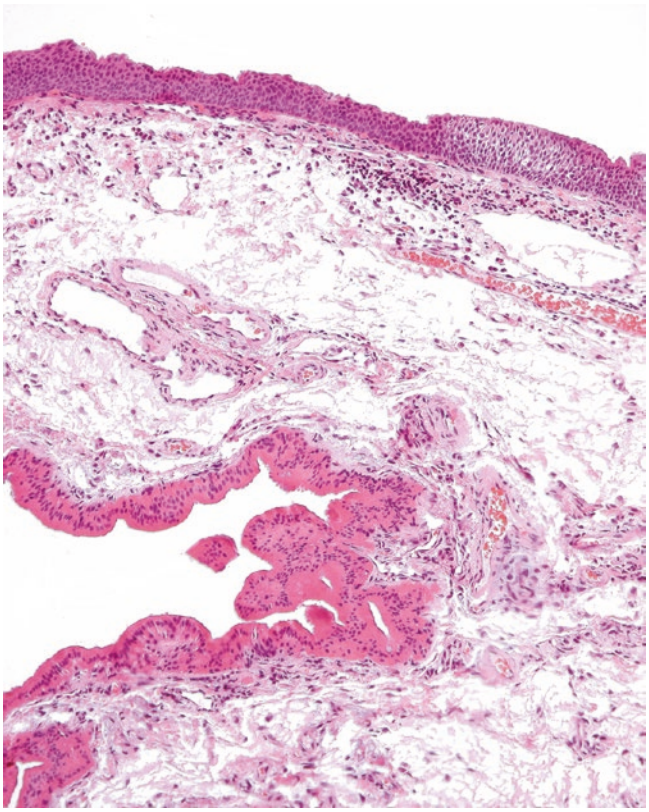


Fig. 7.8 Oncocytic cyst of the false vocal cord. The cyst is lined by metaplastic oncocytic epithelium; oncocytic cells have abundant granular eosinophilic cytoplasm and small dense, darkly stained nuclei

7.3.4 Oncocytic Cyst

Definition A laryngeal oncocytic cyst (OC) is a rare, slow-growing lesion, which develops from an oncocytic metaplasia of the ductal epithelium.

Epidemiology and etiology Oncocytic lesions usually present as supraglottic swellings, predominantly in older females, although some patients are also under 50 years of age. Oncocytic cells are thought to be a result of functional exhaustion and mitochondrial proliferation and may be a compensatory mechanism against mitochondrial injuries [25]. They occur on the false vocal cords and ventricles, with hoarseness or cough as the leading symptoms [26]. An oncocytic metaplasia frequently occurs in epithelial endocrine cells with high metabolic activity; however, it is also related to inflammation, degenerative processes and ageing. A whole spectrum of oncocytic laryngeal lesions has been observed, ranging from a focal to diffuse oncocytic metaplasia and papillary cystic hyperplastic lesions to benign tumors [27]. It has been suggested, however, that the whole spectrum of lesions is more likely to belong to non-neoplastic rather than to true neoplastic lesions. This opinion is supported by the various extent of oncocytic metaplasia in the laryngeal minor salivary glands, as well as by the occasional appearance of multiple cystic lesions [28–30].

Macroscopy OCs are often solitary; they may present as a polypoid sessile or pedunculated lesion or a submucosal swelling.

Microscopy Oncocytes are enlarged cells with a characteristic granular eosinophilic cytoplasm and small dense, darkly stained nuclei. Laryngeal OCs may show focal, inconspicuous or extensive proliferation of oncocytes, mainly with unilocular or multilocular cystic formations with papillary projections, resembling Warthin's tumor. The epithelium of an OC, which shows papillary proliferations or a varied degree of folding of the cystic wall, is typically double layered; the inner layer consists of columnar eosinophilic cells encircling the cystic lumina, while the outer layer is composed of small basal cells (Fig. 7.8).

Treatment Complete endoscopic surgical excision is the recommended treatment, if necessary, by laryngofissure.

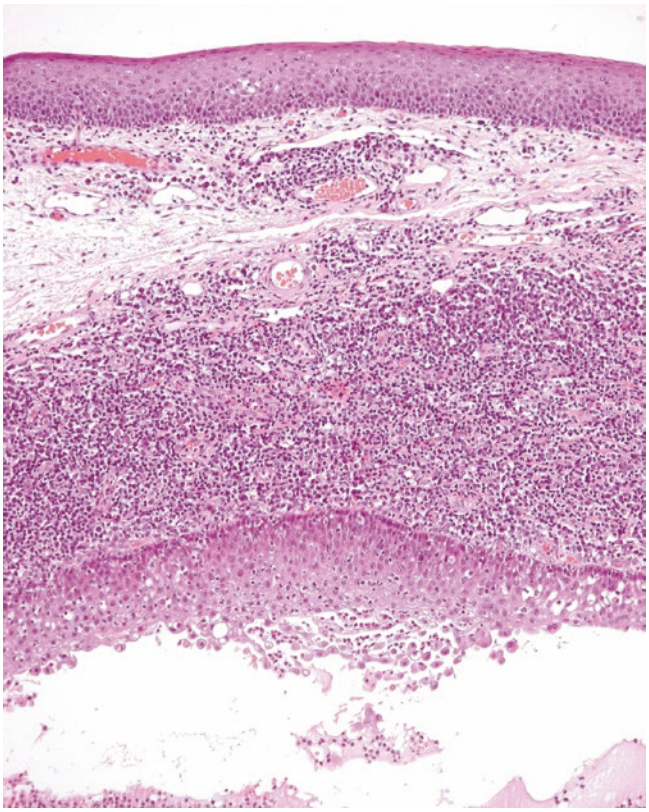


Fig. 7.9 Tonsillar cyst of the epiglottis. The cyst is lined by squamous epithelium; abundant lymphatic tissue is seen under the epithelium

7.3.5 Tonsillar Cyst

Definition Tonsillar cysts (TCs) occur almost exclusively in the epiglottis, vallecula or pyriform sinus; they may be single or multiple.

Microscopy TCs are usually small, lined with a squamous epithelium; the cystic space can be filled with keratin. A prominent amount of lymphatic tissue with germinal centres is seen under the epithelium, resembling a tonsillar crypt (Fig. 7.9).

Treatment Conservative endoscopic removal is recommended.

7.4 Zenker's Hypopharyngeal Diverticle

Definition An outpouching of the dorsal hypopharyngeal wall above the upper oesophageal sphincter is known as Zenker's diverticulum (ZD) [31].

Epidemiology and etiology The site of origin is between the thyropharyngeal and the more horizontal part of the cricopharyngeal muscles. The lesion, which usually occurs in elderly persons, is now widely accepted to be of acquired rather than congenital origin. The aetiological factors of ZD occurrence have not been explained, but an incomplete sphincter opening with an increase of hypopharyngeal pressure during swallowing has to be considered [32].

Clinical aspects The symptoms are virtually pathognomonic: dysphagia, regurgitation of undigested food, weight loss, foetor ex ore, coughing and repeated aspiration [33].

Microscopy ZD is composed of a squamous epithelium and thinned fibrous tissue of the subepithelial stroma, with possible inflammatory changes.

Treatment and prognosis Treatment options for ZD include open surgical, rigid endoscopic and flexible endoscopic therapy [31]. Exceedingly rarely, a squamous cell carcinoma may develop in ZD [33].

7.5 Aberrant Thyroid Tissue

Definition Ectopic thyroid tissue is a rare finding that results from development defects in the early stages of thyroid gland embryogenesis during its route from the foramen cecum to the final pretracheal position [34].

Epidemiology Aberrant thyroid tissue (ATT) may be found in the subglottic area of the larynx and upper trachea, especially between the lower border of the cricoid cartilage and the upper ring of the trachea. It has been reported that two-thirds of patients are middle-aged women from regions of endemic goitre. Intralaryngotracheal thyroid is a rare lesion, which has been divided into "false" and "true" aberrant thyroids. The former is likely to arise in the pre- or neonatal period, when the thyroid gland can grow into incompletely formed laryngotracheal cartilages that remain in continuity with the thyroid gland. The latter, the "true aberrant thyroid", develops during the foetal period as an isolated, misplaced thyroid tissue, when the thyroid gland is encroached upon and divided by the later-developing laryngeal and tracheal cartilages [35].

Clinical aspects The most common symptom of intralaryngeal ATT is slowly progressive dyspnoea, but it may also be asymptomatic. According to various reports, an intraluminal thyroid tissue appears as a broad-based, smooth, rounded mass protruding from the subglottic posterolateral wall [36].

Microscopy The thyroid follicles are usually small and regular, with a well-formed colloid lying close to the seromucinous glands in the laryngeal mucosa. The overlying mucosa

is commonly intact; there may be some evidence of chronic irritation. Finding thyroid tissue in the laryngotracheal wall raises the question as to whether or not it represents ectopic tissue appearing through a developmental defect or a well-differentiated carcinoma. The final decision must be based on an overall clinical and histological finding [35].

Treatment Management of ATT is often not clear-cut, but primarily surgery is proposed [34–36].

7.6 Tracheopathia Osteochondroplastica

Definition Tracheopathia osteochondroplastica (TO) is a rare, slowly progressing lesion, characterised by the presence of cartilaginous and bony submucosal nodules projecting into the lumen of the trachea, larynx and major bronchi [37–39]. This rare disorder was originally described at autopsy; the development of laryngobronchoscopy and airway-imaging techniques has improved its detection and follow-up [38].

Epidemiology and etiology The disease appears predominately in adult life, between the fourth and seventh decade, without gender predominance, but it may also be seen in childhood and early adult life. TO with minimal expression may often be overlooked. The etiology and pathogenesis of TO remain uncertain. Chronic infections, chemical and mechanical irritations, metabolic disorders, ecchondrosis, exostosis and metaplasia of the elastic tissue might be potential causal factors. Bone morphogenetic protein-2 may have a role in nodule formation and act synergistically with transforming growth factor β1 to promote an inductive cascade of the TO submucosal nodules [38, 40]. The role of chronic irritation and cough on the development of TO has not been elucidated [38] nor has an association between TO and atrophic rhinitis caused by *Klebsiella ozaenae*, which was found in 20 % of patients with TO [37].

Clinical aspects Typical florid cases narrow the airways and cause a dry cough, dyspnoea, hoarseness and recurrent infections with sputum production and haemoptysis [38, 40].

Macroscopy Laryngobronchoscopic examination and radiographic studies are decisive for diagnosis and follow-up of the disease. Elevated, hard, whitish nodules bulge into the lumen, with the appearance of a stalactite cave arising from the anterior and lateral walls, while the posterior wall of the trachea is typically spared [38, 41]. The nodules are small, measuring from 1 to 10 mm in diameter; they may be scattered, diffuse or confluent. An intralaryngeal location of TO is very rare; as a rule it appears in the subglottic region [39], exceptionally around the arytenoids.

Microscopy Submucosal nodules of cartilage and lamellar bone with marrow spaces are characteristic findings, usually in relation to the underlying cartilage. Calcifications, ossifications and fatty marrow formations may be seen within the nodules. The surface usually shows squamous metaplasia.

Treatment The majority of patients are discovered incidentally and are only carefully followed up. Symptomatic patients are treated with laser photovaporisation and mechanical debridement [38, 41].

7.7 Inflammatory Lesions

7.7.1 Acute Epiglottitis

Definition Acute epiglottitis (AE), more precisely designated as supraglottitis [42, 43], is a potential risk condition for a fatal airway obstruction in previously healthy persons.

Etiology In the past, AE was mainly a childhood disease caused by *Haemophilus influenzae* type B. Due to the introduction of an immunisation programme in the late 1980s, the disease has been steadily decreasing in children, but there is still an increasing incidence in the adult population, more frequently in a form related to infections with pyogenic cocci [43–45]. Concomitantly, the number of epiglottic abscesses has increased with the rise in the incidence of AE [43].

Clinical aspects The most consistently found presenting symptom is severe pain on swallowing. In children, breathing difficulties are often the predominant symptom. Other symptoms and signs are hoarseness, drooling, fever, tachycardia and toxic appearance [44].

Macroscopy A reddish and evidently oedematous supraglottic area, including the tongue and pharyngeal structures, is observed. Oedematous swelling rarely spreads to the glottic region.

Microscopy AE shows diffuse exudative inflammation with fibrin, neutrophils and erythrocytes involving supraglottic structures. An early complaint of dyspnoea may discriminate between patients requiring invasive airway management with intubation and conservative treatment with close observation.

Treatment and prognosis The essence of AE treatment is to safeguard the patient's airway and prevent obstruction. The course of AE is often benign and can be controlled with conservative treatment, but intensive drooling, history of diabetes mellitus, rapid onset of symptoms and abscess formation may predict airway obstruction and thus require urgent surgical intervention [43].

7.7.2 Laryngotracheobronchitis

Definition and clinical aspects Laryngotracheobronchitis (LTB), also known as subglottic laryngitis, non-diphtheric croup, virus croup, spasmodic croup and fibrinous LTB, often occurs in children between 1 and 3 years. Generally, LTB is of limited duration, caused by influenza, parainfluenza or other viruses. Prolonged infection by other pathogens may be also involved [46]. The onset of the disease is more gradual than with acute epiglottitis. When fully developed, a croupy cough with inspiratory and expiratory stridor is present.

Microscopy Characteristic fibrinous laryngitis is observed, with destruction of the respiratory epithelium.

Prognosis The mortality rate of the disease has remained low for many years.

7.7.3 Diphtheria

Definition and etiology Diphtheria is an acute, highly infectious, vaccine-preventable and previously endemic disease, caused by exotoxin-producing *Corynebacterium diphtheriae* [47, 48]. The bacteria are usually transmitted by direct contact or coughing.

Epidemiology and clinical aspects All age groups can be affected, but nonimmune children before the age of five commonly fall sick with diphtheria. The disease is related to local growth of the bacterium in the pharynx with pseudomembrane formation. After systemic dissemination of the toxin, various distant organs are affected, such as lymph nodes, myocardium and nervous system [48]. The larynx and trachea may also be a primary site of infection or extensions of the pharyngeal infection.

Macroscopy Dirty white, fibrinosuppurative membranes cover the pharyngeal, laryngeal and tracheal mucosa, with a characteristic foul smell.

Microscopy The pseudomembranes are composed of bands of fibrinopurulent exudates, exfoliated epithelial cells and accumulation of the causative agents.

Treatment and prognosis The cornerstone of treatment is diphtheria antitoxin. In the pre-vaccination era, diphtheria was the most common infectious cause of death in several countries. Routine childhood vaccination has virtually eliminated the disease in industrialised countries but it is still endemic in developing Asian countries [47]. However, an epidemic in Russia in the 1990s is an important reminder of the role of vaccination in reducing disease in all age groups of patients [47, 48].

7.7.4 Tuberculosis

Definition Laryngeal tuberculosis (LT) is considered a frequent complication of pulmonary tuberculosis and is one of the most common laryngeal granulomatous diseases [49]. However, since 1990, there have also been reports of patients without pulmonary tuberculosis [50].

Epidemiology An increasing incidence of the disease has been established over the past three decades owing to the spread of HIV infection, immunosuppressive diseases or treatments, immigration from countries in which tuberculosis is still endemic, poor living standards with malnutrition and the emergence of drug-resistant mycobacteria [51–54]. LT currently affects mostly males; the average age of patients is about fifty, with a history of heavy drinking and smoking. The most common presenting symptom is dysphonia, followed by dysphagia, odynophagia, stridor, cough and haemoptysis, generally associated with more or less obvious signs of pulmonary involvement [53, 55, 56]. The true vocal cords are most commonly affected, although the supraglottic region can also be involved [52].

Macroscopy The majority of cases are presented as hypertrophic, exophytic, hyperaemic lesions (Fig. 7.10), sometimes nodular or ulcerated [55].

Microscopy Microscopically, the subepithelial stroma contains granulomas with a central caseous necrosis, surrounded by epithelioid macrophages, Langhans-type giant cells and lymphocytes (Fig. 7.11). The covering epithelium may be normal, ulcerated or show pseudoepitheliomatous hyperplasia. Identification of *Mycobacterium tuberculosis* by special stainings, immunohistochemistry or molecular genetic methods confirms the diagnosis of LB.

Differential diagnosis It includes a large spectrum of granulomatous diseases, such as sarcoidosis, cat-scratch disease, fungal infections, Wegener's granulomatosis and tumorous lesions. Differentiation between sarcoidosis and tuberculosis is difficult. Generally, granulomas in sarcoidosis lack caseation; stainings for mycobacteria are negative. Cat-scratch disease can be ruled out by the presence of rounded or stellate granulomas containing central granular debris and neutrophils. Fungal granulomas can be confirmed by identification of the microorganism. Granulomas in Wegener's granulomatosis are not closely packed, fibrinoid necrosis of collagen is prominent and vasculitis is occasionally present.

Treatment and prognosis The treatment of LT primarily consists of antituberculosis-drug treatment, while surgical procedure is reserved for cases of air compromise [56]. If the

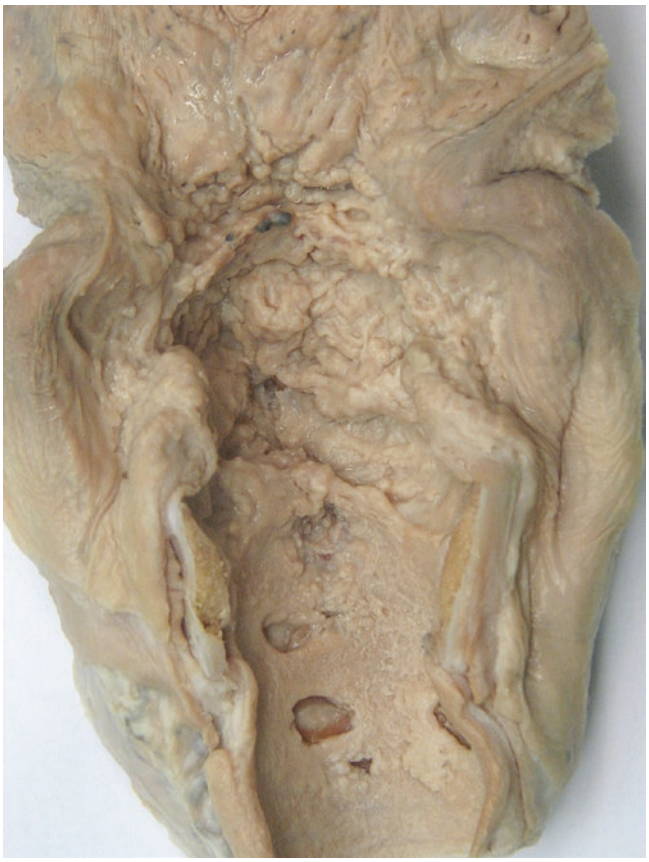


Fig. 7.10 Laryngeal tuberculosis – autopsy specimen without epiglottis; the whole mucosa of the endolarynx is thickened, uneven, corrugated and focally ulcerated

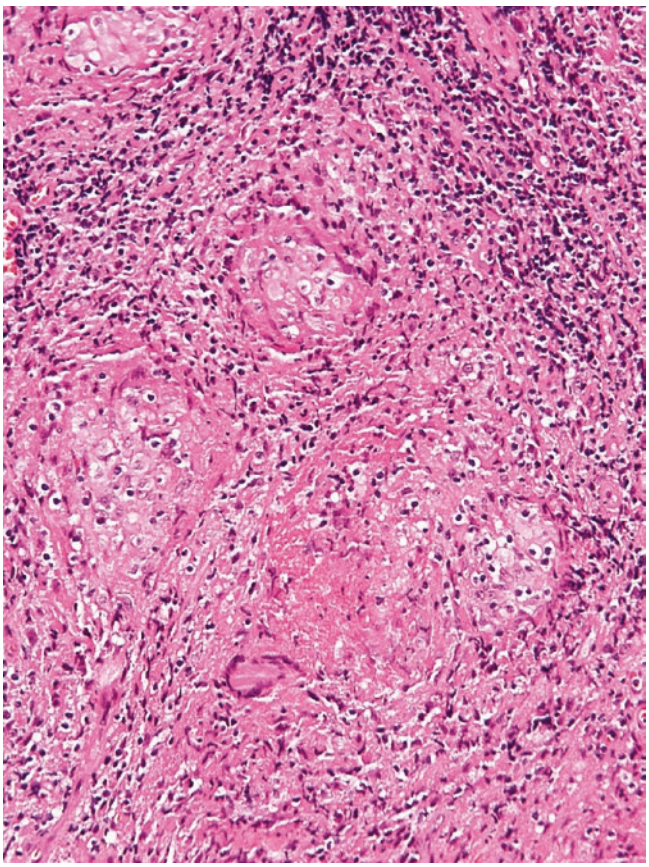


Fig. 7.11 Laryngeal tuberculosis. Laryngeal subepithelial stroma contains granulomas without evident central necrosis; granulomas are composed of epithelioid cells, multinucleated giant cells and lymphocytes

disease is not treated early, LT can cause posterior glottic stenosis, subglottic stenosis, muscular involvement and vocal cord paralysis when the cricoarytenoid joint or recurrent nerve is affected [55].

7.7.5 Fungal Infections

Definition and clinical aspects Fungal infections of the larynx are very rare, but especially laryngeal candidosis (LCN) may be expected to arise in immunocompromised patients during long-term antibiotic use and in patients with diabetes mellitus. Histories of smoking, inhalated steroid use and gastrointestinal reflux disease have been also described as additional aetiological factors [57, 58]. LCN commonly presents with hoarseness, dysphagia and pain. In a few patients, LCN has imitated squamous cell carcinoma (SCC), requiring biopsy to exclude a possible malignant tumor [57].

Macroscopy Endoscopically, the laryngeal mucosa is usually thickened, whitish in colour and of an uneven, stumpy surface with ulcerations.

Microscopy Frequently a hyperplastic squamous epithelium dominates with acute inflammatory infiltrates in the upper epithelial part together with varying amounts of fungal hyphae and budding yeasts consistent with *Candida* (Fig. 7.12). Identification of the causal agent by special silver, periodic-acid-Schiff or mucicarmine stains of the biopsy specimens and/or cultures of microorganisms is crucial for accurate diagnosis and successful treatment.

Treatment LCN is treated by oral antifungal agents and a clear diagnosis can prevent unnecessary surgical intervention [58].

Other types of mycotic infections have been reported, such as laryngeal *histoplasmosis* [59], *cryptococcosis* [60], *coccidioidomycosis* [61], *blastomycosis* [62], *paracoccidioidomycosis* [63] and *aspergillosis* [64–66].

The histological features are similar for each of these infections and range from granulomatous lesions related to histoplasma and cryptoccocus to an abscess formation in blastomycosis and aspergillosis. Pronounced epithelial hyperplasia with prominent ortho-parakeratosis or pseudoepitheliomatous hyperplasia in laryngeal blastomycosis, candidosis and aspergillosis may mimic squamous cell and verrucous cell carcinoma [62, 65, 67].

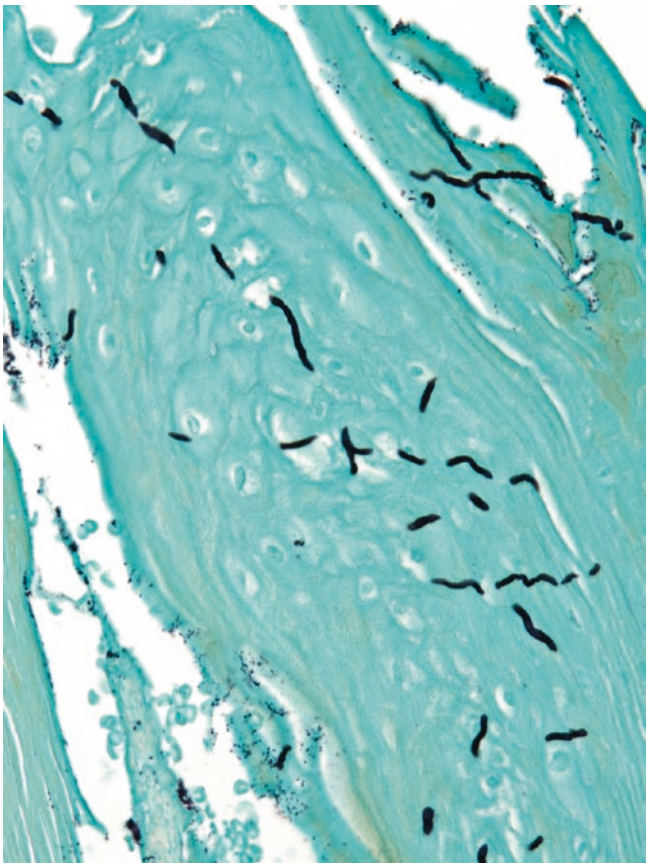


Fig. 7.12 Laryngeal candidosis. The upper part of the squamous epithelium and the parakeratotic layer are infiltrated by fungal hyphae, which are black stained by Grocott's silver staining

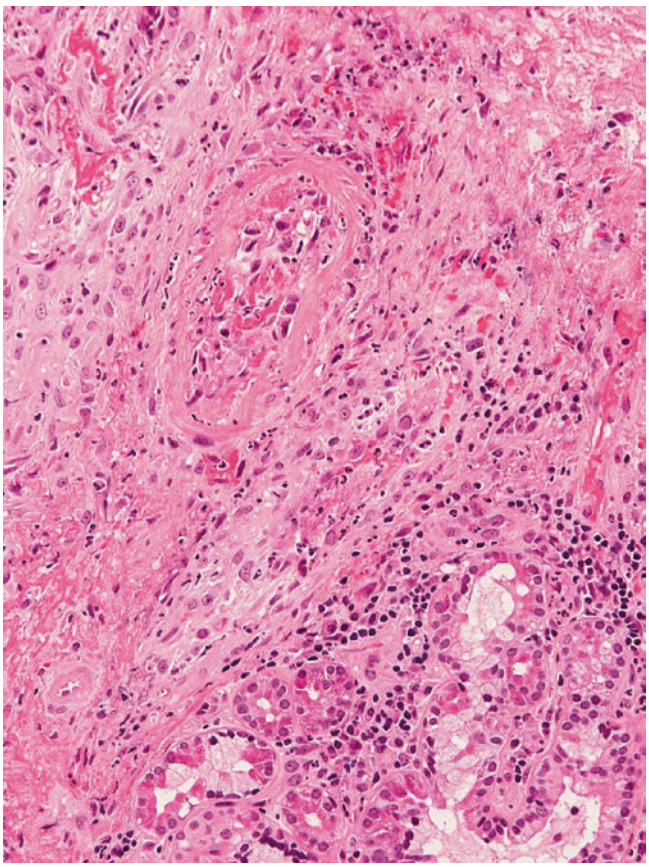


Fig. 7.13 Wegener's granulomatosis. Subepithelial tissue is focally necrotic, mixed chronic inflammatory cells are focally present, vasculitis with fibrinoid necrosis is evident in a small artery

7.7.6 Other Rare Infections

In both European and non-European countries, the larynx is occasionally involved with additional infectious agents, such as *Klebsiella rhinoscleromatis* [68], *Trichinella spiralis* [69] and *Actinomices israelii* [70]. The last two infectious agents have accompanied laryngeal carcinomas.

7.7.7 Wegener's Granulomatosis

Definition Wegener's granulomatosis (WG) is a systemic disease characterised by necrotising vasculitis, formation of granulomas in the upper and lower respiratory tracts and glomerulonephritis. The alternative name for WG is granulomatosis with polyangiitis [71]. Limited forms of WG also occur, often with involvement of the respiratory tract but without kidney involvement.

Epidemiology Upper respiratory tract involvement is the commonest presenting site of WG, mainly affecting the paranasal sinuses, followed by the nose, nasopharynx and larynx [72]. Head and neck manifestations of WG account for the initial presenting symptoms in 73 % of patients who are finally diagnosed with this disease [73].

Clinical aspects Local laryngeal and tracheal clinical symptoms include cough, haemoptysis, stridor and dyspnoea, which may be associated with cranial nerve palsies and isolated subglottic stenosis. Young patients under 30 years are prone to develop airway manifestations [74, 75].

Microscopy Microscopical features include inflammation, necrotising granulomas and vasculitis. Necrosis in WG has a patchy distribution, with serpiginous borders, and is usually basophilic, with a finely granular appearance. Granulomas tend to be loose, not closely packed as in sarcoidosis or tuberculosis [72]. Vasculitis typically involves small- to medium-sized arteries and veins, with any of the following features: fibrinoid necrosis, fragmentation of the elastic lamina, acute and chronic inflammatory cells and granulomas (Fig. 7.13). The lesions may undergo organisation and fibrosis producing subglottic, tracheal and bronchial stenosis [74].

The diagnosis of WG is based on clinical features, biopsy of the related lesions and the cytoplasmic pattern of anti-neutrophil cytoplasmic antibodies (C-ANCA) in the serum, which has a sensitivity of 90–95 % and specificity of 90 % [74, 76]. A positive biopsy of the upper respiratory tract has a high predictive value, up to 100 %, indicating few or even no false positive results [77]. Histology often reveals nonspecific features – inflammation and necrosis, with or without granuloma formation [78]. Vasculitis is only rarely seen on biopsy.

Differential diagnosis WG should be differentiated from other forms of vasculitis, other granulomatous diseases, cocaine abuse and neoplasms, particularly NK/T lymphoma of a nasal type [79]. The presence of C-ANCA proves extremely helpful in differentiating WG from almost all the mentioned diseases [77].

Treatment and prognosis WG was almost universally fatal in the past, usually within a few months of the onset of clinically apparent renal disease. However, with modern immunosuppressive therapy, the prognosis of WG is excellent. The treatment algorithms are based on disease severity and the affection of organ systems. Unlike most other head and neck manifestations of WG, laryngeal and tracheal involvement may prove fatal if untreated [73]; it is five times more frequent when the disease starts in childhood [80]. Subglottic stenosis needs surgical treatment, either endoscopic dilatation or airway reconstruction [81]. Early detection of WG is essential to prevent the fully developed disease. For WG at other sites in the head and neck, see also Chaps. 2 and 3.

7.7.8 Sarcoidosis

Definition Sarcoidosis is an inflammatory, chronic granulomatous disease of unknown etiology that can affect any organ system.

Epidemiology and clinical aspects In addition to the classic involvement of the lungs, hilar and mediastinal lymph nodes, the eyes, skin, liver, bones and nervous system may also be affected. Laryngeal involvement is usually a part of the generalised disease, with an incidence from 1 to 6 % [82, 83]. However, laryngeal sarcoidosis can also appear as an isolated disease. The supraglottic region is mostly affected, especially the epiglottis, aryepiglottic folds and arytenoids, showing oedematous, pale, diffusely enlarged mucosa with occasional nodularity, which has been considered the pathognomonic feature of laryngeal disease [82, 84]. Subglottic and true vocal cord involvement is rare [85].

Microscopy Non-caseating and non-confluent granulomas are a characteristic feature. Granulomas are composed of epithelioid cells and Langhans-type giant cells with no central necrosis. Two structures are often found in giant cells, although they are not pathognomonic for sarcoidosis: asteroid bodies, which are stellate crystalline inclusions, and laminated concretions composed of calcium and proteins, known as Schaumann bodies. Sarcoid granulomas can be transformed into hyaline fibrous scars.

Differential diagnosis Microscopically, proven non-caseating granulomas and the exclusion of other laryngeal granulomatous diseases, such as infectious granulomatous diseases, granulomatous processes of unknown pathogenesis (Wegener's granulomatosis) and inhalant granulomatous processes (berylliosis, asbestosis), must be confirmed [86]. No microorganisms are found in sarcoidal granulomas, with some exceptions, such as cell-wall-deficient forms of mycobacteria [87]. Sarcoidosis lacks vasculitis, characteristic of Wegener's granulomatosis. Inhalant granulomatous diseases, which result in significant pulmonary fibrosis, rarely affect the laryngeal mucosa [86].

Genetics It has been shown that the serum level of YKL-40, a chitinase cartilage glycoprotein, may be considered a novel sarcoidosis marker [88] but is unsuitable as a prognostic marker of the disease [89]. Chitinase 3-like 1 gene (CHI3L-329 G/A) polymorphism contributes to interindividual variations of YKL-40 levels [89].

Treatment and prognosis Early diagnosis and adequate treatment of laryngeal sarcoidosis is important to prevent upper airway obstruction and tracheotomy. Although the course of the disease may be long and require low-dose steroid treatment to maintain remission, spontaneous remissions usually occur. However, for laryngeal sarcoidosis, minimally invasive endoscopic surgery with intralesional corticosteroid injection and laser ablation and/or debulking can successfully improve laryngeal symptoms with minimal morbidity [90].

7.7.9 Rheumatoid Arthritis

Definition Rheumatoid arthritis (RA) is a chronic systemic, presumed autoimmune disorder, characterised by proliferative synovitis that often progresses to destruction of the articular cartilage and ankylosis of the joints.

Epidemiology Extra-articular manifestations may occur, involving various organs, such as the skin, blood vessels, heart, lungs, nervous system, etc. They are common, mostly due to serositis, nodule formation and vasculitis [91]. Laryngeal involvement in RA includes arthritis of the cricoarytenoid and cricothyroid joints and/or the formation of rheumatoid nodules in the soft tissue of the larynx [92].

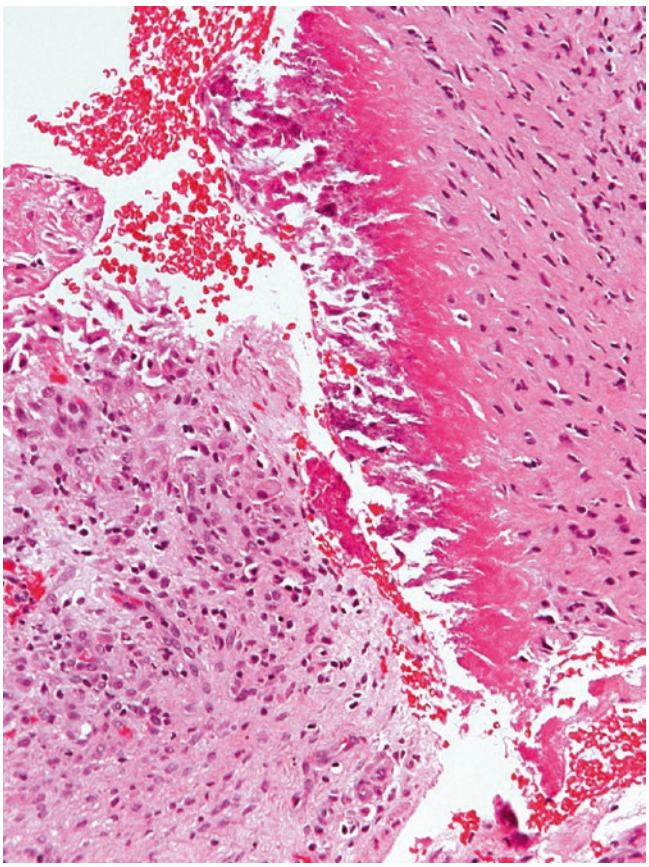


Fig. 7.14 Rheumatoid nodule of the vocal cord. Fibrinoid necrosis is surrounded by macrophages, lymphocytes, plasma cells and by granulation tissue

Clinical aspects In the acute phase, symptoms and signs are usually mild and consist of pain and voice disturbance. In the chronic phase, dyspnoea and respiratory obstruction may develop [92–94].

Microscopy The acute phase of arthritis is characterised by swelling and thickening of the synovia, which is heavily infiltrated by mononuclear cells, resulting in villous hypertrophy. In the chronic phase, there is destruction of the articular cartilage and proliferation of fibrous tissue, with obliteration of the joint spaces, occasionally leading to bony ankylosis. Rheumatoid nodules may develop in the soft tissue adjacent to the joints or in the vocal cords. Microscopically, they consist of a central fibrinoid necrosis, surrounded by palisading macrophages (Fig. 7.14); nodules are not pathognomonic of RA but are also seen in patients with other autoimmune diseases, particularly systemic lupus erythematosus [95].

Treatment RA is treated by anti-inflammatory drugs and local administration of steroids by means of injection or aerosol; in rare cases, surgical therapy is needed to relieve airway obstruction [92, 93, 96].

7.7.10 Relapsing Polychondritis

Definition Relapsing polychondritis (RP) is an uncommon, chronic, multisystem, presumed autoimmune disease against type II collagen, characterised by recurrent attacks of cartilage destruction, both elastic and hyaline, especially in the nose, ears, laryngeal and tracheal cartilages or proteoglycan-rich tissue [97–99].

Epidemiology and etiology The disease is rare, with an estimated annual incidence of 3.5 cases per million [99]. RP commonly affects patients between 40 and 60 years and a female-to-male ratio of 3:1 has been noted. Laryngeal disease affects approximately 50 % of patients with RP [99]. Aetiologically, there is strong indication of an autoimmune origin of RP, which is supported by the finding of antibodies to type II collagen in two-thirds of patients [100].

Clinical aspects Hoarseness, cough, dyspnoea, choking and tenderness over the laryngotracheal cartilages are symptoms of laryngeal and lower respiratory tract involvement [99]. Although airway obstruction may be localised to the glottic and subglottic area, a diffuse involvement of the respiratory tract is more common and occurs frequently in patients with RP.

Microscopy The affected cartilage shows distinct features: the cartilaginous matrix loses its basophilic staining, peri- and intracartilaginous inflammation is evident and inflammatory cells infiltrate the perichondrium and cartilage, leading to a moth-eaten appearance of the damaged cartilage. The chondrocytes become vacuolated and necrotic and cartilage fragmentation is evident. With the progression of the disease, necrotic cartilage is replaced by granulation tissue and later by fibrosis. In the course of the disease, persistent inflammation can destroy the cartilaginous rings and cause luminal collapse.

Differential diagnosis It includes all conditions that essentially show cartilage destruction: various infectious (tuberculosis and other bacterial, fungal and viral infections) and noninfectious diseases (sarcoidosis, Wegener's granulomatosis and other types of systemic vasculitides) and tumors (lymphoma, cartilaginous tumors).

Treatment and prognosis RP treatment is based on systemic steroid and occasionally immunosuppressive therapy [101]. Airway obstruction, as the most serious or even fatal outcome, may be caused by inflammatory swelling during the active faze of RP or by the collapse of the laryngotracheal cartilages. Subglottic stenosis occurs most frequently in younger patients, predominantly in females [99]. Early diagnosis can influence a better outcome, and the survival rate appears more favourable than was previously thought.

7.7.11 Gout

Definition Gout is a disorder of the purine metabolism presenting during the first years by asymptomatic hyperuricaemia, followed by acute attacks of typically mono-articular inflammatory arthritis and precipitation of monosodium urate crystals within and about the joints and eventually chronic arthritis with the formation of tophi, in which monosodium urate crystals serve as the nidus and evoke a granulomatous reaction [102, 103].

Epidemiology The head and neck region is rarely involved, although the classic site is the external ear. In addition, tophi may appear in the intervertebral discs, oropharynx, temporomandibular joint, ear and tongue. There is limited evidence of chronic gouty involvement of the larynx [102–104].

Clinical aspects and macroscopy Acute gouty cricoarytenoid arthritis is most common and may give rise to pain within the larynx, dysphonia, odynophagia, dysphagia or stridor. After repeated attacks, the articular cartilage is gradually destroyed, leading to ankylosis of the joint. The fixed vocal cord may mimic growth of a malignant tumor [104]. Tophi of the laryngeal soft tissue are exceedingly rare [103]; the involved mucosa of the vocal cords shows a granular surface.

Microscopy The microscopical features of tophi are conspicuous, with large aggregates of needle-shaped urate crystals (birefringent crystalline deposits) surrounded by macrophages, foreign-type giant cells, lymphocytes and fibroblasts.

Differential diagnosis In more remote differential diagnostic possibilities, other lesions with deposition of various substances in the laryngeal mucosa, such as amyloid or Teflon, need to be considered.

Treatment The therapy of gout depends on clinical features. Acute arthritis is treated with colchicine and other non-steroid anti-inflammatory drugs, hyperuricaemia should be parallelly treated and tophaceous lesions can be eradicated by endolaryngeal laser surgery [103].

7.7.12 Teflon Granuloma

Definition Injection of Teflon paste (polytetrafluoroethylene) into the lateral thyreoarytenoid muscle has been used in patients with unilateral paralysis of the vocal cord, with the aim of augmenting and medialising the paralysed hemilarynx. The increased bulk of the vocal cord can, therefore, contribute to a more complete glottic closure, prevent aspiration and improve a breathy poor-quality voice.

Epidemiology and etiology The most common cause of vocal cord paralysis is surgical trauma of the laryngeal recurrent nerve and/or malignant tumors [105, 106]. In general, Teflon injection has been well tolerated and, after a short-lived inflammatory reaction, it becomes stable and walled off by surrounding fibrosis. Technical errors during injections, such as over-injection or misplaced injection of Teflon, may, however, cause dysphonia and airway obstruction, as well as the presence of a neck mass, resembling neoplasm, in the case of an escape of Teflon via the cricothyroid membrane [105].

Macroscopy Teflon granulomas (TGs) are submucosal polypoid lesions of the vocal cord. The length of time between the injection of Teflon and development of a clinically significant lesion is variable and unpredictable [107].

Microscopy TGs are composed of a foreign body giant cell reaction with extension to the underlying muscle and cartilage. Teflon is present in foreign body giant cells and also extracellularly as glassy crystalline deposits that are characteristically birefringent under polarised light (Fig. 7.15a, b). A dense fibrotic tissue is evident over time, while surrounding inflammatory infiltrate is not present [106].

Treatment TG is treated by conservative surgery, although the results are unpredictable. There has been no report of cancer development in TG [106]. With the introduction of laryngeal framework surgery and medialisation laryngoplasty, fewer centres nowadays additionally advocate Teflon injection [105, 108].

7.7.13 Idiopathic Subglottic Laryngeal Stenosis

Definition Idiopathic subglottic stenosis (ISS) is a rare, slowly progressive inflammatory disease of unknown etiology mainly involving the region of the cricoid cartilage and the first tracheal ring.

Epidemiology, etiology and pathogenesis ISS has a strong female predilection [109, 110]. The age of females when the symptoms start to appear ranges from 15 to 75 years (average 43.5 years). Diagnosis of the disease is a matter of exclusion and all other possible causes of a subglottic stenosis must first be ruled out. The pathogenesis of the disease remains hypothetical. ISS has recently been associated with various possible causes, such as gastro-oesophageal reflux, autoimmune diseases and previous infections of the respiratory tract [110–112]. Maronian and co-workers suggested that the term ISS should even be replaced with reflux-induced subglottic stenosis if there is no other clear cause of the

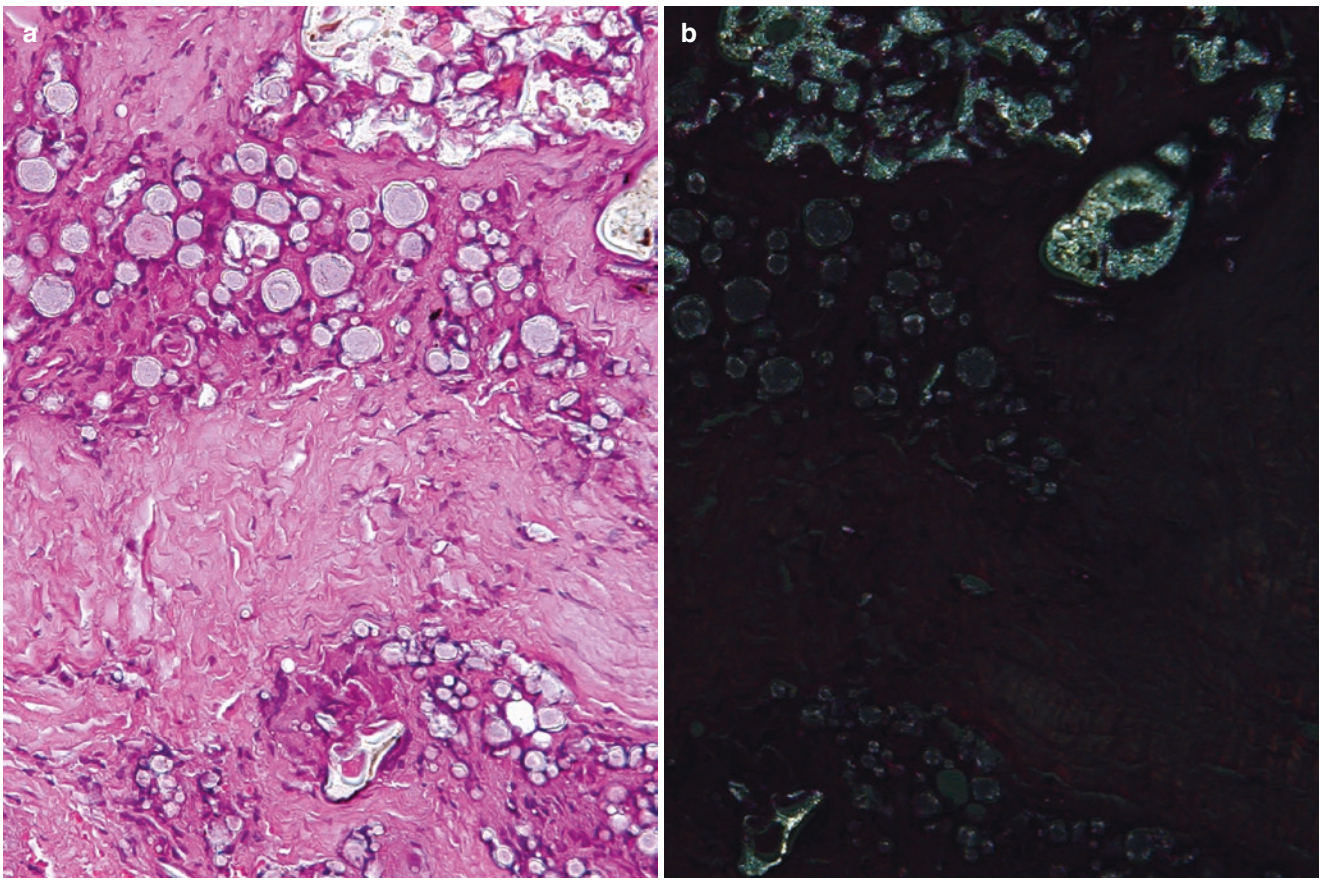


Fig. 7.15 Teflon granuloma of the vocal cord. (**a**) Teflon particles of rounded and oval shape are surrounded by multinucleated giant cells and fibrocytes. (**b**) Teflon particles are clearly evident under polarized light

disease [112]. Aetiologically, subglottic stenoses are most commonly linked to endolaryngeal trauma, especially after prolonged intubation. Other diseases, including infections, laryngotracheal localisation of systemic diseases such as Wegener's granulomatosis and other collagen vascular diseases, amyloidosis and sarcoidosis, are rarely associated with laryngeal stenosis. ISS usually presents with dyspnoea, cough and dysphonia [109, 110] (Fig. 7.16).

Microscopy Histological examination characteristically shows a spreading of dense fibrous tissue, extending up to the surface of the epithelium. Fibrosis is usually poorly cellular, with prominent augmentation of thick collagenous fibres. Some inconspicuous chronic inflammatory infiltrate may be present around blood vessels, without evidence of vasculitis. The covering epithelium, squamous or respiratory, may be reactively hyperplastic.

Treatment and prognosis There is no cure for ISS; the treatment is to maintain adequate space for breathing without tracheostoma. Evaluation for laryngopharyngeal reflux disease should be performed with pharyngeal pH testing in all patients in an attempt to clarify the etiology of ISS [112]. Therapy with endoscopic laser radial incisions with mitomycin-C application has recently been proposed [113]. More severe cases are managed with laryngotracheal resection and reconstruction [109].

7.7.14 Angio-oedema

Definition Angio-oedema (AO) is the end result of deep dermal, subcutaneous and/or mucosal swelling and is a potential life-threatening condition when the larynx and pharynx are affected [114]. Several forms of AO are recognised: (a) IgE dependent, caused by pollens, foods, drugs, fungi, cold, sun or exercise; (b) complement mediated, hereditary and acquired through a deficiency of C1 esterase inhibitor of the complement cascade; (c) non-immunologic, direct mast cell-releasing agents caused by various drugs, aspirin and nonsteroidal anti-inflammatory drugs that alter the arachidonic acid metabolism and (d) idiopathic [115].

Epidemiology and pathogenesis Hereditary AO is an autosomal dominant disease caused by mutations affecting

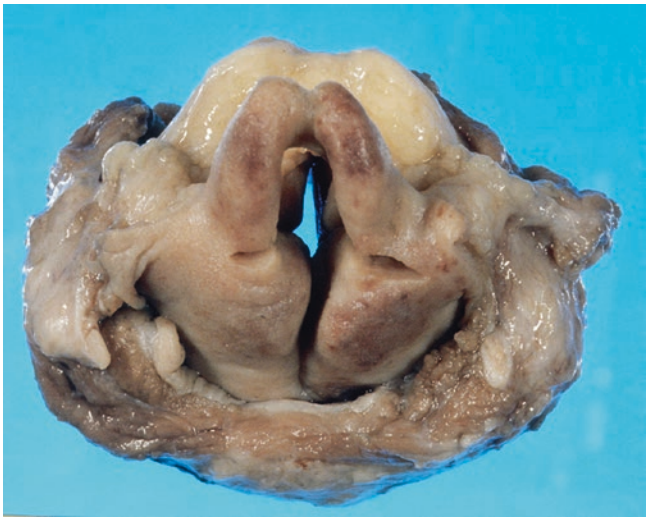


Fig. 7.16 Idiopathic subglottic stenosis. Horizontal section of the larynx. The subglottic space is extremely narrowed. (Courtesy of the late Prof. J. M. Rivera, Bilbao, Spain)

the C1 inhibitor gene, *SERPING1*, resulting in low levels of C1 inhibitor or normal levels of ineffective C1 inhibitor [116]. Surveys of patients suggest that hereditary AO affects about 1 in 50–100,000 of any ethnic group [117] while the prevalence of aspirin or other nonsteroidal anti-inflammatory drugs is 0.3–0.9 % [114]. The typical time pattern of hereditary AO shows an onset of clinical symptoms in the first or second decade of life. The following years are characterised by recurrent attacks and only a minority of patients have symptom-free years in between [118]. AO, either acquired or hereditary, is characterised by sudden onset with full development within a few hours and fades over the course of 48–72 h. The gastrointestinal mucosa is frequently affected, mainly in the hereditary disease, causing severe abdominal pain, nausea, vomiting and oedematous bowel obstruction [117].

Clinical aspects Various degrees of laryngeal oedema may be present, affecting mainly the anterior surface of the epiglottis, aryepiglottic folds, base of the tongue and hypopharynx. Laryngeal oedema is a rare but potentially fatal clinical manifestation of hereditary AO. Approximately half of all patients with hereditary AO have a laryngeal attack at some point in their lives [118].

Treatment Hereditary AO is currently treated by C1 inhibitor with plasma-derived products, while drug-induced AO is treated with anti-allergic and anti-inflammatory drugs [114, 117, 119]. Generally, AO resolves without harm but laryngeal and tracheal oedema may cause asphyxiation and remains a considerable cause of death [120]. Emergency treatment is required if the process leads to respiratory distress because of laryngeal involvement.

7.8 Degenerative Lesions

7.8.1 Oculopharyngeal Muscular Dystrophy

Definition Oculopharyngeal muscular dystrophy (OPMD) is a predominantly inherited neuromuscular disorder, usually presenting in middle years, associated with progressive eyelid ptosis, dysarthria, dysphagia and proximal limb weakness [121, 122].

Genetics The disease is caused by the expansion of a DNA repeat sequence, from ten to between 12 and 17 repeats, containing GCG and GCA codons in exon 1 of the *PABPN1* gene on chromosome 14q11 [122]. Although OPMD has a worldwide distribution, its prevalence is highest in patients of French-Canadian origin [123].

Clinical aspects The disease progresses slowly but the dysphagia may be severe and has been reported as a cause of death by starvation in several cases [124].

Microscopy Muscle biopsy reveals various changes in muscle fibres, such as atrophy and regeneration with an increased number of myocyte nuclei and their centripetal orientation. Some findings, such as intracytoplasmic rimmed vacuoles, found by light microscopy, and intranuclear filament inclusions, seen by electron microscopy, are pathological hallmarks [124].

Treatment Simple procedures, such as blepharoplasty or cricopharyngeal myotomy, considerably improve the quality of life of these patients [123].

7.9 Exudative Lesions of Reinke's Space

The special anatomic framework of Reinke's space is considered essential for the development of a group of so-called exudative benign lesions of the vocal cords, including Reinke's oedema (RO), vocal cord polyps (VCPs) and nodules (VCNs) [4, 125, 126]. Each of the three entities has its own clinical and morphological specificities [127], but most of them overlap (Table 7.1). The common basic pathogenetic mechanism is blood vessel injury, with an accumulation of oedematous fluid in Reinke's space [126, 127].

7.9.1 Reinke's Oedema

Definition RO is a chronic, diffuse, mainly bilateral, oedematous swelling of the membranous part of the vocal cords [4]. Several synonyms for RO have been used, such as polyp-

Table 7.1 Characteristics of the exudative lesions of Reinke's space

Clinical and histological characteristics	Reinke's oedema	Polyp	Nodule
Etiology	Phonotrauma, voice abuse, smoking	Phonotrauma, voice abuse, smoking	Phonotrauma, voice abuse, smoking
Age	20–40 years	20–50 years	Children 5–10 years Middle-aged females
Gender	Females predominate	Male:female = 2:1	Children – both genders equally Adults – females predominate
Macroscopy	Bilateral diffuse oedema of vocal cords	Unilateral pedunculated or sessile polyp between anterior and middle third of the vocal cord	Fusiform bilateral swelling of the vocal cords
Microscopy	Basophilic oedematous stroma, thickened epithelial basement membrane, reactive changes of squamous epithelium	Oedematous stroma, dilated vessels, leakage of fibrin and erythrocytes, ingrowth of blood vessels, fibrosis	Oedematous stroma, distended blood vessels, fibrous tissue
Therapy	Voice therapy, surgical removal	Voice therapy, surgical removal	Disappearance in puberty Adults – voice therapy, surgical removal
Prognosis	Excellent	Excellent	Frequent recurrences

oid vocal fold, polypoid degeneration, chronic polypoid chorditis and chronic oedematous hypertrophy.

Epidemiology and etiology It is a common disease; the prevalence varies from 5.5 to 7.7 % in patients with a voice disorder [128]. Various mechanical and chemical aetiological factors are related to the development of RO, including overuse or abuse of the voice, cigarette smoking, laryngopharyngeal reflux and unfavourable microclimate at work; the role of constitutional and hormonal disturbances, such as hypothyroidism, remains uncertain [4, 126, 129, 130]. A recent study of RO revealed that males with RO, all were also smokers, had significantly higher serum levels of testosterone and progesterone than controls without laryngeal pathology. The authors hypothesise that the possible role of sex hormones is through enzymatic activity of nitric oxide synthase in the endothelial cells [129].

Clinical aspects RO appears most commonly in females between 20 and 40 years of age, with hoarseness as the leading symptom. Women's voices with RO are lower and gradually adopt male characteristics [128].

Macroscopy Laryngoscopically, the surface of the swollen vocal cords in their entire length is smooth, translucent and jelly-like, with a clearly visible capillary network (Fig. 7.17a). Incision yields a characteristic yellowish or gelatinous fluid [4, 131]. The specific morphologic features of Reinke's space, such as sparse lymphatic drainage and its sharply demarcated borders, except the lateral one, contribute to the development of RO [2, 4, 126].

Microscopy An excessive accumulation of oedema is a leading microscopic feature. Increased thickness of the walls of the telangiectatic blood vessels and thickening of the epithelial basement membrane supplement the classical triad of morphologic changes. Sulphated glycosaminoglycans are probably responsible for the characteristic abundant blue-coloured amorphous material in the subepithelial stroma in haematoxylin- and eosin- (H&E) stained slides (Fig. 7.17b–d). Fragility and alterations in the walls of blood vessels, such as thin endothelium with many fenestrae and vesicles and thickened basement membrane revealed by electron microscopy, are considered important in the development of RO. Disarrangement of elastic system fibres and collagen disruption of the extracellular matrix have been stressed as important factors in the pathogenesis of RO [128]. Connective tissue proliferation, especially with ageing of the lesion, makes the lesion irreversible without surgical removal. Changes in the covering squamous epithelium of all three exudative lesions are generally only reactive (squamous cell hyperplasia, basal and parabasal cell hyperplasia) and may turn with ageing and enlargement of the lesions into atrophic epithelium.

In a review of two comprehensive studies of RO, only 12 (1.7 %) patients were found with potentially malignant lesions (atypical hyperplasia and LIN I and II) of the covering epithelium [4, 131].

Genetics In a complementary DNA microarray study of RO, it was found that genes involved in protection against oxidative stress (*MAP2K3*, *SOD1*, *GPX2* and *GTSA2*) and apoptosis (*CASP9*) showed increased expression. Interestingly, in

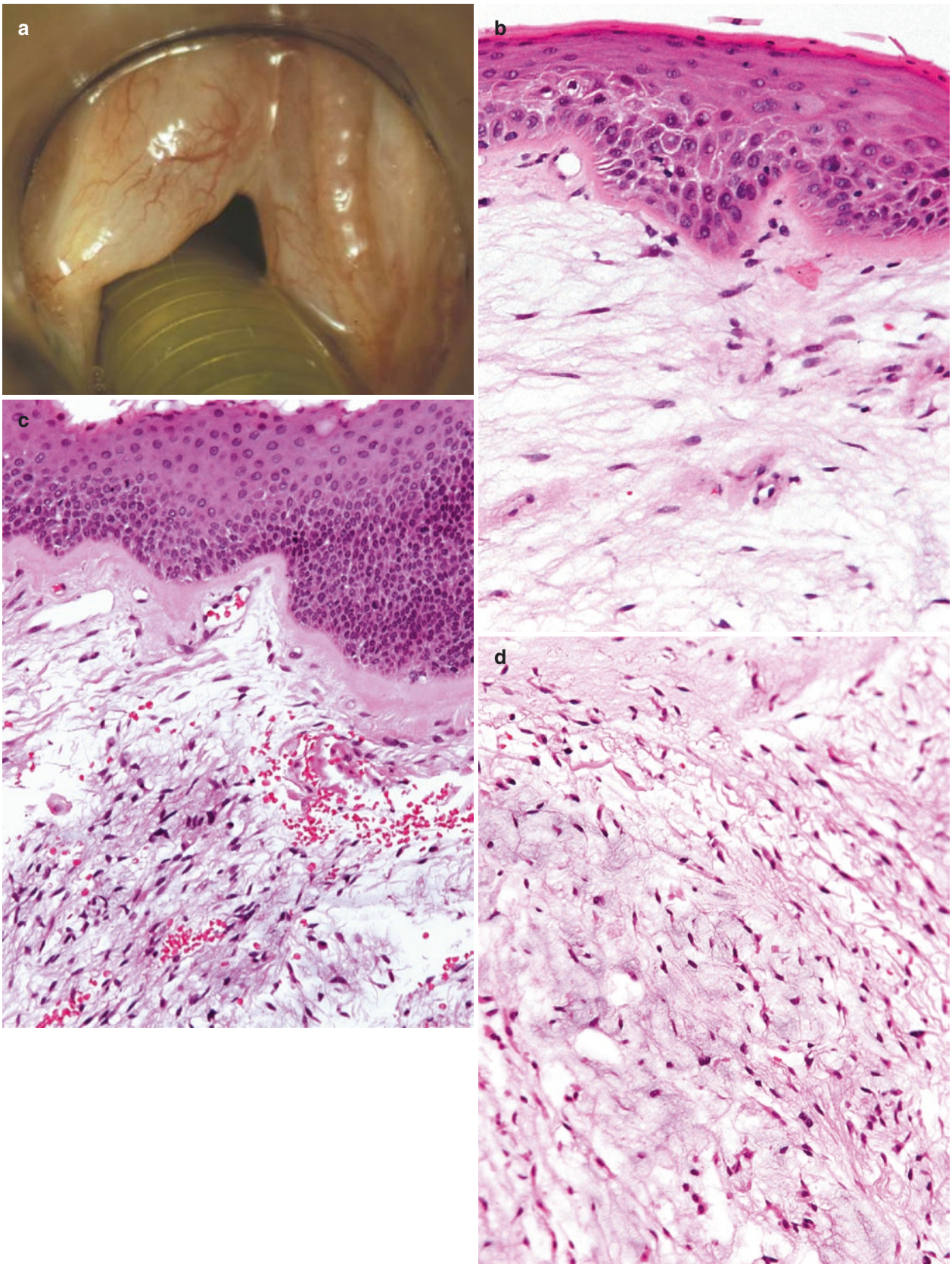


Fig. 7.17 Reinke's oedema. (**a**) Diffuse oedematous swelling is seen in the entire length of both vocal cords with prominent capillary network. (**b**) and (**c**) Covering squamous epithelium is both atrophic and hyperplastic with focal basa-parabasal cell hyperplasia. Basement membrane is thickened. (**d**) Subepithelial stroma is characteristically diffusely oedematous and blue-coloured due to abundant sulphated glycosaminoglycans

the same study, the authors reported that RO and vocal cord polyps (VCP) can be discriminated by means of their gene expression patterns, since VCP do not appear to be induced by oxidative stress; significantly, overexpressed genes involved in extracellular matrix remodelling were characteristic for VCP [132].

Treatment and prognosis Treatment of RO involves different phases. In the early stage, only voice rehabilitation and avoidance of irritating factors, such as smoking and control of reflux disease, are important in prevention. A combined use of a CO2 laser and a cold instrument provides a reliable and safe method for RO surgical treatment. Following surgery, voice therapy is often indicated [4, 133].

7.9.2 Vocal Cord Polyp and Nodule

Definition Vocal cord polyp (VCP) and vocal cord nodule (VCN) are fairly common benign reactive lesions caused by multifactorial noxious events, such as phonotrauma and vocal abuse, smoking, iatrogenic or functional disorders, unfavourable occupational exposure, endocrine dysfunction and infections [4, 125–127, 134]. The distinction between a VCP and VCN is probably only a matter of terminology, since both exhibit similar aetiologies, pathogeneses and, to a certain degree, histological characteristics.

Epidemiology and etiology VCP and VCN are common lesions; about 1.5 % of the general population has hoarseness and the two lesions are the most frequent causes of this clinical symptom. About 2.5 % of children have VCN; the ratio between boys and girls is 2:1 [135]. Damage of the subepithelial blood vessels is the initial event in the evolution of both VCP and VCN. However, the morphology of the two exudative lesions depends on the severity of the initial damage and repeated injuries [4, 125, 126]. A VCP is a pedunculated or sessile, mono- or multilobulated lesion, measuring up to 10 mm in diameter, and located between the anterior and middle third of the vocal cord [4]. Bilateral polyps are found in only 15 % of cases. They primarily affect adults between 20 and 50 years of age, although they may also occur in other age groups. Men are affected at least twice as frequently as women [4, 125].

VCNs are smaller, often bilateral, sessile, fusiform swellings of the vocal cords, positioned symmetrically and rarely exceeding 2 mm in diameter. They usually occur in children, with a peak between the ages of 5 and 10 years. In adults, the highest incidence of these lesions is in young to middle-aged women. VCNs are considered to be the most common benign lesions of the vocal cords [4, 136].

Clinical aspects Hoarseness is the predominant clinical symptom in both lesions. A great variety of voice changes, ranging from mild hoarseness to complete aphonia, is found, depending on the location and size of the lesions [4, 125].

Macroscopy The gross appearance of a VCP varies from a glassy translucent gelatinous formation, to congested and purple red in teleangiectatic variants and, finally, to whitish, firm and opalescent in the predominant fibrous forms at the end stage of the lesions (Fig. 7.18a). VCNs start as a soft reddish swelling; gradually, as the fibrous tissue proliferates, the VCNs become firmer, whitish in colour and conical in shape.

Microscopy Microscopically, different stages of VCP development are noted. Initially, the subepithelial stroma is diffusely oedematous with dilated vessels. After severe or repeated injuries, massive leakage of oedema, mainly fibrin as amorphous hyaline pink material, and erythrocytes are the predominant features in the vicinity of the angiectatic vessels, which may also be thrombotic (hyaline forms the VCP). Evidently dilated vessels, haemorrhages with consequent haemosiderosis and conspicuous ingrowths of new blood vessels create the angiectatic or vascular stage of a VCP (Fig. 7.18b, c). Finally, the lesions may be transformed into a fibrous variant containing an increased amount of fibrous tissue and blood vessels. Not infrequently, mixed-type VCPs are seen, composed of two or more different histological patterns [4, 126].

Rarely, scattered atypical stromal cells, not associated with increased mitotic activity, may be found within the core of the VCP. This finding, as in nasal polyps, only represents reactive changes and must not incorrectly lead to a diagnosis of malignancy [137, 138].

In the initial stage, VCNs show diffusely oedematous tissue with distended capillaries and venules and tiny perivascular haemorrhages surrounded by a minimal or moderate inflammatory reaction (Fig. 7.19). In time, the loose connective stroma is replaced by a mild to moderate cellular fibrous tissue, changing in the various stages of evolution.

However, it has been suggested that the overlapping features of oedema, inflammation and fibrin exudation preclude a definitive distinction between VCP and VCN, although ectatic vessels are more common in polyps [137, 139].

As previously mentioned, the covering squamous epithelium in both lesions shows predominantly benign reactive changes. In four (0.8 %) patients, potentially malignant changes (atypical hyperplasia) were noted in the covering epithelium but with no evidence of malignant alteration [4, 131].

Genetics Microarray analysis revealed that genes involved in extracellular matrix remodelling, as well as cell growth

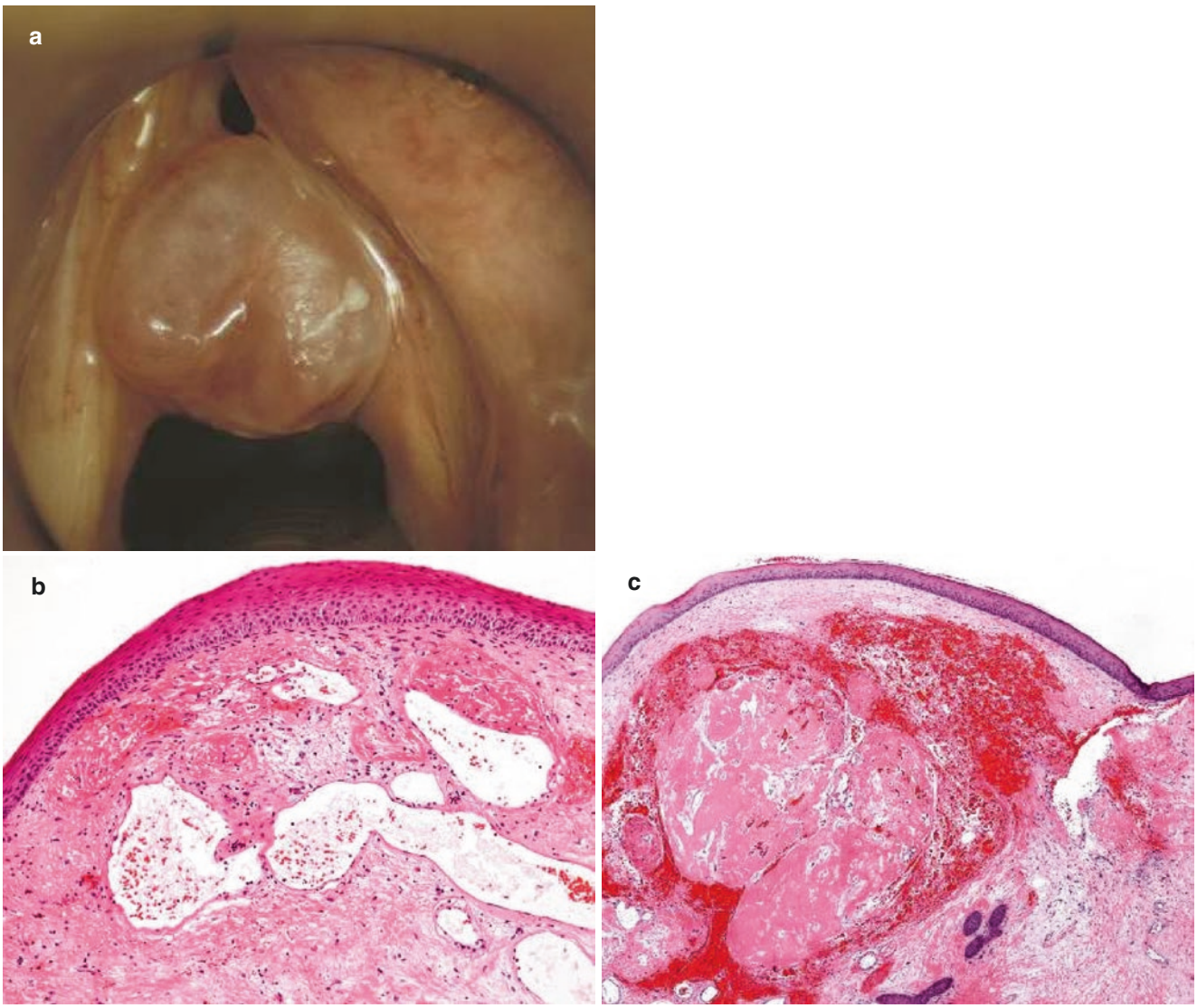


Fig. 7.18 Vocal cord polyp. (**a**) Huge vocal cord polyp arising from the middle third of the right vocal cord. (**b**) Polyp is covered by atrophic and normal squamous epithelium, abundant subepithelial stroma is oedematous, around dilated vessels blood is evident abundant fibrin deposition. (**c**) Abundant fibrin deposition and extravasations of erythrocytes are predominant morphologic changes in the polyp's stroma

and repair proliferation (S*PARC*, *SPARCL1 and Co16A3*), were significantly overexpressed in VCP. Additionally, four genes involved in forming fibroblasts and inflammatory processes (*Col A2, Col3A1, Col5A2* and *PECAM1*) were also overexpressed; these data suggest a high presence of fibroblasts and inflammatory processes in VCP [132].

Differential diagnosis The main differential diagnosis includes amyloidosis and myxoma [135]; the former lesion shows lobular, perivascular, extracellular accumulation of an acellular, eosinophilic matrix with specific staining characteristics: a positive reaction with Congo red and green birefringence with polarised light. The latter is a very rare laryngeal tumor showing rare spindled and/or stellate cells, sparse small vessels and an abundant basophilic, gelatinous matrix.

Treatment and prognosis The treatment of choice of VCPs is microlaryngoscopic surgical removal. Childhood VCNs may disappear in puberty. Small incipient VCNs in adults may also vanish spontaneously or after voice rehabilitation. Surgical intervention is appropriate for longstanding VCN when there is no improvement after conservative treatment. However, recurrences are frequent and may occur late after a mean interval of 5 years. Voice therapy is the only significant factor associated with a lower recurrence rate [140].

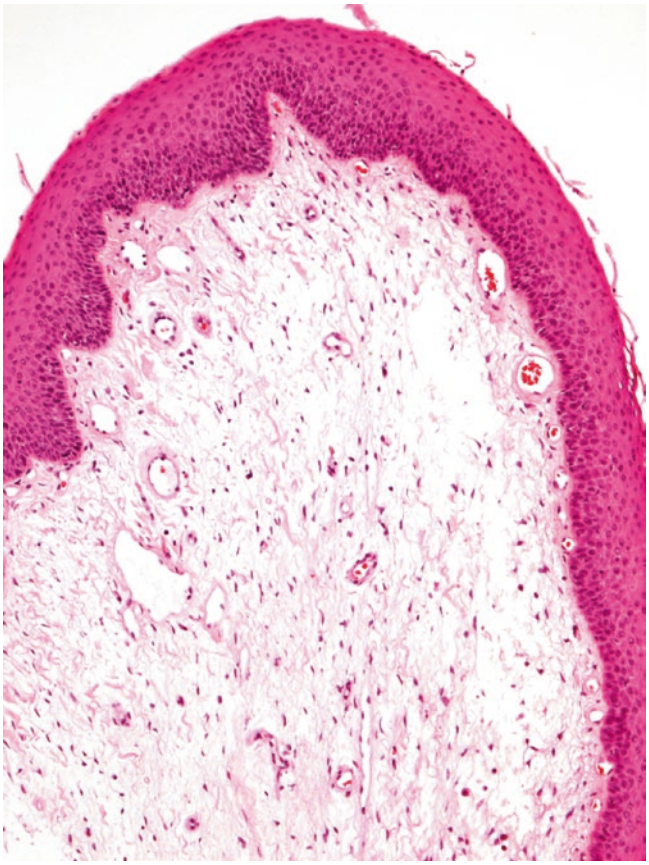


Fig. 7.19 Vocal cord nodule. Nodule's surface is covered by mildly hyperplastic squamous epithelium, subepithelial stroma is diffusely oedematous, and number of small blood vessels is slightly increased

7.10 Pseudotumours

7.10.1 Contact Ulcer and Granuloma and Intubation Granuloma

Definition Contact ulcer (CU), contact granuloma (CG) and intubation granuloma (IG) are benign, inflammatory, exophytic or ulcerative lesions, usually located in the posterior third of the glottic area.

Epidemiology and etiology IGs are more common in females, while hyperacidity and CU/CGs are predominant in males [4, 138, 141]. Aetiologically, the lesions arise in response to various mechanical and chemical injuries, such as voice abuse or protracted forceful coughing, acid regurgitation or intubation injuries. They display similar symptomatology and clinical appearance and more or less identical histopathological features and prognosis [4, 138, 141, 142]. It has recently been hypothesised that idiopathic cases of CG may result from underlying glottal insufficiency caused by paresis, scar or atrophy. A patient with glottic insufficiency makes an increased effort to close their vocal cords and may subsequently cause undue pressure on the vocal processes of the arytenoids [143]. Excessive shouting or coughing cause repeated microtraumas of the thin mucosa of the vocal cord processes. They strike each other in phonatory adduction of the arytenoids, which leads to the development of ulcerative or exophytic lesions of one or both vocal cords [142]. Acid regurgitation due to hiatal hernia or gastritis may also cause the same type of lesion in the posterior glottic area. IG is an undesired sequel of intubation tube pressure during anaesthesia or intensive care treatment.

Macroscopy Clinically, ulceration or exophytic lesions can be found, mono- or multilobular, frequently bilateral, measuring up to 15 mm in diameter, which range from pale grey to dark red, sometimes with an ulcerated surface (Fig. 7.20a). Hoarseness, the sensation of a foreign body, coughing, a sticking sensation, pain in the throat and a sensation of acidity are the prevailing symptoms in all three types of lesions.

Microscopy An ulceration of the posterior mucosa, covered by necrotic tissue and fibrin, is initially seen. The depth of the ulcers can vary from superficial to deep lesions extending down to the perichondrium of the arytenoid cartilage. The localised necrosis of the epithelial and subepithelial tissue triggers an acute inflammatory reaction, with proliferation of granulation tissue initially infiltrated by neutrophils and later by macrophages, lymphocytes and plasma cells (Fig. 7.20b, c). Macrophages usually phagocytise a large amount of haemosiderin. Endothelial cells are usually plump but not atypical, with increased mitotic activity. The basis of the lesion is usually associated with prominent fibrosis, which is more exuberant in chronic lesions. The marginal epithelium starts to proliferate, some regenerative atypias of epithelial cells, such as plump nuclei and increased mitoses, may be present [138, 144].

An exuberant proliferation of granulation tissue forms an exophytic polypoid lesion. New vessels are characteristically arranged radially from the base to the fibrin-covered surface of the lesion. Approximately 1 week after the initial injury, connective cells and collagenous fibres become more abundant and, finally, the predominant elements in the granuloma. In the end stage, granulomas are entirely covered by squamous epithelium, which is usually considerably thickened due to hyperplasia of the prickle cell layer or, rarely, of the basal and parabasal layer [4, 141].

Differential diagnosis It includes rare laryngeal vascular tumors such as angiosarcoma and Kaposi sarcoma. The former is characterised by a proliferation of pleomorphic, plump spindle endothelial cells, which encompass anastomosing vascular channels. The latter shows small, atypical endothelial cells around small vessels and slit-like vascular spaces containing erythrocytes.

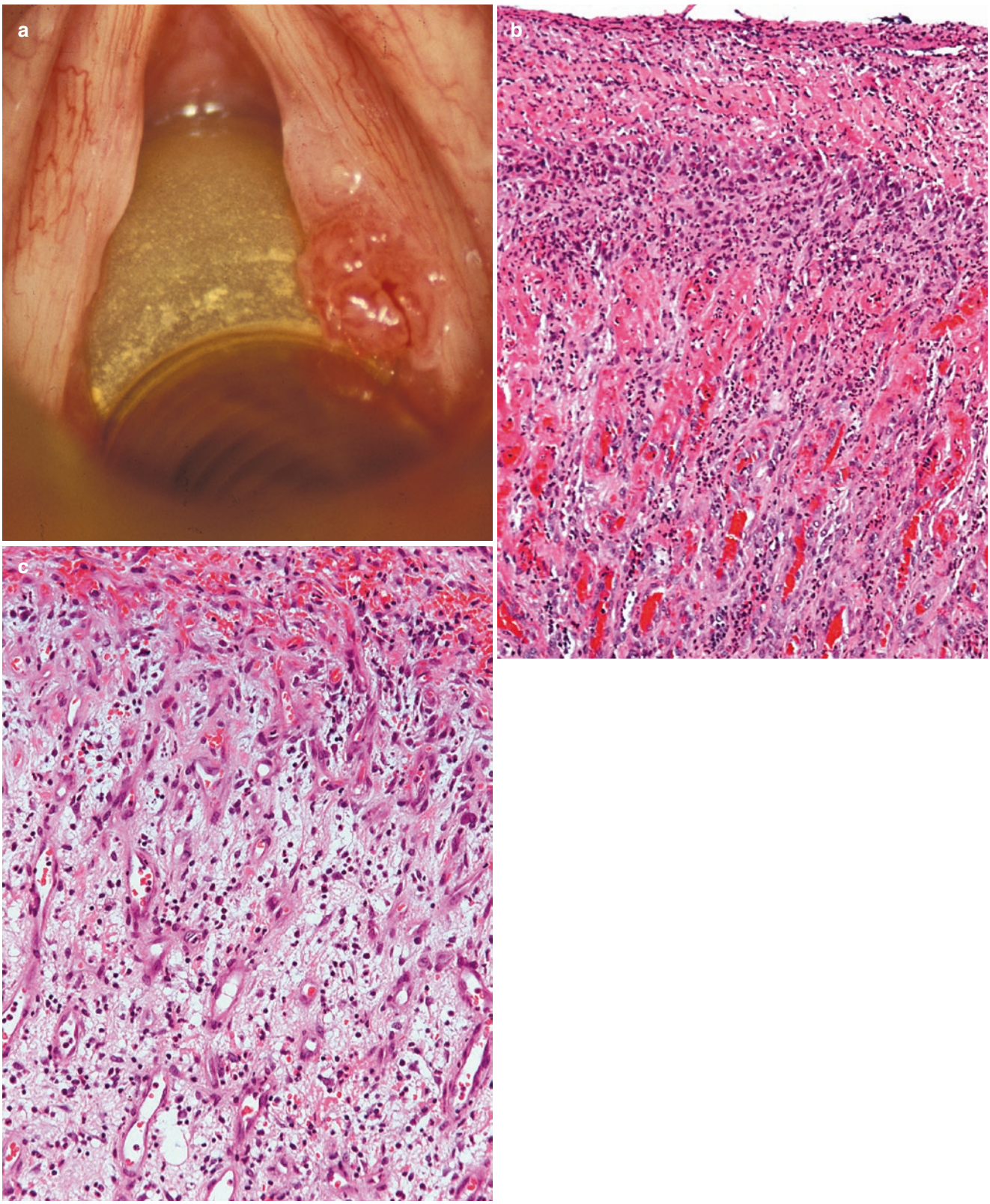


Fig. 7.20 Intubation granuloma. (**a**) Red exophytic lesion with lobular surface is located on the posterior third of the right vocal cord. (**b**) Abundant proliferation of granulation tissue shows radially oriented capillaries from the base to the fibrin covered surface. (**c**) Higher magnification of radially oriented capillaries

Immunohistochemistry Both sarcomas are positive for CD34, CD31 and other endothelial markers, while a positive reaction for human herpes virus 8 (HHV-8) is mandatory for a diagnosis of Kaposi sarcoma. A more remote differential diagnosis is spindle cell carcinoma, which usually shows a transition from the conventional form of squamous cell carcinoma to spindle-shaped atypical cells with increased mitotic activity.

Treatment and prognosis The basis of therapy in CU/CGs and hyperacidic granulomas is elimination of the causative factors, voice rest, voice re-education, dietary measures, cessation of smoking and alcohol abuse and medical therapy such as antacids and corticosteroids. IG often does not require treatment due to its self-limiting nature. In refractory cases, surgical treatment is indicated, either by microsurgery or CO_2 laser.

7.10.2 Necrotising Sialometaplasia

Definition Necrotising sialometaplasia (NS) is a rare benign self-healing inflammatory lesion involving the minor salivary glands, primarily of the hard palate. The lesion is discussed in detail in Chap. 5. Here, some specificities of the rare appearance of NS in the larynx are presented [138, 145–147]. According to previous reports, as well as our own experience, NS occurs in the larynx secondary to trauma or concomitantly with other non-neoplastic or neoplastic lesions [147]. The pathogenesis is probably associated with ischaemia.

Macroscopy Laryngeal NS appears in the supraglottic and subglottic regions, where seromucinous glands are present, as a deep-seated ulcer, that may or may be not painful, or a submucosal nodular lesion.

Microscopy The most prominent microscopical characteristics that help to distinguish the lesion from various forms of laryngeal carcinomas are: pseudoepitheliomatous hyperplasia, lobular infarction with or without mucin spillage, preservation of the lobular architecture, the appearance of epithelial-myoepithelial islands, no cellular atypias or occurrence of pathologic mitoses in the rest of the cellular part, retention of the lumina in preserved ductal formations, acute and chronic inflammatory reaction, and granulation tissue in and around the glands (Fig. 7.21) [146]. The appearance of the surface may cause additional problems in differential diagnosis with laryngeal cancers, especially when frozen sections are performed.

Differential diagnosis Two entities, squamous cell carcinoma and mucoepidermoid carcinoma, are of utmost importance in differential diagnosis. A properly oriented haematoxylin and eosin section remains the gold standard for diagnosis; the use of Ki67, calponin and CK7 may be helpful, as long as they are interpreted in relation to each other.

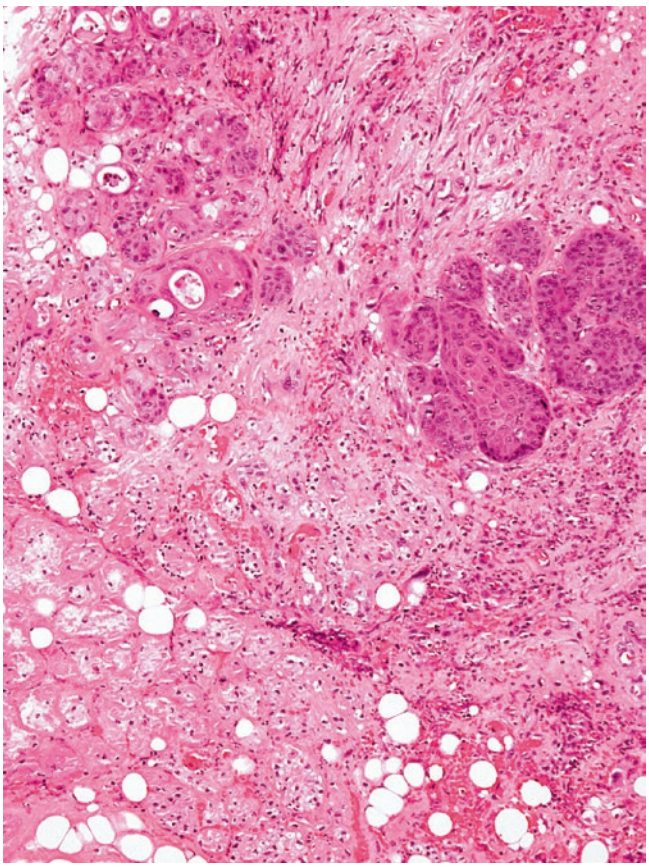


Fig. 7.21 Necrotising sialometaplasia. Acinar coagulative necrosis and residual salivary gland ducts with squamous metaplasia

Treatment NS is usually a self-healing lesion; the duration of the healing process is related to the size of the lesion [147].

7.10.3 Nodular Chondrometaplasia

Definition Nodular chondrometaplasia (NC) are small (less than 1 cm) fibroelastic lesions, most frequently in the posterior and middle portions of the glottis and ventricular bands.

Epidemiology and etiology There are only few reports on NC in the literature, although postmortem foci of metaplastic cartilage may be found in 1–2 % of all examined larynges [148]. NC is probably associated with laryngeal trauma.

Macroscopy Clinical endolaryngeal examination may reveal a nodular lesion up to 1 cm in diameter, covered by a smooth mucosa.

Microscopy NC shows a smooth transition from the initial accumulation of acid mucopolysaccharides between the collagen bundles and their separation, to transition of fibroblasts to enlarged, rounded cells resembling chondrocytes. Aggregates of elastic fibres are present in the centre of the lesions.

Differential diagnosis NC is rarely clinically relevant [148]. One should be aware of its possible existence and distinction from chondroma and low-grade chondrosarcoma. Chondroma has a characteristic lobular pattern and low cellularity, which is not the case with NC. Low-grade chondrosarcomas differ mainly from NC in their locations, as well as in cellular and structural atypias [148].

7.10.4 Amyloidosis

Definition Amyloidosis is a heterogeneous group of disorders associated with extracellular deposition of an abnormal fibrillar protein with pathognomonic tinctorial properties. It may be hereditary or acquired and localised or systemic in distribution. The current classification of amyloidosis is based on the biochemical composition of its peptide subunits.

Epidemiology and etiology Laryngeal amyloidosis (LA) primarily affects patients between 40 and 60 years of age, more frequently males [149]. All parts of the larynx can be affected [150], but the supraglottis was the most common site of involvement in some studies [151]. It can affect the larynx multifocally and can also extend to the tracheobronchial tree. Laryngeal amyloidosis (LA) is rare and is mostly a localised disease. It accounts for less than 1 % of all benign tumorous lesions [149]. In the majority of LA cases, the amyloid is composed of immunoglobulin light chains (AL amyloid). LA may occasionally be part of a systemic disease or may be associated with a tumor, such as neuroendocrine carcinoma of the larynx or medullary carcinoma of the thyroid [150].

Clinical aspects The main symptom is hoarseness, in some patients accompanied by cough, dysphagia, dyspnoea, stridor and rarely haemoptysis [149, 151].

Macroscopy The affected area of the larynx is swollen, sometimes polypoid, covered by an intact mucosa. On a cut surface, it is firm, pale, waxy, tan yellow to red grey [150].

Microscopy H&E staining shows the deposition of an amorphous, eosinophilic, hyaline, extracellular substance in the subepithelial stroma, blood vessel walls and along the basement membranes of the seromucinous glands, with intact covering epithelium. The deposits may be discrete or may appear as large rounded masses of variable size. They stain with Congo red and display green birefringence in polarised light; this property remains the diagnostic gold standard (Fig. 7.22a–c).

Immunohistochemistry Immunohistochemical or in situ hybridisation analyses must be performed to determine the amyloid type, such as amyloid P and light-chain (κ and λ) restriction in some cases.

Treatment and prognosis Most patients can be successfully treated by conservative surgical excision to preserve the laryngeal function for as long as possible [152]. Multiple procedures may be necessary in some patients and recurrences may occur. A fatal outcome has been described in patients with progressive tracheobronchial involvement but association with systemic amyloidosis is rare [153]. Clinical examination is advised to exclude the possibility of a systemic disease.

7.10.5 Sinus Histiocytosis with Massive Lymphadenopathy and Other Rare Pseudotumours

Definition Sinus histiocytosis with massive lymphadenopathy (SHML) or Rosai-Dorfman disease is an idiopathic, relatively rare benign lesion, based on a nodal and/or extranodal histiocytic proliferative disorder that usually resolves spontaneously [154].

Epidemiology and etiology It affects all age groups, including paediatric patients. The exact etiology and pathogenesis of SHML is still not known. Proposed mechanisms include occult infection, such as Epstein-Barr virus or human herpes virus 6, as well as an aberrant exaggerated immune response to an infective agent or antigen, causing proliferation of histiocytes [154].

Clinical aspects The most frequent clinical manifestation of the disease is cervical, bilateral and painless lymphadenopathy. However, extranodal sites may also be involved and the head and neck region is one of the most commonly affected areas [155]. Extranodal disease may be the initial, and sometimes the sole, manifestation of the disease. Foucar and coworkers reported that 43 % of patients had at least one site of extranodal location of SHML [156]. Within the head and neck, the nasal cavity, paranasal sinuses and orbit are commonly involved. Some cases of laryngeal lesions have also been reported [156–158]. Typically, SHML begins insidiously and progresses to a protracted course of the active stage and ends with spontaneous remission. SHML occasionally appears with subsequent recurrence and serious consequences, occasionally

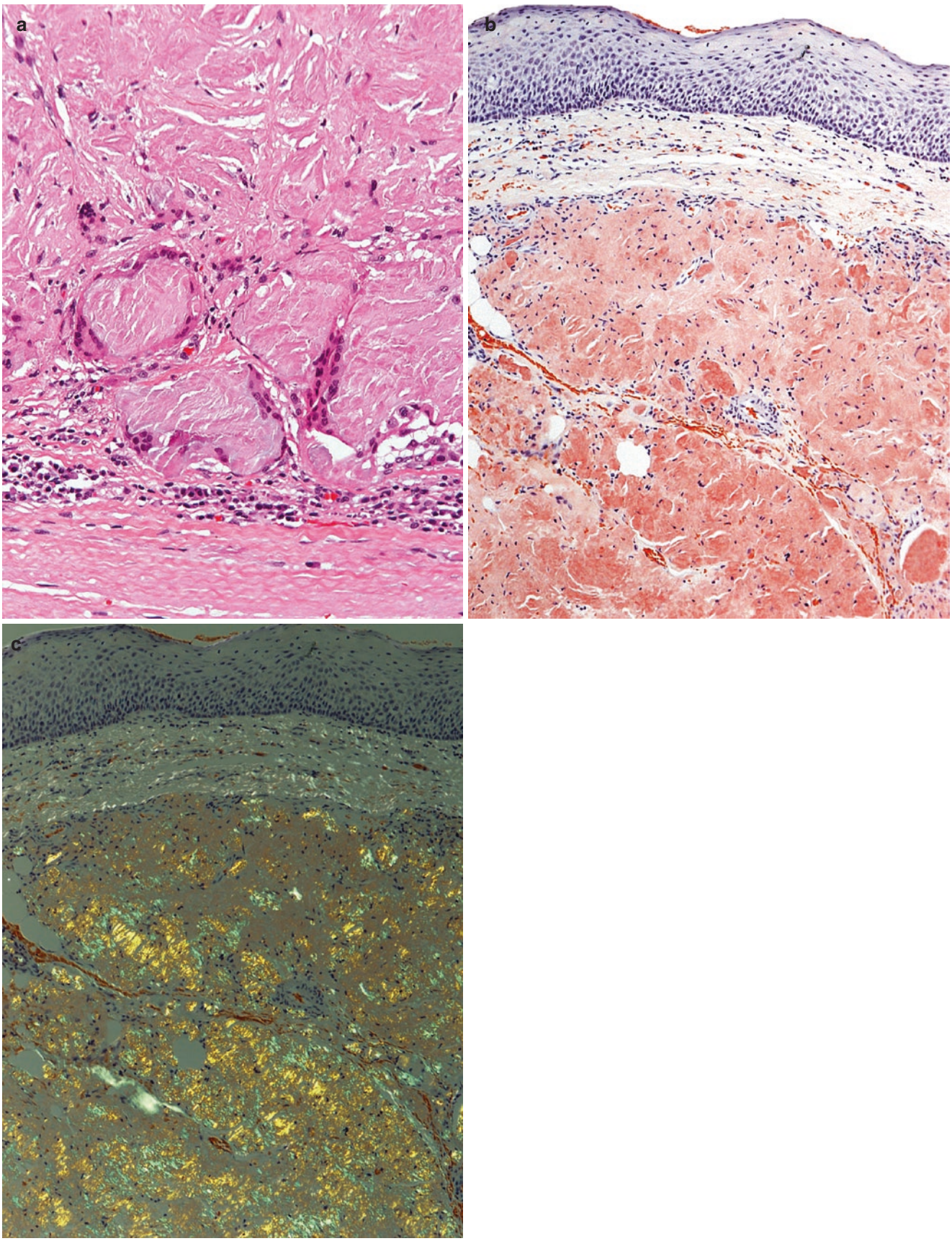


Fig. 7.22 Amyloidosis of the larynx. (**a**) Abundant homogenous eosinophilic deposits in the subepithelial stroma. (**b**) Reddish masses of amyloid stained by Congo red stain. (**c**) Amyloid deposits show green birefringence with polarized light

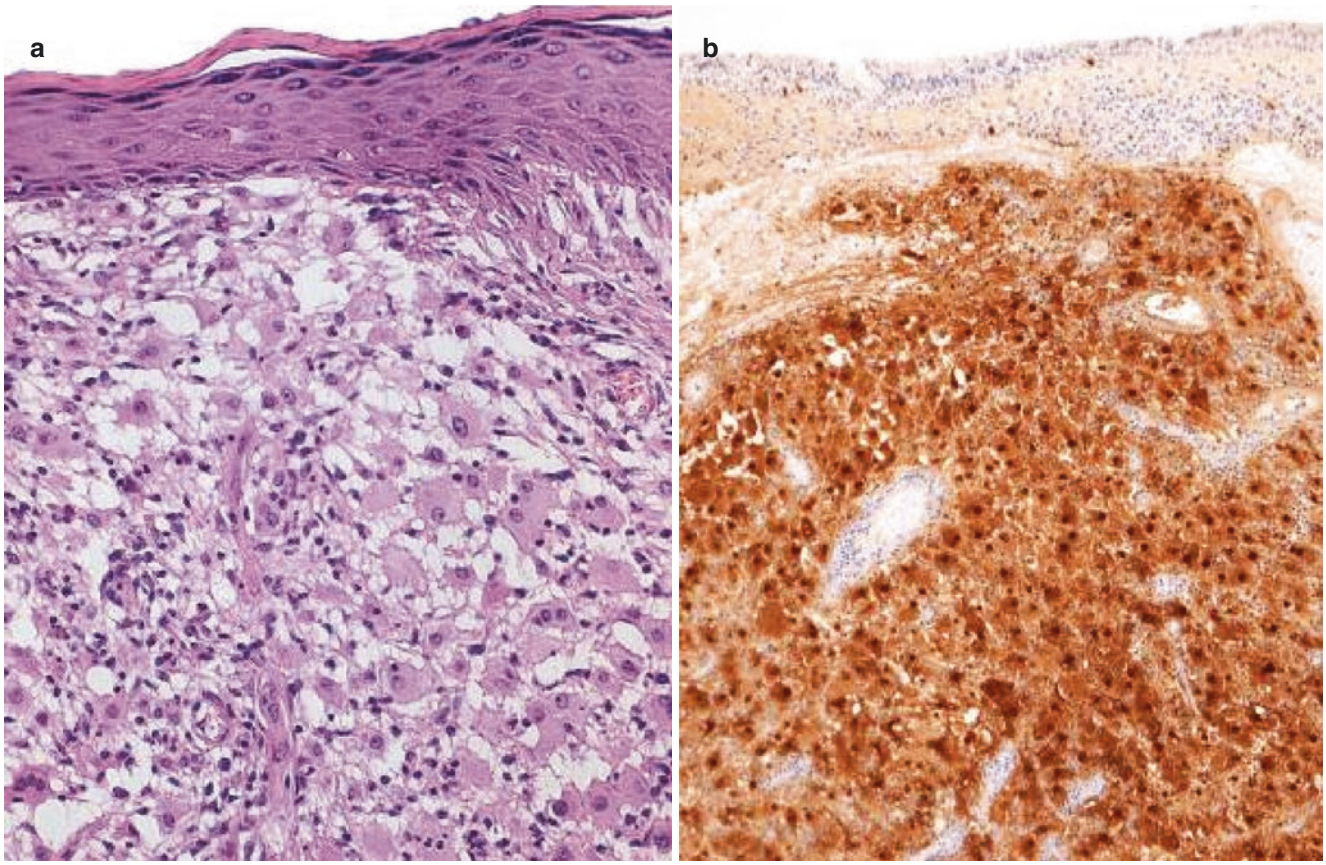


Fig. 7.23 Rosai-Dorman disease. (**a**) Subepithelial diffuse proliferation of histiocytes with pale or eosinophilic cytoplasm intermingled with lymphocytes and plasma cells. Histiocytes characteristically show phagocytosis of lymphocytes (emperipolesis). (**b**) The histiocytes are immunoreactive with S-100 protein

even death, if vital organs are affected. Exclusive extranodal disease is more frequent in elderly persons.

Macroscopy Laryngeal SHML usually manifests as a circumferential narrowing or polypoid mucosal lesion of a tan-white to yellow appearance. Vocal cord involvement results in impaired mobility [157]. The lesion can be also presented as a vocal cord ulceration [158].

Microscopy The laryngeal mucosa is almost diffusely infiltrated by lymphocytes, plasma cells, neutrophils and clusters of histiocytes. Histiocytes show various sizes, with a focally vacuolated pale to pink cytoplasm but ill-defined borders. Their nuclei are round or oval, sometimes vesicular, with well-defined central nucleoli. Lympho- and granulophagocytosis is evident in the cytoplasm of these histiocytes; the phenomenon is termed emperipolesis. No nuclear and cytoplasmic atypias are observed (Fig. 7.23a).

Immunohistochemistry The histiocytes are strongly positive for S-100 protein and Leu-M1 and variously positive for CD68 and CD163 (Fig. 7.23b).

Differential diagnosis It includes infectious diseases (rhinoscleroma), Wegener's granulomatosis, NK/T lymphoma of a nasal type, eosinophilic granuloma, Hodgkin lymphoma and fibroinflammatory disorders. Rhinoscleroma is characterised by a proliferation of large macrophages (Mikulicz cells) in which *Klebsiella rhinoscleromatis* can be identified. The phenomenon of emperipolesis is not found in this disease. Histologically, S-100-positive histiocytes are lacking in Wegener's granulomatosis, while NK/T lymphoma of a nasal type shows an infiltration of malignant lymphoid cells. Eosinophilic granuloma is histologically similar to Rosai-Dorfman disease but differentiation is possible with the morphologic specificities of Langerhans cells, characteristic of eosinophilic granuloma: their nuclei show lobulation, indentation or longitudinal grooving. However, a report has recently been published of a co-occurrence of Langerhans cell histiocytosis and SHML, which raises the question of whether these two disorders are related or perhaps represent a spectrum of the same disease [159].

Treatment There is no ideal treatment of the disease. Half of patients have spontaneous resolution. Surgical debulking using CO_2 laser is a treatment option for laryngeal obstruction.

7.11 Benign Neoplasms

7.11.1 Squamous Cell Papilloma

Squamous cell papillomas (SCPs) are the most common benign epithelial tumors of the larynx, causally related to low-risk human papillomavirus (HPV) infection. SCPs are discussed in detail in Chap. 1.

7.11.2 Benign Salivary Gland-Type Tumors

Benign salivary gland tumors are extremely rare in the larynx; there have been some reports of pleomorphic adenoma (PA) of the larynx [160]. Men predominate slightly over women; the patients' age ranges from 15 to 82 years [160]. The supraglottis is by far the most common site of origin. PA, which may grow up to several centimetres in diameter, occurs as a submucosal mass without ulceration. Histologically, PA shows all the characteristics of a tumor arising in the major salivary glands (see Chap. 5). The diagnosis of laryngeal PA should be considered very carefully due to its rarity in this location. Other differential diagnostic possibilities, such as chondrosarcoma, adenoid cystic carcinoma and mucoepidermoid carcinoma, need to be excluded.

7.11.3 Hemangioma (Infantile and Adult Types)

Definition Laryngeal hemangiomas (LH) are uncommon lesions of vascular origin, defined as benign proliferation of the blood vessels. They are divided into two distinct clinicopathologic entities: infantile (ILH) and adult forms (ALH).

Epidemiology ILH is a rare tumor characteristically involving the subglottic area. Subglottic ILH appears more frequently in girls. Coexistence of hemangiomas of the skin and mucosa of the oral cavity and pharynx, as well as in other organs [161], may also be an important indicator of the disease.

ALHs are more common than infantile forms; they are usually localised in the glottic and supraglottic region and are seen as inconspicuous submucosal, reddish blue lesions. Common symptoms are hoarseness, dyspnoea and/or foreign body sensation.

Clinical aspects Symptoms are present at birth and most children are symptomatic by 6 months of age. A progressive croup-like disease with inspiratory stridor turns into biphasic stridor as the obstruction progresses. Characteristically, the symptoms are intermittent, accentuated during crying, when the vessels are filled up under increased pressure [162, 163].

Macroscopy The gross appearance of the lesion ranges from a flat to polypoid, soft, compressible submucosal mass, pink to reddish to blue in colour. The lesion is usually one-sided, located in the posterolateral subglottic area. However, some hemangiomas are horseshoe shaped and present as a bilateral subglottic reddish swelling. Endoscopic biopsy is generally avoided because of an increased risk of excessive bleeding.

Microscopy The clinical evolution of ILH has a microscopic counterpart: early lesions consist of highly cellular aggregates of plump endothelial cells with developing vascular lumina. Endothelial cells show increased mitotic activity: with time, the vascular spaces enlarge, endothelial cells become more spindled and elongated, the number of mitoses decreases and, gradually, a proliferative fibrous tissue may dominate within the lesion. ALHs are more often of a cavernous type, consisting of large vascular spaces filled with erythrocytes, surrounded by flattened endothelial cells, usually devoid of mitotic activity (Fig. 7.24). Vascular tissue is intertwined with fibrous tissue. The surface epithelium is usually intact.

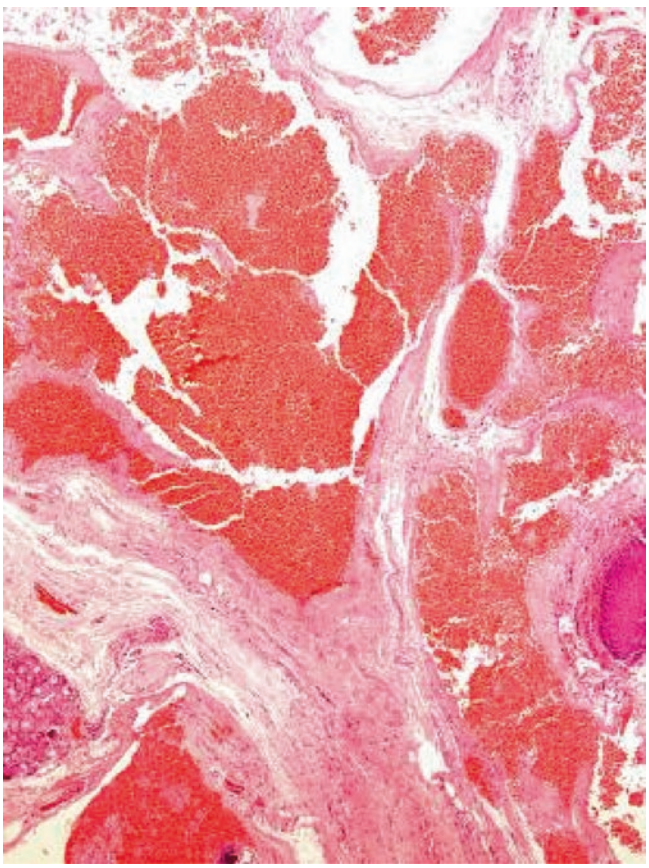


Fig. 7.24 Adult cavernous hemangioma. Proliferation of dilated, thin-walled vascular channels filled with erythrocytes and lined by flattened endothelial cells

Immunohistochemistry Endothelial cells classically express positivity for CD31, CD34, ERG transcription factor and factor XIIIa antigens. A recent multi-institutional study confirmed that most ILH are histologically and immunohistochemically similar to infantile hemangiomas in other locations and that the glucose transporter protein isoform 1 (GLUT 1) is an important diagnostic tool for discriminating vascular tumors from malformations [161, 164].

Differential diagnosis Various lesions of vascular origin, such as the vascular type of vocal cord polyp (VCP), intravascular papillary endothelial hyperplasia and even angiosarcoma, must be considered. The vascular variant of VCP is usually characterised by highly angiectatic vascular spaces surrounded by a massive leakage of fibrin as amorphous hyaline pink material, a feature not characteristic of capillary hemangioma. Papillary endothelial hyperplasia is an organisation of thrombosis with papillary proliferation of the endothelial cells, which is not a striking histological feature of hemangioma. The distinction between the cellular variant of hemangioma and angiosarcoma may sometimes be problematic. However, anastomoses of the vascular channels, lined by considerably pleomorphic endothelial cells with evident pathologic mitoses, certainly favour a diagnosis of angiosarcoma.

Treatment and prognosis Subglottic ILH is generally a self-limiting lesion but potentially fatal, causing progressive airway obstruction. Treatment is reserved for ILH that pose functional problems; several treatment modalities have been proposed, such as systemic steroids and interferon alfa-2a applications, CO_2 laser excision and tracheostomy. A case of successful treatment of an isolated subglottic ILH with propranolol was recently reported [165]. Various treatment modalities can be used for ALH, depending on the age of the patient, as well as the size and site of the tumor, including laser excision, systemic steroids and local sclerosing agents.

7.11.4 Paraganglioma

Definition Laryngeal paragangliomas (LPs) are rare benign tumors of the dispersed neuroendocrine organs (paraganglia) and are morphologically and cytochemically similar to the neural-crest-derived cells of the parasympathetic nervous system [166].

Epidemiology and etiology Patients are usually of middle age and women are more frequently affected. The predominant site is the supraglottic area in 82 %, followed by the subglottic in 15 % and the glottic in 3 % of cases. Signs and symptoms are mainly related to the localisation and size of the tumor. Laryngeal paraganglia are paired: superior and inferior ones. The former are localised in the false vocal cords, the latter in the vicinity of the cricoid cartilage [167]. LPs are rarely functional, multicentric head and neck paraganglioma, which are usually the result of hereditary or familial paraganglioma syndrome, which is transmitted in an autosomal dominant pattern with variable penetrance.

Macroscopy LPs usually present as a rounded submucosal mass with an intact covering mucosa, ranging in size from 0.5 to 6 cm. The tumors are firm; on the cut surface, they may be homogenous or nodular, from pink to tan and dark red in colour. A prominent vascularity of the tumor may cause abundant bleeding during biopsy.

Microscopy LPs are composed of two cell types: chief and sustentacular or supporting cells. The chief cells of epithelioid appearance are packed into round nests showing an organoid pattern, surrounded by highly vascular fibrous tissue (i.e. Zellballen) (Fig. 7.25a). However, the characteristic cell nests may be squeezed and not apparent in a small biopsy specimen. The chief cells typically have an eosinophilic, finally granular cytoplasm and central vesicular nuclei. Cellular pleomorphism may be present and is occasionally prominent but prognostically unimportant. Rare mitoses can be found, usually less than two to three per ten high-power fields. The supporting cells are inconspicuous, spindle shaped and usually found at the edge of the cell balls [168].

Immunohistochemistry Immunohistochemical findings are characteristic and decisive for the diagnosis. The chief cells are positive for neuroendocrine markers, such as chromogranin, synaptophysin and neuron-specific enolase (Fig. 7.25b).

Paragangliomas usually stain negatively for epithelial markers, such as cytokeratin, epithelial membrane antigen, carcinoembryonic antigen and calcitonin. Sustentacular cells are positively stained with S-100 protein and glial fibrillary acid protein [168].

Genetics The importance of *SHDB*, *SHDC* and *SHDD* gene mutations has been highlighted in head and neck familial paraganglioma syndromes [169]. LPs, in contrast to carotid body paraganglioma, are exceptionally associated with multicentric or familial paraganglioma [170]. However, it was postulated that sporadic head and neck paragangliomas have deletions at the same or closely related loci (11q13 and 11q22–23) as their family counterparts [171].

Differential diagnosis LPs must be primarily differentiated from typical and atypical carcinoids. The most reliable aid is positivity for both epithelial (cytokeratin, epithelial membrane antigen and carcinoembryonic antigen) and neuroendocrine markers in both types of carcinoids [172]. Other,

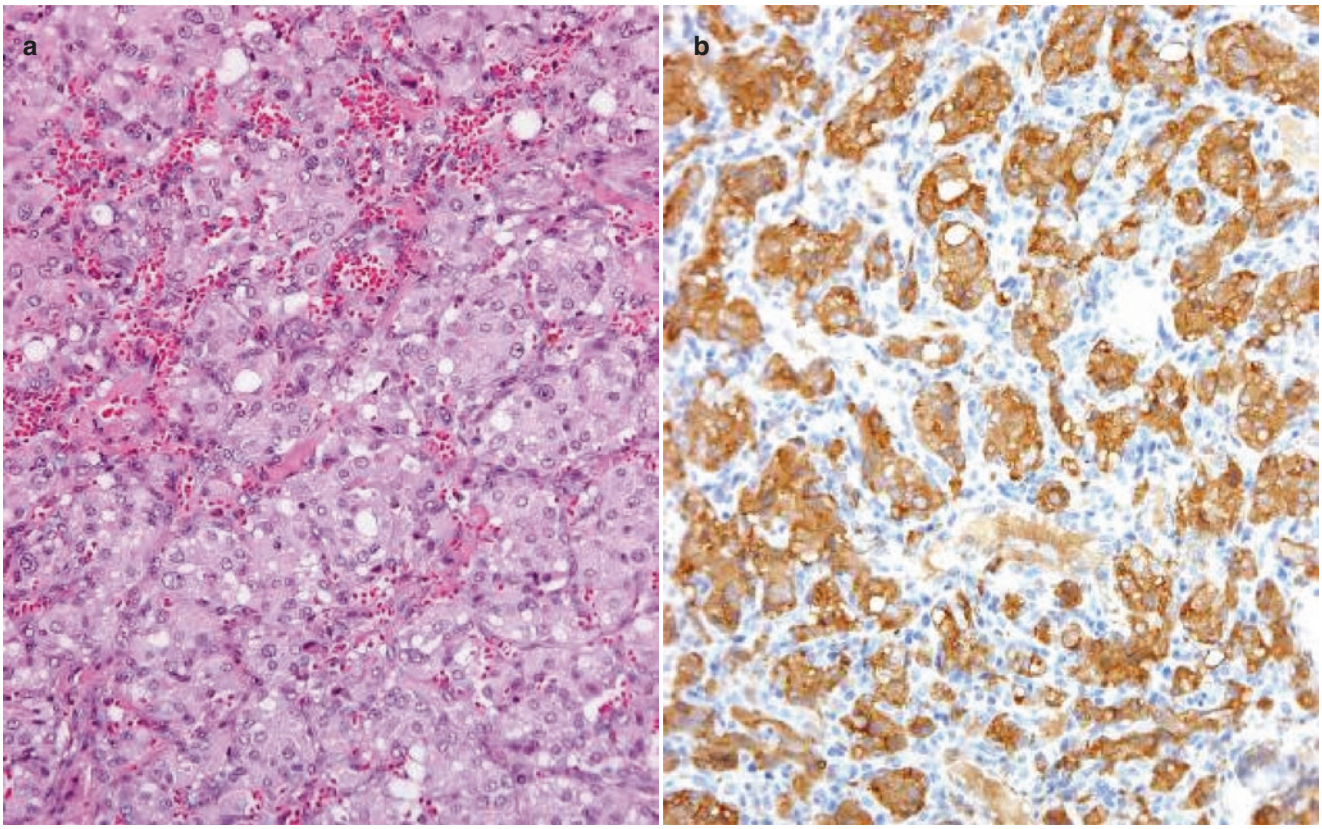


Fig. 7.25 Paraganglioma. (**a**) Characteristic alveolar pattern (Zellballen) of central chief and peripheral sustentacular cells; the nest of cells are surrounded by a thin fibrovascular stroma. (**b**) Chief cells stain immunohistochemically for synaptophysin

more remote differential diagnostic possibilities include malignant melanoma, metastases of renal cell carcinoma and medullary thyroid carcinoma. Melanoma can be confirmed by S-100, melan-A and HMB-45 positivity. Renal cell carcinoma, in contrast to paraganglioma, does not express neuroendocrine markers. Medullary carcinoma expresses positive staining for calcitonin, amyloid and CEA [168].

Treatment and prognosis Since paragangliomas are only exceptionally malignant, conservative surgical treatment is suggested. There are no histological criteria that can reliably predict the biological behaviour of the lesion.

7.11.5 Granular Cell Tumor

Definition Granular cell tumor (GCT) is an uncommon benign, slowly growing tumor, presumably of Schwann cell origin [173].

Epidemiology and etiology The tongue and subcutaneous tissue of the head and neck are the most common sites of the tumor, while laryngeal involvement, less frequent [174–176], comprises about 10 % of all cases [175]. GCT typically appears between the fourth and fifth decades; the average age for laryngeal forms is 36 years [176]. The tumor rarely occurs in children.

Clinical aspects GCT most commonly appears in the posterior area of the true vocal cords and half of them extend into the subglottis as a smooth, polypoid and sessile lesion [175, 176]. Hoarseness, stridor and dysphagia are the most common complaints.

Microscopy GCT is poorly circumscribed and consists of clusters and sheets of rounded and polygonal cells with indistinct cellular borders and small, bland-looking and central nuclei. A mild degree of nuclear pleomorphism may be present but mitotic activity is low. Cytoplasm of tumorous cells is abundant, characteristically coarsely granular and eosinophilic. Cytoplasmic granules are periodic-acid-Schiff positive and resistant to digestion. Marked desmoplasia is often present in older lesions, thereby masking the presence of granular cells. In about 50–60 % of cases, the covering epithelium shows pseudoepitheliomatous hyperplasia of the overlying squamous epithelium. This curious histological

feature, with an infiltrative growth of islands of squamous epithelium, may be mistaken for squamous cell carcinoma (Fig. 7.26a, b). However, the coexistence of GCT and true squamous cell laryngeal carcinoma has also been reported. Malignant variants of GCT should meet three out of six criteria: a high nuclear and cytoplasmic ratio, pleomorphism, increased mitotic activity, vesicular nuclei with large nucleoli, spindling and necroses [176].

Immunohistochemistry Positivity for S-100 protein, vimentin, CD68 and NSE and negativity for keratin is in accordance with the proposed theory of origin (Fig. 7.26c).

Differential diagnosis It should include benign lesions such as rhabdomyoma, paraganglioma or histiocytic proliferations. In contrast to GCT, rhabdomyoma does not show infiltrative growth; its cells are larger, with well-defined cellular borders and evidence of cross-striation. Paraganglioma typically shows an organoid pattern (i.e. Zellballen) and positivity for neuroendocrine markers. Proliferation of histiocytes is usually related to inflammatory reaction. Sheets of histiocytes are characteristically intermingled with inflammatory cells not commonly found in GCT. Covering pseudoepitheliomatous hyperplasia of the GCT may lead to incorrect diagnosis of squamous cell carcinoma. An identification of the underlying granular cells may resolve this, sometimes difficult, diagnostic problem.

Treatment and prognosis Complete surgical excision, with an attempt to preserve the normal structures, is the advised treatment option.

7.11.6 Inflammatory Myofibroblastic Tumor

Definition and epidemiology Inflammatory myofibroblastic tumor (IMT) is clinicopathologically a well-defined fibroinflammatory proliferative lesion with unpredictable biological behaviour. Lung, gastrointestinal and genitourinary tract systems are the commonest sites for IMT, although the lesion has been reported throughout the body [177]. It rarely affects the head and neck region and only a few well-documented IMTs have been found in the larynx and pharynx [177–182].

Most reported laryngeal IMTs are polypoid or pedunculated lesions that occur in the true vocal cords or in the subglottic area. Hoarseness, foreign body sensation, dyspnoea and stridor are presenting symptoms. Patients with laryngeal IMTs are mainly adult males [177].

Microscopy IMT is composed of myofibroblastic spindle cells, admixed with a prominent infiltrate of lymphocytes, plasma cells and neutrophils. The nuclei of the spindle cells are elongated and slightly polymorphous, containing one or more small nucleoli; the cytoplasm is pale eosinophilic (Fig. 7.27). Occasional regular mitoses are seen. Inflammatory cells are unevenly distributed within the lesion.

Three basic histological patterns have been described: (a) myxoid/vascular pattern, resembling inflammatory granulation tissue, (b) compact spindle cell pattern with fascicular and/or storiform areas of various cellular density and (c) hypocellular pattern, densely collagenised and reminiscent of a fibrous scar.

Immunohistochemistry Immunohistochemistry confirms the myofibroblastic phenotype of the spindle cells, which are typically reactive to vimentin, smooth muscle actin and muscle-specific actin [178]. Additionally, ALK1 and/or p80 have been reported in a cytoplasmic pattern in 40 % of cases of IMT [182]. Both markers are useful indicators of a 2p23 abnormality, suggesting the neoplastic nature of positive cases of IMT. However, it must be interpreted in the context of histologic and other clinicopathologic data if used as an adjunct to differential diagnosis [182].

Genetics The etiology of the lesion is unknown. It is now considered a true neoplasm of unpredictable biological behaviour, with various chromosomal abnormalities involving 2p.23, or fusion of the ALK gene with tropomyosin 3 (*TPM3-ALK*) or tropomyosin 4 (*TPM4-ALK*) [180]. Comparative genomic hybridisation in one case also revealed losses on 13q14–22, which is the location of the tumor-relevant retinoblastoma gene [180].

Differential diagnosis It includes mainly spindle cell carcinoma and low-grade myofibrosarcoma. The former tumor is composed of a population of pleomorphic spindle cells with increased mitotic activity; a transition from conventional squamous cell carcinoma to spindle-shaped cells is frequently present. The latter is a highly cellular tumor; spindle cells are arranged in non-alternating fascicles, nuclei are mostly uniform, although focally enlarged and pleomorphic with increased mitoses, and an infiltrative growth pattern, nuclear atypias and possible necroses favour a diagnosis of low-grade myofibrosarcoma.

Treatment and prognosis Radical excision of the lesions has been reported to be curative in more than 90 % of extrapulmonary IMTs, including head and neck lesions. Six of seven patients with laryngeal IMTs in Wenig's series were free of disease over periods from 12 to 36 months after complete excision. In one patient, laryngectomy was required after recurrences of the disease [177]. A metastatic potential has been exceptionally noted in patients with abdominal and mediastinal IMTs. However, a fatal outcome of a patient with IMT of the paranasal sinuses has been recently

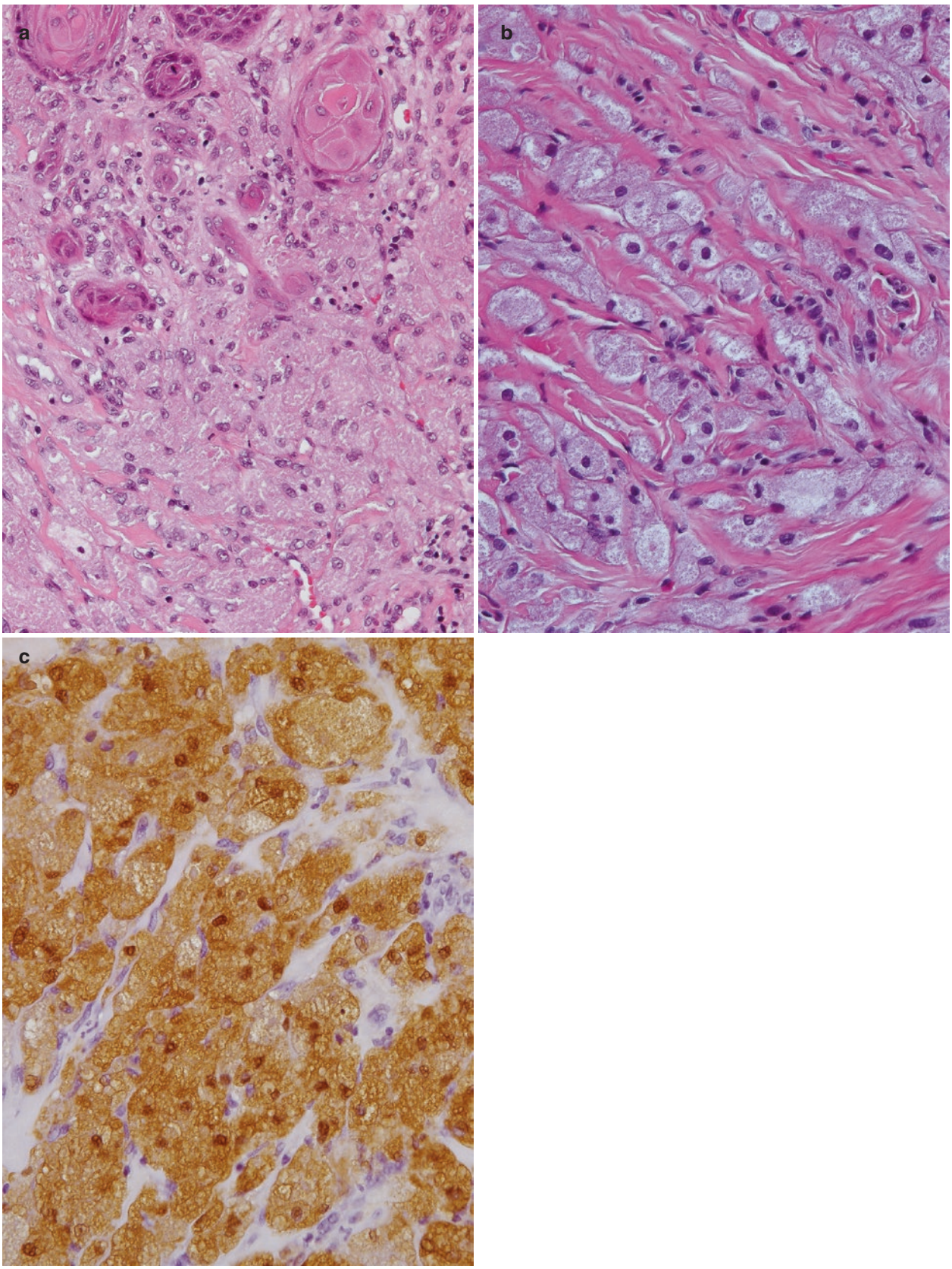


Fig. 7.26 Granular cell tumor. (**a**) Diffuse proliferation of large polygonal cell with granular cytoplasm. The covering squamous epithelium shows pseudoepitheliomatous hyperplasia, mimicking invasive squamous cell carcinoma. (**b**) Higher magnifications of polygonal cells with prominent granular cytoplasm, the cells are surrounded by collagen fibres. (**c**) The neoplastic cells are strongly positive for S-100 protein

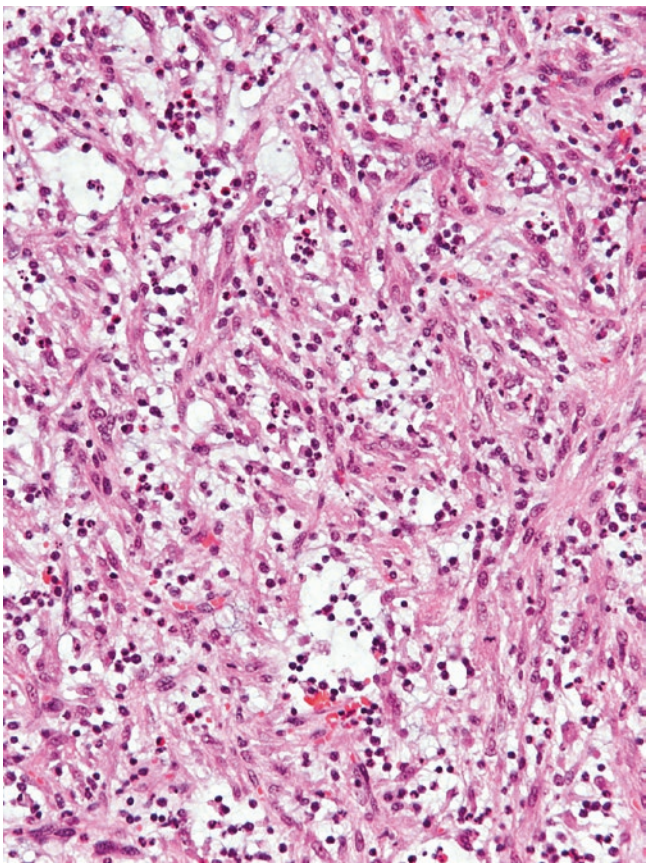


Fig. 7.27 Inflammatory myofibroblastic tumor. Oedematous myxoid stroma is diffusely infiltrated by variable inflammatory cells and spindle shaped myofibroblasts with oval nuclei and abundant pale eosinophilic cytoplasm

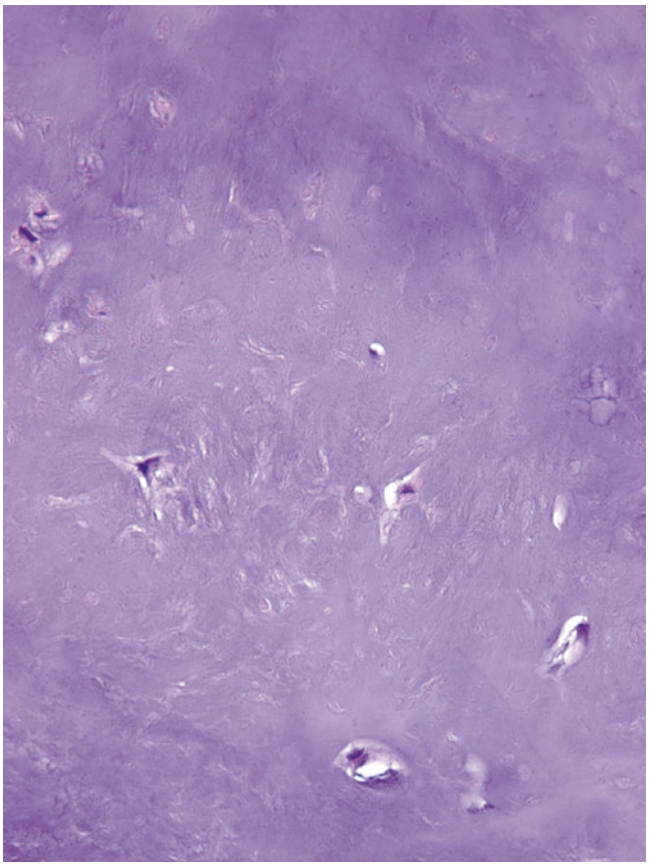


Fig. 7.28 Chondroma. Chondroid tissue with bland-looking chondrocytes resembling normal cartilage

reported. Aggressive behaviour supports recent observations that IMT is a true neoplasm rather than a reactive myofibroblastic proliferation [181].

7.11.7 Chondroma

Definition Chondromas of the larynx are exceedingly uncommon, well-circumscribed, small (less than 2 cm) cartilaginous tumors, which most commonly originate from the posterior lamina of the cricoid (70–78 %) and thyroid cartilage (15–20 %) [183–187] and exceptionally from the epiglottis [185].

Epidemiology and etiology They are more common in men than in women; the peak incidence rate is in the fifth decade [184]. The development of chondromas in the older population is probably related to an alteration of the ossification process, which starts in the cricoid and thyroid cartilages in the third decade and increases with advanced years [187].

Clinical aspects Hoarseness, dyspnoea and dysphagia are the usual complaints of patients, but the tumors may be also asymptomatic. Computed tomography is the method of choice for radiological evaluation [186].

Microscopy Chondromas show a characteristic well-defined lobular pattern, with benign-looking and evenly distributed chondrocytes, which lack nuclear pleomorphism and mitotic activity (Fig. 7.28). The cellularity is judged to be low when a given high-power (40×) field is unlikely to contain more than 40 nuclei of chondrocytes [185].

Differential diagnosis Pathologic diagnosis of laryngeal chondroma, especially from a small biopsy specimen, should be reported with due reservation. It has become apparent that many of the so-called chondromas from the past, which recurred locally, were actually misdiagnosed low-grade chondrosarcomas [187]. It is obvious that the distinction between chondroma and low-grade chondrosarcoma remains a very difficult task. Increased cellularity, nuclear pleomorphism and hyperchromasia, as well as the appearance of clusters of malignant-looking chondrocytes in a single lacuna, are the most conspicuous histological features of

chondrosarcoma. A thorough examination of the entire specimen is suggested to try to avoid an incorrect diagnosis of a given tumor. Chondromas should also be distinguished from laryngeal chondrometaplasia, which appears as small nodules of the fibroelastic cartilage in the submucosal tissue of the glottic region.

Treatment Local conservative excision is the preferred treatment of laryngeal chondromas. Each recurrence of the lesion should be considered a low-grade chondrosarcoma [187].

7.12 Malignant Neoplasms

Squamous cell carcinoma is the most common malignant tumor of the larynx, hypopharynx and trachea. Neuroendocrine carcinoma, adenocarcinoma, lymphoma, malignant melanoma, sarcoma and metastatic tumors may also occur at these locations.

7.13 Squamous Intraepithelial Lesions

Squamous intraepithelial lesions of the larynx (SILs) represent a wide spectrum of epithelial morphologic changes, ranging from benign, reactive lesions to potentially malignant (risky epithelium) and intraepithelial carcinoma. SILs are discussed in detail in Chap. 1.

7.14 Invasive Squamous Cell Carcinoma

Definition Squamous cell carcinoma (SCC) is by far the most common malignant tumor of the larynx and hypopharynx, accounting for about 95–96 % of all malignant tumors at this location. The majority are conventional type SCC.

Epidemiology SCC of the larynx and hypopharynx is the second most common respiratory cancer after lung cancer [188]. It accounts for 1.6–2 % of all malignant tumors in men and 0.2–0.4 % in women [189–191]. Its incidence is increasing in much of the world, being slightly higher in urban than in rural areas. It is also higher among blacks than whites [188, 192].

Laryngeal and hypopharyngeal SCC occur most frequently in the sixth and seventh decades of life. It rarely occurs in children and adolescents [193, 194]. It is more common in men, with a male-to-female ratio of about 5:1 worldwide [188, 192]. The male-to-female ratio is decreasing in some countries, reflecting a greater incidence among women. The increasing incidence of laryngeal cancer in women has been attributed to the increased incidence of smoking over the last two decades [195].

Etiology Cigarette smoking and alcohol consumption are the most important risk factors in laryngeal and hypopharyngeal cancer. Among them, smoking is more important. Epidemiological studies have shown that the relative risk of laryngeal cancer associated with cigarette smoking is approximately ten for all subsites of the larynx and hypopharynx. An independent role of alcohol from that of tobacco is less striking, although plausible [196] and evidenced in some studies [197]. Smoking and drinking combined have a multiplicative rather than additive effect [198–202]. Avoiding of smoking and alcohol consumption could prevent about 90 % of current incidence of laryngeal cancer, detecting cigarette smoking as the main risk factor [203].

Some other factors, such as gastroesophageal reflux, diet and nutritional factors [196, 204–208] have been also related to increased risk of laryngeal cancer development, particularly in patients who lack the major risk factors [209, 210].

Much attention has been recently paid to the possible role of infection with human papillomavirus (HPV) in the pathogenesis of laryngeal and hypopharyngeal cancer but the results are conflicting. HPV, mainly type 16, has been found in 3–85 % of laryngeal cancers [211, 212] and also in 12–25 % of individuals with a clinically and histologically normal larynx [213, 214]. Recent studies have shown expression of p16 and p21 proteins in a subset of laryngeal SCC [215], which are considered as robust markers of HPV-driven tumors. In some of them, HPV DNA was detected by in situ hybridisation or RT-PCR. However, using an additional methodologic approach, i.e. RT-PCR for HPV E6/E7 mRNA, no evidence of transcriptionally active high-risk α-PV was found. It appears, therefore, that HPV infection plays little role, if any, in laryngeal carcinogenesis [4, 211, 216, 217].

Macroscopy SCC of the larynx and hypopharynx may present as a polypoid exophytic lesion, as a flat tumor with raised edges, or as an ulcerative endophytic lesion.

Microscopy The main microscopic feature of invasive SCC of the larynx and hypopharynx is the evidence of squamous differentiation, i.e. keratinization with or without keratin pearl formation and/or intercellular bridges, and the evidence of invasive growth. Invasion is manifested by interruption of the basement membrane of the surface epithelium and the downward growth of tumor islands or isolated tumor cells in the underlying tissue. Invasive growth is usually accompanied by desmoplastic stromal reaction which consists of proliferation of myofibroblasts, excessive deposition of an extracellular matrix, and neovascularisation [218].

Tumor cells may invade the lymphatic and blood vessels or spread along the nerves; the presence of vascular and perineural invasion is a reliable proof of invasive tumor growth.

SCC is usually graded into well-, moderately, and poorly differentiated SCC according to the degree of differentiation,

cellular pleomorphism and mitotic activity, as described in details in Chap. 1.

7.14.1 Histological Variants of Squamous Cell Carcinoma

Several variants of SCC occur in the larynx and hypopharynx, including verrucous carcinoma, spindle cell carcinoma, basaloid SCC, papillary SCC, lymphoepithelial carcinoma, adenoid (acantholytic) SCC and adenosquamous carcinoma, which are dealt with separately in Chap. 1. Their recognition is important because most of them are true clinicopathologic entities, with an important prognostic implication: basaloid SCC, adenosquamous carcinoma and lymphoepithelial carcinoma are more aggressive than conventional SCC, while verrucous SCC and, arguably, papillary SCC have a better prognosis than conventional SCC.

It seems that in the larynx, basaloid and papillary SCC are not aetiopathogenetically related to the infection with HPV as it has been proven for these variants of SCC at some other locations, particularly in the oropharynx [215, 219]. Recent studies, using sensitive and specific PCR-based assays, suggest that even verrucous carcinoma is not a HPV-associated tumor, not only in the larynx but also at other sites in the head and neck region [220–222].

7.14.2 Anatomic Sites and Its Impact on Clinical Features, Treatment and Prognosis

Larynx is anatomically divided into three compartments: supraglottic, glottic and subglottic. Superiorly and posteriorly, it is continuous with the hypopharynx. Because of this anatomic proximity, the tumors of the larynx often extend to the hypopharynx, as well as vice versa, so that in large tumors, it is impossible to determine whether it originates from the larynx or hypopharynx.

There are geographic differences in the topographic distribution of the laryngeal SC [193, 223]. In France, Spain, Italy, Finland and the Netherlands, supraglottic SCC predominates, while in the USA, Canada, England and Sweden, glottic SCC is more common. In Japan, SCC is approximately equally distributed between the two sites. The rarest localisation of laryngeal cancer is the subglottis (1–5 %) [193, 224].

Determining the primary site of origin of laryngeal/hypopharyngeal cancer is important as it has a significant impact on the clinical presentation, spread, behaviour and prognosis [225, 226]. This, however, is not always possible, especially in large tumors.

Supraglottic SCC The most common location of supraglottic SCC is the epiglottis (45–55 % of supraglottic cancer) (Fig. 7.29a), followed by the false vocal cords (12–33 %) and the aryepiglottic folds (8–21 %). The remaining cases arise from the ventricles and the arytenoids [193]. Supraglottic SCC tends to spread to the oropharynx and pyriform sinus, but it rarely invades the glottis and thyroid cartilage.

The most common symptoms in supraglottic cancer are dysphagia, change of the quality of voice, a sensation of foreign body in the throat, haemoptysis and odynophagia.

The lymph node metastases are present in 30–40 % of patients. The overall 5-year survival rate in supraglottic SCC is 65–75 % [193, 223].

Glottic SCC arises mostly from the anterior half of the vocal cord or from the anterior commissure (Fig. 7.29b); posterior origin is rare. Because of poor lymphatic supply, glottic SCC tends to remain localised for a long period. As SCC progresses, it invades the vocal muscle resulting in the fixed vocal cord which is an ominous clinical sign [227]. In late stages of the disease, it may extend to the opposite true vocal cord and to the supraglottis and subglottis; it may also extend through the thyroid cartilage and invade the soft tissue of the neck.

The most common early symptom in glottic cancer is hoarseness. Other symptoms include dysphagia, change of the quality of voice, a sensation of foreign body in the throat, haemoptysis and odynophagia.

The incidence of lymph node metastases in early stages is low (0–11 % for T1 and T2) and increases to 14–40 % in advanced stages. The overall 5-year survival rate is 80–85 % [193, 223].

If SCC crosses the ventricles and involves the supraglottis and glottis, it is termed *transglottic SCC* (Fig. 7.29c). Transglottic carcinoma is rare, accounting for 5 % of all laryngeal SCC, and is associated with a high incidence of lymph node metastases and a poor prognosis [228].

Subglottic carcinoma is rare, involving the region extending 1 cm below the true vocal cord between the lower edge of the true vocal cord and the first tracheal cartilage. The most common presenting symptoms are dyspnoea and stridor [229] often requiring an emergency tracheotomy [230]. The subglottic SCC may spread to the thyroid gland, cervical oesophagus, hypopharynx and trachea.

About 20–25 % of patients have cervical lymph node metastases at presentation, but about 50 % of patients have clinically undetectable metastases in the paratracheal lymph nodes. It has been therefore suggested that paratracheal and superior mediastinal nodes should be removed in patients with subglottic cancer. A frequent complication in the course of the disease is a stomal recurrence, which is defined as a recurrent SCC in the mucocutaneous junction of the tracheostomy after laryngectomy [231–233]. The overall 5-year survival rate in subglottic SCC is 40–47 % [193, 224].

Hypopharyngeal SCC occurs most frequently in the pyriform sinus (60–85 % of hypopharyngeal SCC) (Fig. 7.29d)

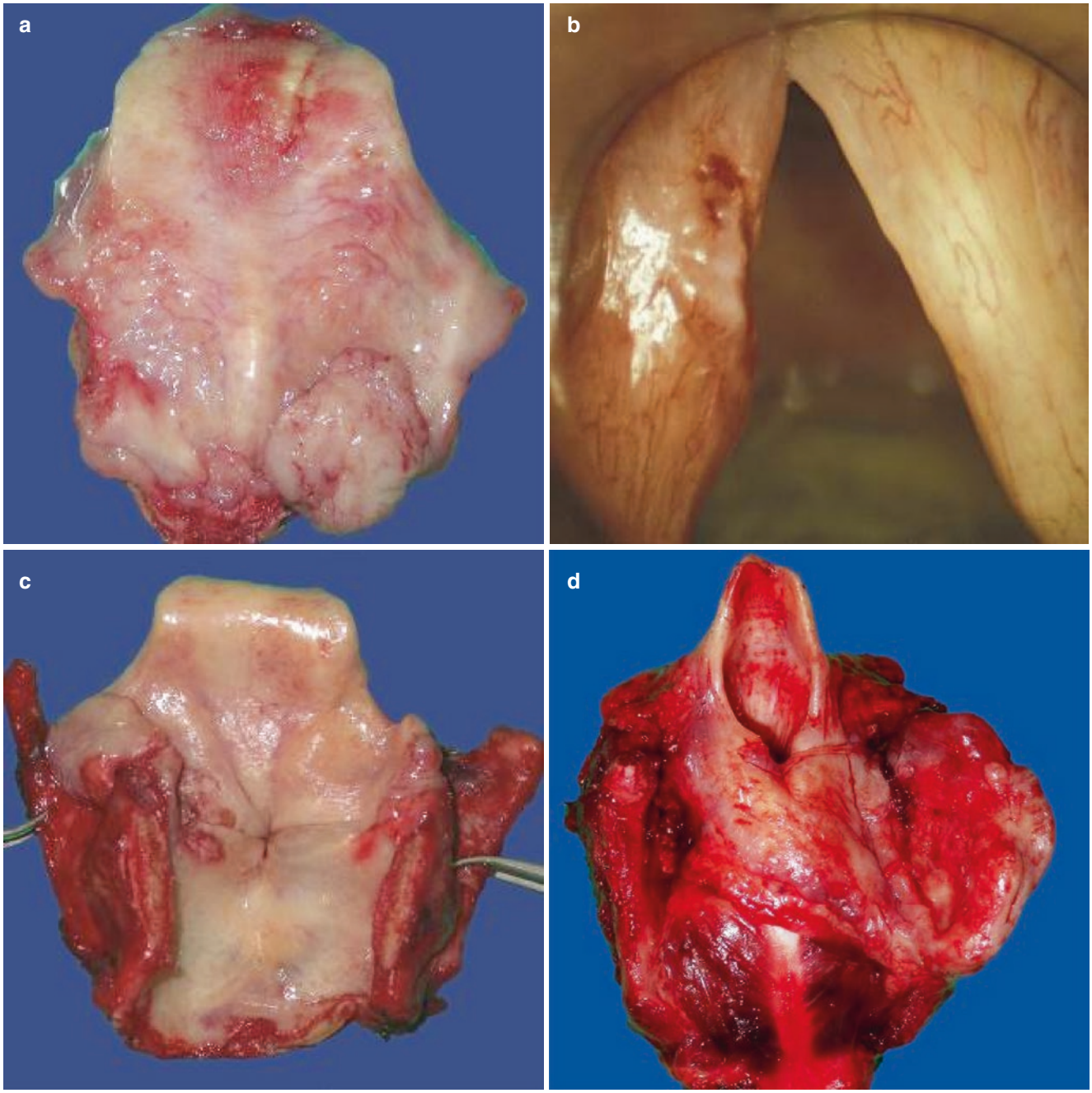


Fig. 7.29 Macroscopic appearance of the squamous cell carcinoma of the larynx. (**a**) Supraglottic carcinoma: an exophytic tumor at the base of the epiglottis. (**b**) Carcinoma of the vocal cord, endoscopic view. (**c**) Transglottic carcinoma crossing the ventricles, involving glottis and supraglottis. (**d**) Carcinoma of the hypopharynx: a large, exophytic, ulcerated tumor in the pyriform sinus

and rarely in other localisations, such as the posterior pharyngeal wall (10–20 %) and postcricoid area (5–15 %) [234]. Hypopharyngeal cancer frequently extends to the larynx. The most frequent symptoms in hypopharyngeal SCC are odynophagia, dysphagia and neck mass. Other symptoms include voice changes, otalgia and constitutional symptoms.

Prognosis of hypopharyngeal cancer is poor. Because of the rich lymphatic supply, unrestricted area for tumor growth and late symptoms, patients mostly present in advanced stages. About 70 % of patients have lymph node metastases at presentation. Haematogenous dissemination is rather frequent (in 20–40 % of patients) [235]. The overall 5-year survival rate in hypopharyngeal SCC is 62.5 % [235].

Treatment of laryngeal cancer depends on the stage of disease, location and size of the tumor and the patient's health

status and age. Treatment strategies are focused on surgical and nonsurgical procedures with the main aim of organ and function preservation. However, there is no widely accepted scheme on how to treat laryngeal cancer. Total laryngectomy was in the past, and is still in the present, the golden standard for advanced laryngeal cancer. Organ-preserving treatment modalities include cordectomy, partial laryngectomy and hemilaryngectomy as well as radiotherapy, chemotherapy and targeted molecular therapies. Recently, transoral endoscopic procedures, such as robotic surgery and laser surgery, have been introduced and are often superior to open approach in early cancers with less morbidity [236].

There is also no unified strategy for early glottic cancer (T1 and T2) treatment. Some centres prefer radiotherapy while others use laser excision. The reported rates of local control of early glottic cancers treated with radiotherapy alone range from 84 to 95 % [236].

Transcutaneous open partial laryngectomy (horizontal and vertical) is applied for treatment of early and advanced laryngeal cancer or a combination of radiotherapy and chemotherapy. Partial laryngectomies are also used for salvage surgery when radiotherapy and chemotherapy fail. Total laryngectomy still remains an efficient treatment modality for advanced cancers as an initial therapy or for recurrent laryngeal cancers following unsuccessful nonsurgical treatment [236].

Prognostic significance of cervical nodal status is dealt with in Chap. 1 and surgical procedures used for removal of evident or occult cervical metastases in Chap. 9.

7.14.3 TNM Grading

For the staging of laryngeal cancer, the TNM system (T-tumor, N-node, M-metastasis), established by the International Union Against Cancer (UICC), is widely used [237]. The stage remains the most significant predictor of survival. The size of the tumor and the presence of regional and distant metastases are independent predictors of survival. Consistently, the prognosis varies from excellent for patients with early disease (stages I and II), with more than 90 % 5-year survival rate, to poor for patients with advanced disease (stages III and IV), with less than 60 % 5-year survival [191].

An important parameter ignored in the TNM system is the extracapsular spread. Several studies have shown that the presence of extracapsular spread in lymph nodes is strongly associated with both regional recurrence and distant metastases resulting in a decreased survival [238–241]. Some studies, on the contrary, have not confirmed the independent prognostic significance of the extracapsular spread [242, 243].

Immunohistochemistry, genetics and differential diagnosis of SCC are discussed in details in Chap. 1.

7.15 Neuroendocrine Carcinoma

Neuroendocrine carcinomas (NECs) are malignant epithelial neoplasms with neuroendocrine differentiation. This can be proven by positive immunohistochemical reaction against neuroendocrine markers, e.g. chromogranin A, synaptophysin, neural cell adhesion molecule (CD56), CD57, Leu-7 and neurofilament protein, and/or by electron microscopical demonstration of dense-core granules in the cytoplasm measuring 100–275 nm.

NECs of the larynx are uncommon, accounting for less than 1 % of laryngeal tumors. Their classification and grading have been recently the subject of debate, mostly due to reluctance to abandon old terminology (carcinoid, atypical carcinoid) instead of the newly proposed term “neuroendocrine carcinoma”. In the WHO classification [244], both old and new terminologies are included.

NECs are divided into well-differentiated NEC (WD-NEC) (carcinoid, NEC grade 1), moderately differentiated NEC (MD-NEC) (atypical carcinoid, NEC grade 2) and poorly differentiated NEC (PD-NEC) (NEC grade 3). MD-NEC is the most common type of NEC in the larynx, followed by PD-NEC and WD-NEC [193, 245].

The putative cells of origin are the Kultschitzky-like argyrophilic cells which have been described in the human laryngeal mucosa and are similar or identical to the Kultschitzky cells in the bronchial mucosa [246]. Other possible cells of origin are the pluripotent stem cells of the surface or glandular epithelium.

7.15.1 Well-Differentiated Neuroendocrine Carcinoma

Definition, epidemiology and clinical aspects Well-differentiated neuroendocrine carcinoma (WD-NEC) is the least common type of laryngeal NEC. In a critical review of the world literature, El-Nagar and Batsakis found only 12 well-documented cases of laryngeal WD-NEC [245]. Since then, few more cases have been described in the English literature [247–253].

WD-NEC occurs predominantly in males, the average age is 58 years, and the majority (83 %) are located in the supraglottis [245]. They present clinically with dyspnoea, hoarseness and/or sore throat.

Macroscopy Macroscopically, laryngeal WD-NEC is typically a submucosal nodule or a polypoid lesion measuring up to 2 cm in diameter.

Microscopy Like WD-NEC elsewhere in the body, they are composed of small uniform cells growing in islands, ribbons and cords, occasionally forming gland-like structures. Mucin

is occasionally present. The nuclei are round, with finely dispersed chromatin and inconspicuous nucleoli; the cytoplasm is scant, clear or eosinophilic. Mitoses are sparse or absent, and there is no necrosis or cellular pleomorphism.

Immunohistochemistry Laryngeal WD-NEC expresses markers of neuroendocrine differentiation (such as chromogranin, synaptophysin, CD56, neuron-specific enolase, Leu-7) and markers of epithelial differentiation (such as cytokeratins and epithelial membrane antigen). Electron microscopy reveals dense-core neurosecretory granules [245].

Differential diagnosis It includes moderately differentiated NEC, paraganglioma and adenocarcinoma and is discussed in the next section.

Treatment and prognosis The treatment of choice is a complete but conservative surgical excision. Neck dissection is not indicated. Radiotherapy and chemotherapy have not proven effective [254].

Prognosis is favourable, though metastases to the lymph node, liver, bones and skin have been reported in one-third of patients. Only one patient has died of the disease [245]. These data suggest a more aggressive behaviour of laryngeal WD-NEC compared to bronchial WD-NEC but the number of patients is too small to draw conclusions [193].

7.15.2 Moderately Differentiated Neuroendocrine Carcinoma

Definition, epidemiology and clinical aspects It is the most frequent type of NEC in the larynx constituting 54 % of all laryngeal NECs, with approximately 300 cases described in the literature [249].

Similarly to other types of NEC, MD-NEC is more common in males, with a wide age range from 20 to 83 years. The majority of patients are heavy smokers. It arises mostly in the supraglottic region. Hoarseness and dysphagia are the most common symptoms; 20–30 % of patients also experience pain [193, 255]. MD-NEC is rarely associated with carcinoid syndrome [256]. Some patients with MD-NEC have elevated level of the serum calcitonin [245, 257].

Macroscopy Macroscopically, it presents as a submucosal nodule or as a polypoid lesion measuring up to 4 cm in diameter (average 1.6 cm), with or without surface ulceration.

Microscopy Microscopically, the tumor grows in rounded nests, trabeculae, cords, ribbons and glandular structures; the tumor cells are round, with round nuclei and a moderate amount of cytoplasm which is slightly eosinophilic or occasionally oncocytic. Mucin production may be present [258].

In contrast to WD-NEC, cellular pleomorphism, increased mitotic activity and necroses are frequently present in MD-NEC. Vascular and perineural invasion may be present.

Immunohistochemistry MD-NEC usually expresses synaptophysin, chromogranin, CD56 and cytokeratin; they may also express calcitonin, carcinoembryonic antigens and rarely serotonin [249, 256, 258, 259].

Differential diagnosis It includes paraganglioma, adenocarcinoma, other neuroendocrine carcinomas and medullary carcinoma of the thyroid gland.

The differentiation between paraganglioma and MD-NEC is important because the former usually behaves as a benign tumor, while the latter behaves as an aggressive tumor. The correct diagnosis is usually possible with the use of immunohistochemistry: MD-NEC expresses cytokeratin and carcinoembryonic antigen (CEA), while paraganglioma does not. Both tumors express markers of neuroendocrine differentiation [249]. Adenocarcinoma can be distinguished from carcinoid by the absence of neuroendocrine markers. The presence of cellular pleomorphism, increased mitotic activity and necroses helps to distinguish MD-NEC from WD-NEC.

Differentiation from thyroid medullary carcinoma may be difficult, especially when dealing with cervical metastases, as tumor cells in both medullary carcinoma and MD-NEC express calcitonin by immunohistochemistry. The most important distinguishing feature is the different location of the primary tumors. Additional useful information may be obtained by measuring the serum level of CEA, which is elevated in metastatic medullary carcinoma of the thyroid and normal in MD-NEC [193]. The elevated serum level of calcitonin should not be considered as a reliable feature of medullary carcinoma, as it has been reported in patients with MD-NEC [245, 257].

Treatment and prognosis MD-NEC is an aggressive, potentially lethal tumor. Lymph node metastases have been reported in 43 % of patients, cutaneous metastases in 22 % and distant metastases in 44 % of patients, mostly to the lungs, liver and bones [256, 258, 259].

Surgery is the treatment of choice. Neck dissection is also advised because of the high incidence of cervical lymph node metastases. Radiation and chemotherapy have not been effective [254]. The 5- and 10-year survival rates are 48 % and 30 %, respectively [256].

7.15.3 Poorly Differentiated Small-Cell Neuroendocrine Carcinoma

Definition, epidemiology and clinical aspects Poorly differentiated neuroendocrine carcinoma (PD-NEC, small cell) is the least differentiated and the most aggressive type of

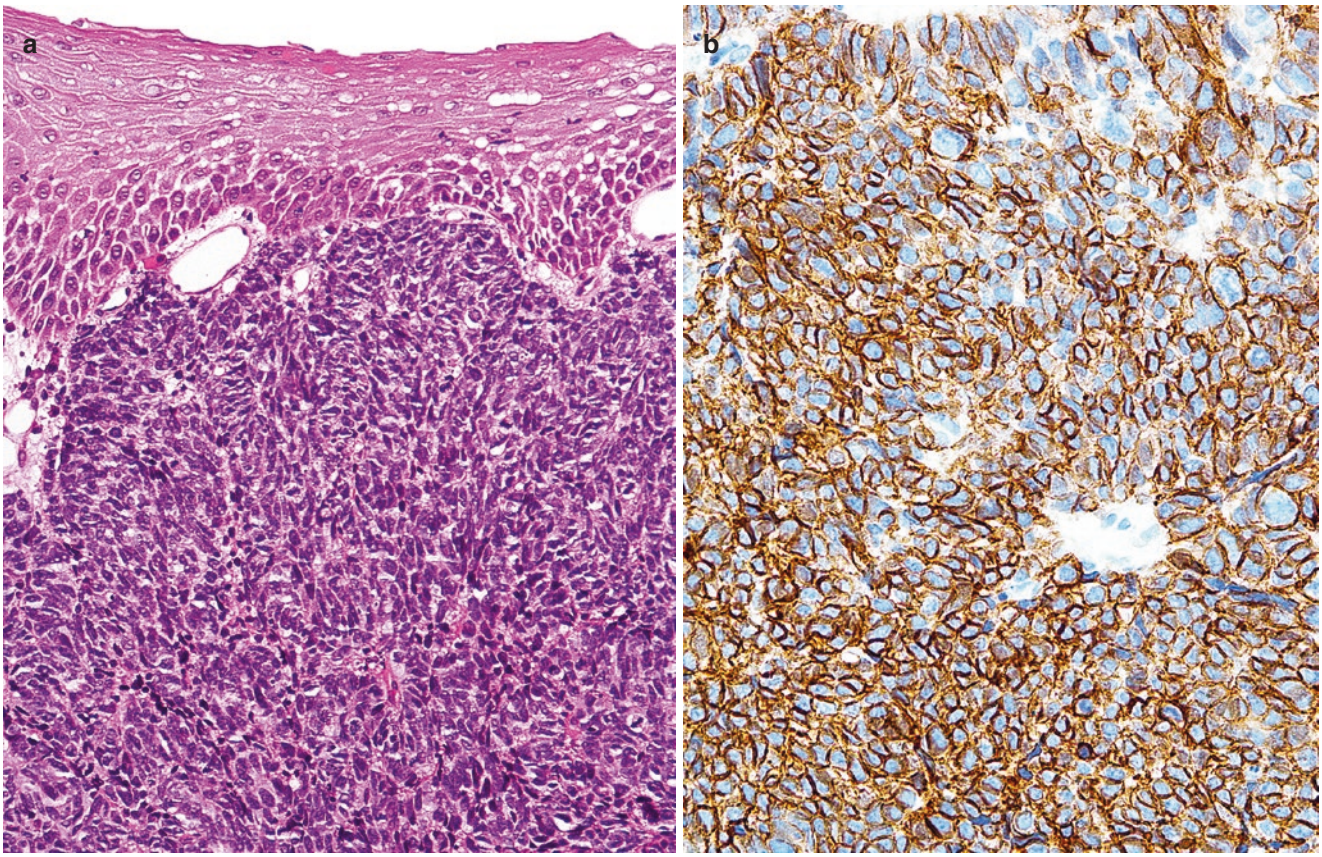


Fig. 7.30 Poorly differentiated neuroendocrine carcinoma of the larynx. (**a**) Islands of closely packed small cells with hyperchromatic nuclei beneath the surface epithelium. (**b**) Immunohistochemical expression of CD56 in the tumor cells

NEC. It is rare, accounting for less than 0.5 % of all laryngeal carcinomas. Approximately 160 cases have been described in the literature [260–262].

PD-NEC arises most often in the supraglottis, but it also occurs in other parts of the larynx. It affects men more frequently than women, mostly between 50 and 70 years of age; most patients are heavy smokers. The most common presenting symptoms are hoarseness and dysphagia, frequently associated with painless enlarged cervical lymph nodes due to metastases. It may be associated with a paraneoplastic syndrome [260].

Macroscopy PD-NECs are submucosal nodular or polypoid masses, frequently ulcerated and cannot be distinguished from other laryngeal carcinomas.

Microscopy Laryngeal small-cell PD-NECs are identical to their pulmonary counterparts [255]. They are composed of closely packed small cells with hyperchromatic round or oval nuclei with inconspicuous nucleoli and very scant cytoplasm (Fig. 7.30a). Necroses, mitoses and vascular and perineural invasion are frequently present. The mucosa is often ulcerated but there is no carcinoma in situ or significant atypia of the surface epithelium.

Immunohistochemistry The tumor cells variably express cytokeratins and neuroendocrine markers, such as synaptophysin, neuron-specific enolase, chromogranin, S-100 protein and CD56 (Fig. 7.30b).

By electron microscopy, sparse neurosecretory granules are occasionally found, but they may be absent.

Differential diagnosis In the differential diagnosis, the possibility of a metastasis from the lung must be excluded. PD-NEC must also not be confused with the basaloid squamous carcinoma, malignant lymphoma and malignant melanoma. Basaloid squamous carcinoma is composed of larger cells, contains areas of squamous differentiation, tends to stain for high-molecular-weight cytokeratins and is frequently associated with atypia of the overlying squamous epithelium. Malignant lymphomas characteristically express leukocyte-common antigen and B- or T-cell markers which are absent in PD-NEC. Malignant melanoma occasionally consists of small undifferentiated cells, thus resembling

PD-NEC but, in contrast to PD-NEC, it typically expresses S-100 protein, melan-A and/or HMB45.

Treatment and prognosis The clinical course is aggressive, characterised by early metastases to the regional lymph nodes and distant sites, especially to the lungs, bones and liver. In contrast to lung small-cell PD-NEC, laryngeal small-cell PD-NEC does not frequently metastasise to the brain.

Radiation with chemotherapy is the treatment of choice. Surgical therapy is not indicated because most patients have disseminated disease at presentation. Prognosis is poor; the 5-year survival rate is 5 % [262].

7.15.4 Poorly Differentiated Large-Cell Neuroendocrine Carcinoma

Definition, epidemiology and clinical aspects Some cases of laryngeal NEC are composed of larger cells with prominent nucleoli than small-cell PD-NEC, resembling closely the pulmonary large-cell NEC. In the WHO 2005, they were included in the category of the MD-NEC. However, there is merging evidence that these tumors behave aggressively, similar to large-cell NEC in other organs, including the lungs, and should be better regarded as a variant of PD-NEC or NEC grade 3 [263–265].

Laryngeal large-cell PD-NECs are rare, affecting older men and heavy smokers, and arise most frequently in the supraglottis [265].

Macroscopy Macroscopically, PD-NECs are usually ulcerated tumors which cannot be distinguished from other laryngeal carcinomas.

Microscopy Large-cell PD-NEC usually grows in a solid or basaloid pattern, with occasional rosette formation and nuclear palisading. It is composed of large, polygonal cells with a low nuclear-to-cytoplasmic ratio, coarse nuclear chromatin and prominent nucleoli. There is a high mitotic rate (>10 mitoses/mm^2 or ten per high-power field). Extensive necroses are usually present [265].

Immunohistochemistry The diagnosis of large-cell NPD-NEC must be confirmed by positive immunohistochemistry for at least one neuroendocrine marker.

Differential diagnosis Similarly to small-cell PN-NEC, the differential diagnosis in large-cell PD-NEC includes metastasis from the lung, poorly differentiated squamous cell carcinoma, malignant lymphoma and malignant melanoma. It was discussed in the previous section.

Large-cell PD-NEC must also be differentiated from small-cell PD-NEC. In contrast to small-cell PD-NEC, it has vesicular nuclei and prominent nucleoli, whereas small-cell PD-NEC has finely granular chromatin and inconspicuous nucleoli. Mitotic activity is the main criterion for distinguishing large-cell PD-MEC and MD-NEC: the former has at least ten mitoses per ten high-power fields (magnification 40×, or area of 2 mm^2), and the latter has less than ten mitoses per ten high-power fields [263].

Treatment and prognosis Large-cell PD-NECs of the larynx are rare, and there is no standardisation regarding therapy. Some authors recommend primary chemotherapy and radiation rather than primary surgery with postoperative chemotherapy. The prognosis is poor. The majority of reported patients developed metastases and died within 2 years [263].

7.16 Adenocarcinoma

In spite of rather prominent salivary gland tissue in the supraglottic and subglottic larynx, laryngeal adenocarcinoma is rare, accounting for 1 % of all laryngeal neoplasms [266–268]. The majority of laryngeal adenocarcinomas are of the salivary gland type. The most common types are adenoid cystic carcinoma and mucoepidermoid carcinoma. Rare examples of other types of adenocarcinoma have been also described in the larynx, such as acinic cell carcinoma [269], clear cell carcinoma [270], malignant myoepithelioma [271], epithelial-myoepithelial carcinoma [272], salivary duct carcinoma [273], etc.

The etiology is unknown, though exposure to asbestos or lead, alcohol abuse, viral infections, ionising radiation and genetic risk factors have been implicated as possible aetiologic factors [274].

7.16.1 Adenoid Cystic Carcinoma

Definition, epidemiology and clinical aspects In contrast to other laryngeal carcinomas, adenoid cystic carcinoma (ACC) occurs at a younger age, with no gender predominance, and is more common in the subglottis [275]. Symptoms are similar than in other tumors in the same localisation. In addition, pain is frequently present, probably because of the tendency of ACC for perineural invasion.

Macroscopy Macroscopically, the tumor usually grows as a submucosal mass covered by normal mucosa.

Microscopy The microscopic features of laryngeal ACC are the same as in other locations.

Treatment and prognosis The treatment of choice is complete surgical excision. Laryngeal ACC is characterised by a slowly progressive course, with a high incidence of local

recurrence, long survival and a low cure rate. ACC has a tendency for haematogenic spread, mostly to the lungs and less frequently the bones, liver and other organs [275, 276]. It does not usually metastasise to the regional lymph nodes. The 5-year survival rate is 30 % [274, 275].

7.16.2 Mucoepidermoid Carcinoma

Definition, epidemiology and clinical aspects Mucoepidermoid carcinoma (MEC) occurs at all ages, even in childhood, but it usually presents in the sixth and seventh decades, predominantly in males. The majority occurs in the supraglottis but it has been described also in the glottis and subglottis, as well as in the hypopharynx [274, 277]. The clinical picture correlates with the localisation and size of the tumor.

Macroscopy Macroscopically, they usually present as submucosal masses [277]. Macroscopical appearance may also be nonspecific, resembling that of other carcinomas (Fig. 7.31a).

Microscopy Microscopically, they are similar to MEC in other sites and are composed of varying proportions of mucinous, intermediate, squamous and clear cells (Fig. 7.31b, c). On the basis of morphologic and cytologic features, MECs are classified as low-, intermediate- and high-grade tumors. The suggested criteria for grading include the relative proportion of cell types, proportion of a tumor containing cysts, degree and pattern of invasion, mitotic rate, presence of vascular and perineural invasion, necrosis and degree of nuclear and cellular atypias.

Treatment and prognosis The behaviour is unpredictable and is related to the grade and stage of the disease. The best treatment is complete surgical excision. Radiotherapy has been reported to be successful in a limited number of patients [277]. Neck dissection may be necessary, as 50 % of patients with MEC have metastases in the regional lymph nodes. The 5-year survival is 90–100 % for low-grade MEC and 50 % for high-grade MEC [278].

7.17 Sarcoma

Sarcomas of the larynx are uncommon, accounting for 1–2 % of all laryngeal neoplasms. Among them, chondrosarcoma is the most frequent type, comprising 75 % of all laryngeal sarcomas [184].

7.17.1 Chondrosarcoma

Definition and etiology Chondrosarcoma (CS) is the most common non-epithelial neoplasm in the larynx. It appears that laryngeal CS behaves less aggressively than its counterpart in the rest of the body. The majority of laryngeal CS are low-grade CS [184, 279].

Etiology is unknown, though disordered ossification of the laryngeal cartilages and ischaemic changes in a chondroma have been suggested as possible predisposing risk factors [279]. Other possible risk factors include previous radiation exposure [280] and Teflon injection [281].

Epidemiology and clinical aspects Laryngeal CS affects men more frequently than women, mostly in the seventh decade [184]. It usually presents with hoarseness; other symptoms include dyspnoea, dysphonia, cough, neck mass, airway obstruction and pain [184, 279]. The symptoms are frequently present for a long time before the diagnosis is established.

CS arises predominantly in the cricoid cartilage, especially at the inner posterior plate; it can also arise in the thyroid and arytenoid cartilages. It very rarely arises in the epiglottis [184, 279].

Macroscopy CS is characteristically a lobulated, submucosal mass covered by normal mucosa; on cut surface, it is glassy, firm white or grey (Fig. 7.32a). Radiographic findings are characteristic showing coarse or stippled calcifications [279, 282].

Microscopy Microscopically, laryngeal CS is indistinguishable from CS of bone origin elsewhere in the body and is graded according to the histologic criteria proposed by Evans and co-workers for CS of the bones [283]. Low-grade CS (grade I) has slightly increased cellularity, binucleation in the lacunar spaces, slight nuclear pleomorphism and hyperchromasia (Fig. 7.32b). High-grade CS (grade III) has remarkable cellularity, multinucleation in the lacunar spaces, nuclear pleomorphism, nuclear hyperchromasia, necrosis and mitotic activity, whereas the intermediate-grade CS (grade II) has medium cellularity and less nuclear pleomorphism [284].

The vast majority of laryngeal CS are low or intermediate grade. High-grade CS are considered rare; in a large series of 111 laryngeal CS, only six (6 %) were high grade [279]. Dedifferentiated (mesenchymal) CS characterised by the presence of both well-differentiated CS and a high-grade non-cartilaginous sarcoma is even rarer [279, 284–286].

Immunohistochemistry Immunohistochemically, CS expresses S-100 protein and vimentin [285].

Differential diagnosis Differential diagnosis includes chondroma and chondrometaplasia. Differentiation between low-grade CS and chondroma can be extremely difficult and is only possible with adequate tumor sampling. Chondromas are considered to be exceedingly rare in the larynx; they are

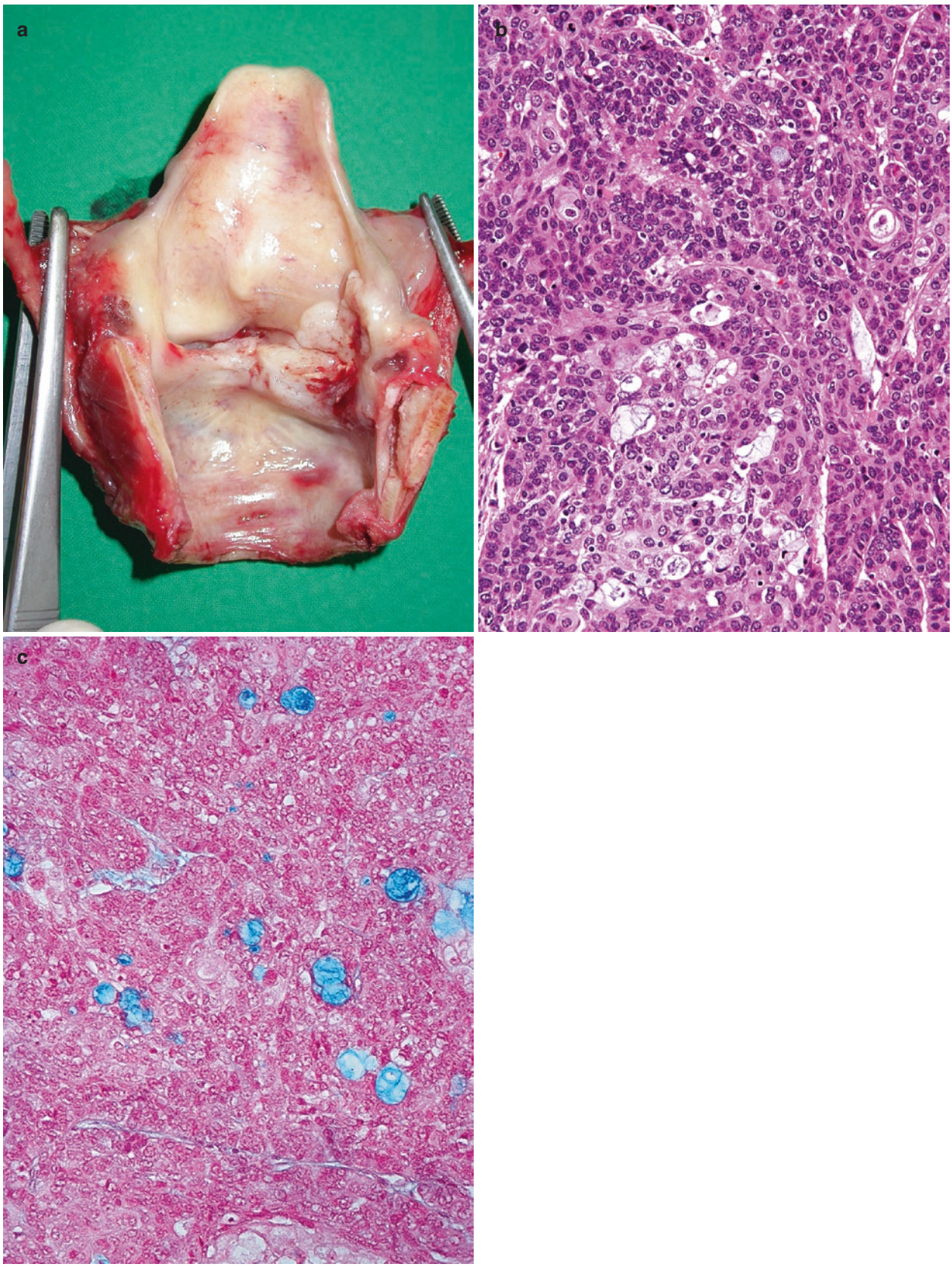


Fig. 7.31 Mucoepidermoid carcinoma of the larynx. (**a**) Macroscopic appearance: the tumor infiltrates the right ventricular fold, Morgagni sinus and the right vocal cord, and extends to the anterior comissure. (**b**) Solid growth of moderately pleomorphic squamous cells with high mitotic rate, and few goblet cells. (**c**) Alcian blue staining shows mucin production in tumor cells

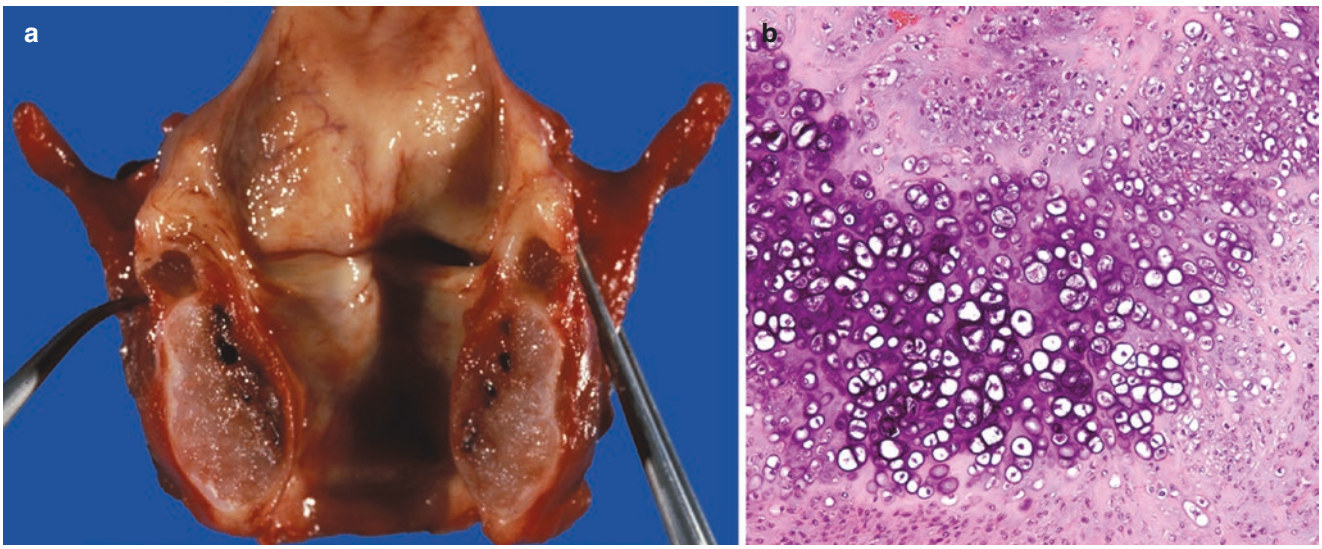


Fig. 7.32 Chondrosarcoma of the cricoid cartilage of the larynx. (**a**) typical macroscopic appearance of the tumor located in the cricoid cartilage: cut section reveals a glistening, lobulated, glassy tumor. (**b**) Microscopically, there is slightly increased cellularity and binucleation in the lacunar spaces, mild nuclear pleomorphism and hyperchromasia

smaller than CS and less cellular, with less pleomorphism, lacking mitoses and necrosis.

Treatment and prognosis The treatment of choice is conservative surgery [184, 285, 287]. Total laryngectomy should be avoided as long as possible, even in recurrent CS. Radiation therapy is generally regarded as ineffective, though few cases with a favourable response to radiation have been reported [288].

Prognosis is favourable. CS is characterised by a slowly progressive growth, with frequent recurrences (18–40 %) which are related to incomplete surgical excision and/or higher tumor grade [279]. Metastases from laryngeal CS are unusual, reported in approx. 10 % of patients, most commonly to the lungs and lymph nodes [279, 286]. The 5- and 10-year survival rates are 90 % and 80.9 %, respectively.

7.17.2 Other Sarcomas

Rare examples of other sarcomas have been described in the larynx and hypopharynx, such as liposarcoma [289], osteosarcoma [290–292], angiosarcoma [293], synovial sarcoma [294], malignant fibrous histiocytoma [295], Kaposi sarcoma (Fig. 7.33a, b) [296, 297], leiomyosarcoma [298, 299], etc.

The etiology of laryngeal sarcomas is unknown, though exposure to radiation [300] has been implicated as a possible aetiologic factor for osteosarcoma [292] and malignant fibrous histiocytoma [295] and infection with HIV for Kaposi sarcoma [296, 297].

7.18 Other Malignant Neoplasms

Larynx may be rarely involved by disseminated systemic lymphoma or leukaemia [301]. It can also be the primary site of a haematopoetic or lymphoid neoplasm. Extramedullary plasmacytoma seems to be the most common primary lymphoid neoplasms of the larynx. Various types of non-Hodgkin lymphoma of B-cell type and T-cell type have also been reported in the larynx, as well as rare cases of granulocytic sarcoma and mast cell sarcoma [301].

7.18.1 Malignant Lymphoma

Primary non-Hodgkin lymphoma (NHL) of the larynx is rare, accounting for less than 1 % of all laryngeal neoplasms [302] and approximately 1 % of all primary extranodal lymphomas [244]. By definition, the bulk of the disease should occur in the larynx [244]. About 65 cases have been reported in the English literature [302–305].

The majority of laryngeal NHL are of B-cell type, especially diffuse large B-cell lymphoma, and extranodal marginal lymphoma of MALT type. Rare cases of T-cell NHL have been reported, such as NK/T-cell lymphoma of a nasal type and peripheral T-cell lymphoma [244]. All regions of the larynx may be involved, with the exception of the extranodal marginal lymphoma of MALT type which has been described in the supraglottis only [304, 306], presumably because mucosa-associated lymphoid tissue has been found mostly in the supraglottic region [306].

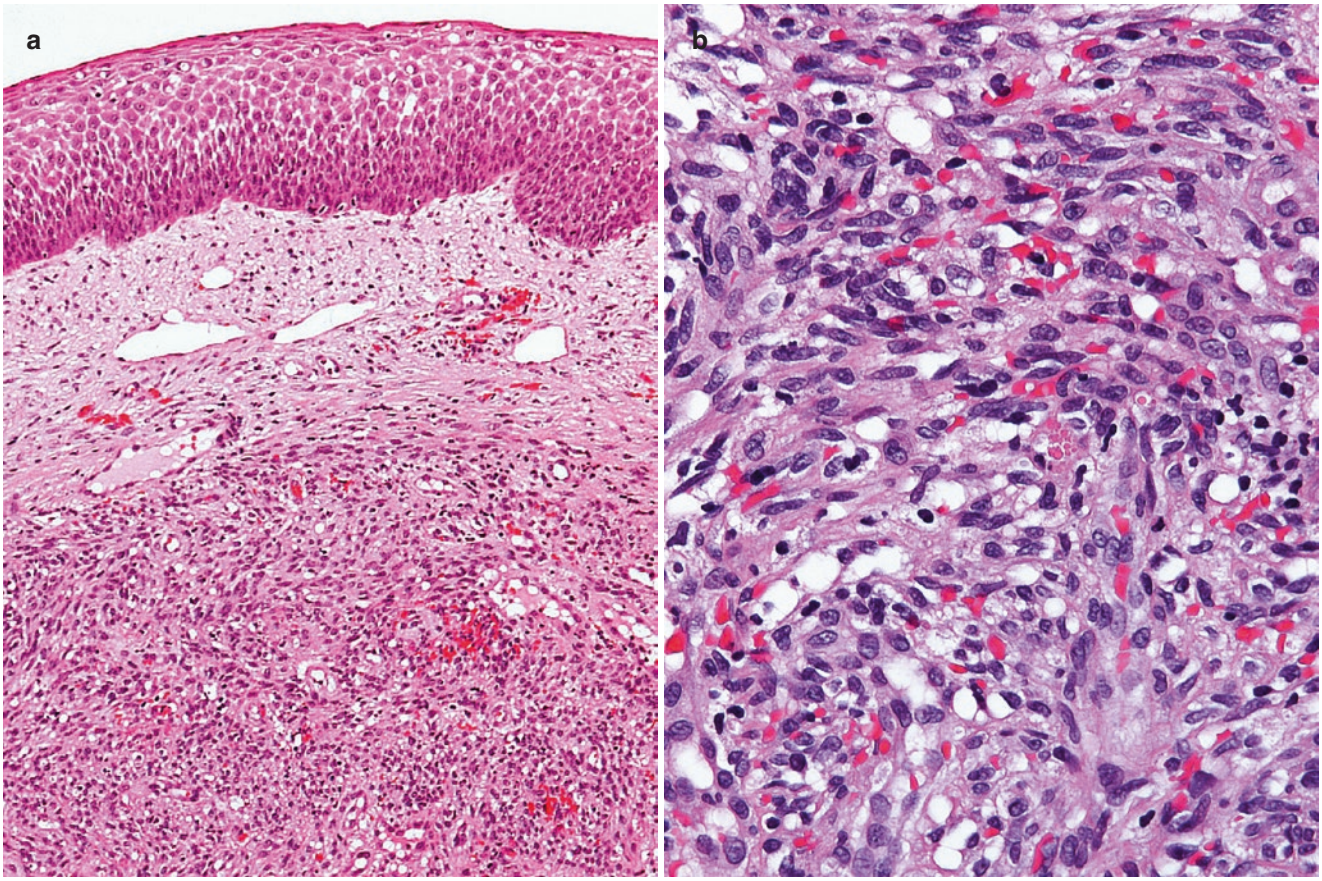


Fig. 7.33 Kaposi sarcoma of the larynx. (**a**) and (**b**) Highly cellular proliferation of spindle cells beneath the surface epithelium, forming small vascular spaces. There is little cellular pleomorphism, mitoses are rare. Extravasation of erythrocytes is present

They present mostly in stage I or II, limited to the larynx, with or without regional lymph node involvement. The most common symptoms are hoarseness, foreign body sensation and airway obstruction.

The prognosis is favourable. Laryngeal NHL should be treated according to the histologic type of NHL. It usually responds well to radiation therapy. Systemic chemotherapy is indicated for recurrent or disseminated disease [302].

7.18.2 Extraosseous (Extramedullary) Plasmacytoma

Definition Extraosseous (extramedullary) plasmacytoma is a clonal proliferation of plasma cells arising in tissues other than the bone. By definition, there is no evidence of plasma cell myeloma on bone marrow examination or by radiography. The malignant plasma cells express monotypic cytoplasmic immunoglobulins, and plasma cell-associated antigens, with absence of immature B-cell antigens [244, 307].

Epidemiology The majority of extraosseous plasmacytomas occur in the upper respiratory tract; among them, larynx is involved in only 6–18 %. An incidence of 1–5 plasmacytomas in 1,000 laryngeal tumors has been reported [308].

Laryngeal plasmacytoma is more frequent in males, with a mean age of 60 years.

Macroscopy The epiglottis is the most common site of involvement, followed by the vocal cords, false cords, ventricles and subglottis [308–312]. It generally presents as a solitary, submucosal lesion or as a polypoid lesion (Fig. 7.34). It may occasionally involve multiple sites in the larynx [308, 311].

Microscopy Microscopically, the tumor consists of sheets of plasma cells which vary in differentiation from well differentiated (Fig. 7.35a) to poorly differentiated. They may contain Russell bodies or grape-like inclusions of retained immunoglobulin (Mott cells), which are also found in reactive plasma cells and do not help in establishing the diagnosis of plasmacytoma [307].

Immunohistochemistry Immunohistochemically, neoplastic plasma cells usually express CD79a, CD138 and CD38. Other immunohistochemical and genetic features of plasmacytoma are presented in Chap. 13.

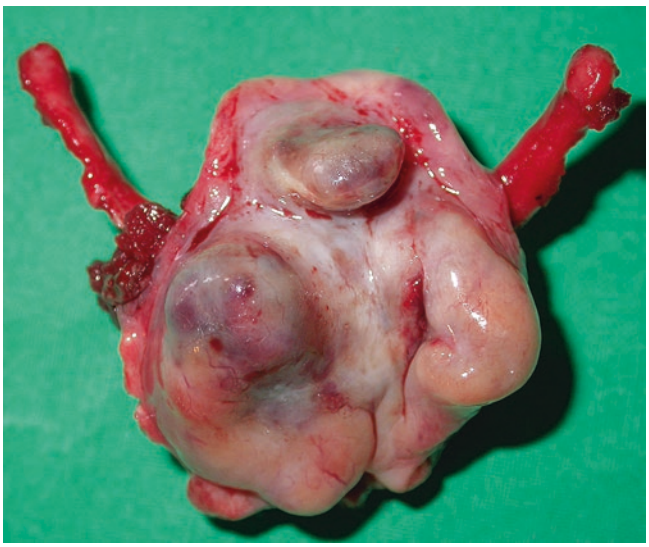


Fig. 7.34 Plasmacytoma of the supraglottis. Supraglottic laryngectomy specimen with a polypoid tumor of the epiglottis

Differential diagnosis Well-differentiated plasmacytoma cannot be distinguished morphologically from reactive (polyclonal) proliferation of plasma cells. Therefore, the monoclonality of plasma cells must be proven which is best achieved by demonstrating the cytoplasmic immunoglobulin heavy- and/or light-chain restriction. Besides immunohistochemistry, non-isotopic paraffin section in situ hybridisation is useful in the assessment of clonality for kappa or lambda light-chain mRNA (Fig. 7.35b, c) [307].

Poorly differentiated plasmacytoma must be differentiated from other lymphoid neoplasms and from other malignant tumors, such as malignant melanoma and carcinoma. This is achieved by appropriate immunohistochemical analysis; plasmacytoma, in contrast to lymphoma, does not express CD45 and immature B- and T-cell markers [313]. It also does not express antigens characteristic for malignant melanoma (i.e. S-100 protein, HMB-45 and melan-A), carcinoma (cytokeratins) and neuroendocrine neoplasms (i.e. synaptophysin, chromogranin).

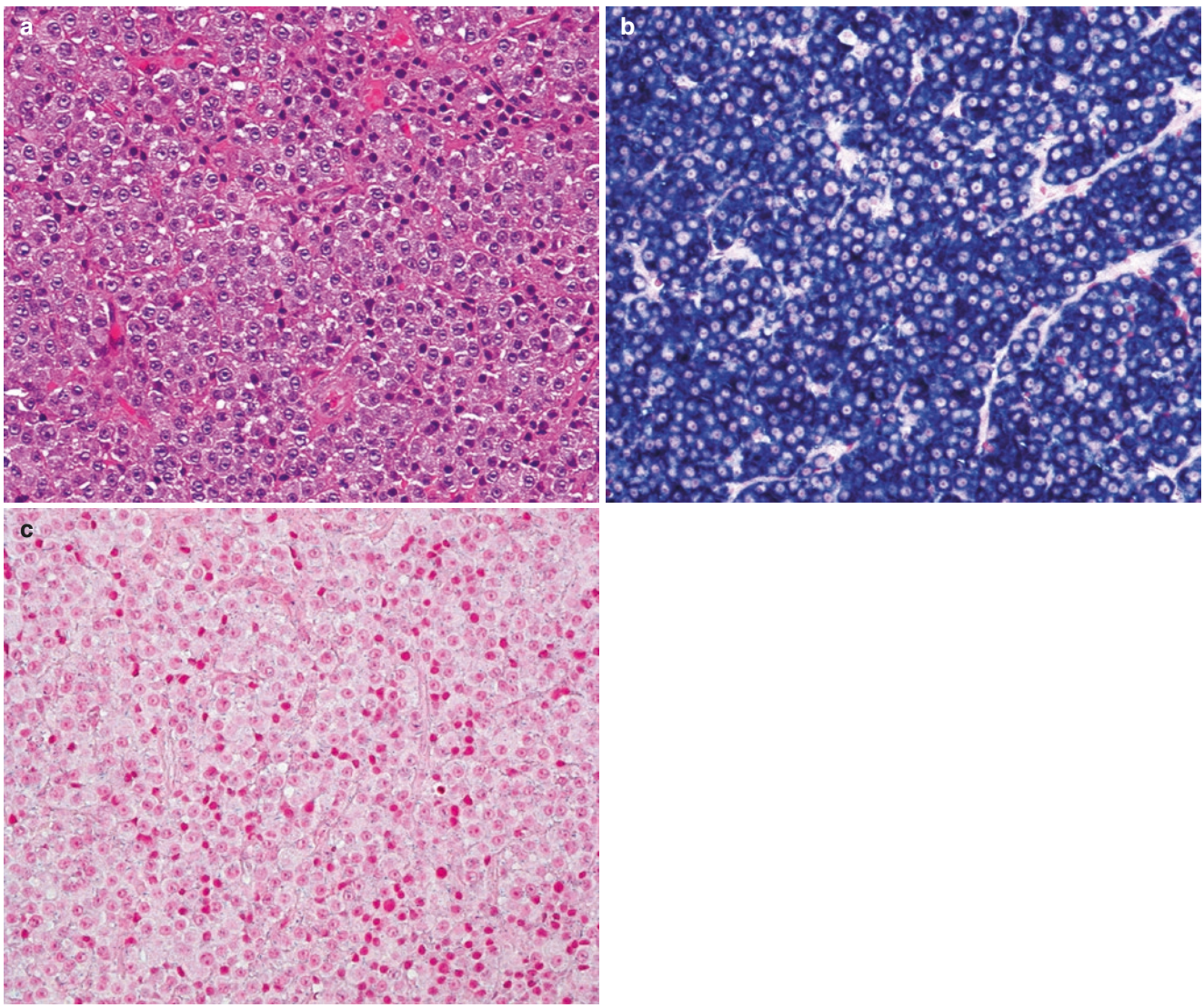


Fig. 7.35 Plasmacytoma of the larynx. (**a**) Diffuse infiltration of the laryngeal mucosa by neoplastic plasma cells. (**b**) In situ hybridization shows lambda light chain in neoplastic cells. (**c**) In situ hybridization for kappa light chain is negative

Treatment and prognosis Plasmacytoma is radiosensitive, and complete eradication by radiation and/or surgery is potentially curative [308–312]. Prognosis is favourable, though the development of a plasma cell myeloma may occur in 15 % of patients with extraosseous plasmacytoma. This indolent course indicates that many cases of extraosseous plasmacytoma may be related to MALT lymphoma [307].

7.18.3 Primary Mucosal Melanoma

Definition, epidemiology and clinical aspects Primary malignant melanoma (MM) of the larynx is extremely rare; less than 60 cases have been described in the literature. They represent 3.6–7.4 % of all mucosal melanomas of the head and neck [314–317].

Primary laryngeal MM is more common in men, mostly in the sixth and seventh decades. It occurs primarily in the supraglottic region and less often in the glottic region, but it hasn't been described in the subglottis yet. The symptoms vary according to the site of involvement and generally occur over a short period of time [317].

Macroscopy Macroscopically, it may present as a polypoid, exophytic, nodular, sessile or pedunculated lesion, with or without surface ulceration, varying in colour from black or brown to tan grey or white.

Microscopy Microscopically, primary laryngeal MM is indistinguishable from MM of the skin and other mucous membranes. It may be composed of epithelioid cells, spindle cells or both. Nuclear and cellular pleomorphism, nuclear pseudoinclusions, mitoses and necroses are usually prominent.

The diagnosis is based on histological examination together with special stainings for melanin, such as Warthin-Starry and immunohistochemistry.

Differential diagnosis The differential diagnosis must always include the possibility of a metastatic MM because in the larynx, metastatic MM is considerably more common than primary MM. Histologic features that favour the diagnosis of primary MM are junctional activity and/or an in situ component. However, as melanocytes are also normally present in the subepithelial compartment, junctional changes are not required for the diagnosis of primary MM [317].

Apart from metastatic MM, the differential diagnosis includes carcinoma (especially spindle cell carcinoma), sarcoma and lymphoma. Positive staining for well-known MM markers, such as S-100 protein, HMB-45, melan-A and vimentin, and negative staining for CD45, B- and T-cell markers and markers of epithelial differentiation (cytokeratins, epithelial membrane antigen), are diagnostic for MM.

Treatment and prognosis The treatment of choice is complete surgical excision. The prognosis of primary laryngeal MM is poor, similarly to primary mucosal malignant melanoma in general, with an average survival of less than 3.5 years [317, 318].

7.18.4 Metastases to the Larynx

Definition and epidemiology Metastases to the larynx from distant primary tumors are uncommon, accounting for less than 0.5 % of all laryngeal neoplasms. Metastases to the hypopharynx and trachea are even less common. The most common source is malignant melanoma (Fig. 7.36), followed by renal cell carcinoma. Other tumors with proven laryngeal metastases include cancer of the kidney, breast, lung, prostate, colon, stomach and ovary [319–324]. The rare occurrence of metastases to the larynx seems to be related to the terminal location of this organ in the lymphatic and vascular circulation.

Laryngeal metastases are most commonly located in the supraglottic and subglottic regions, presumably due to the

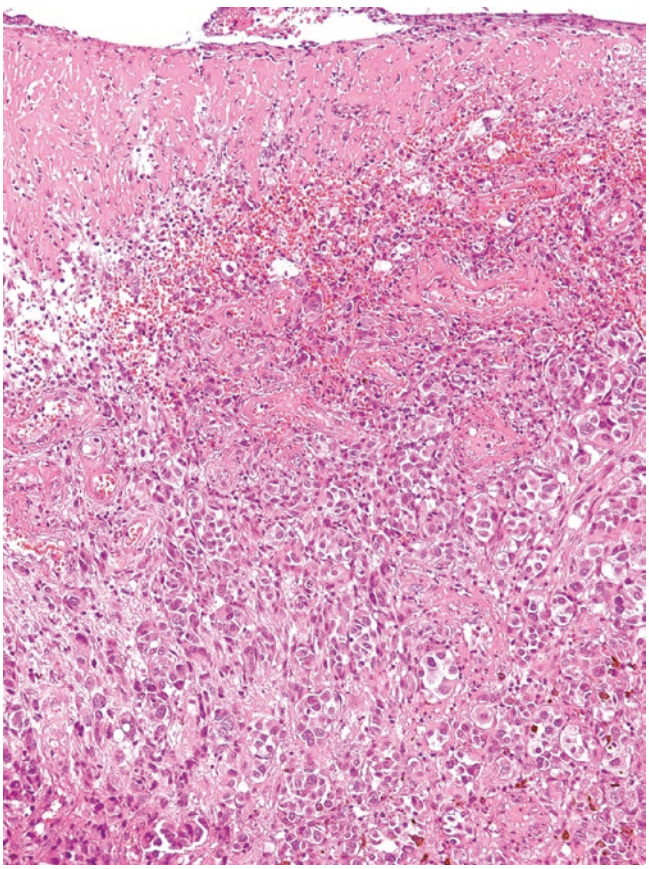


Fig. 7.36 Metastastic malignant melanoma of the larynx. An ulcerated tumor composed of large, atypical epithelioid cells with focal melanin pigment. Primary tumor was located on the left heel

their rich vascular supply [319]. They can be divided into those located in the soft tissue (metastases from melanoma and renal cell carcinoma) and those located primarily in the marrow spaces of the ossified laryngeal cartilage (metastases from breast, prostate, and lung cancers).

Clinical features The signs and symptoms of metastatic laryngeal tumors do not differ from those of other laryngeal tumors and vary according to the site of involvement. Haemoptysis may be present, especially in highly vascularised metastatic renal cell carcinoma.

Macroscopy The macroscopic appearance of metastatic tumors of the larynx and hypopharynx is nonspecific, but they tend to present as submucosal lesions covered by an intact epithelium.

Microscopy The microscopical appearance is related to the type of the primary tumor.

Immunohistochemistry Immunohistochemical analyses are usually needed for the correct diagnosis. Clinical data about the location of the primary tumor is helpful and allows pertinent, selective immunohistochemistry for the diagnosis. In cases of unknown primary tumors, more extensive analyses are needed, as described in Chap. 9.

Treatment and prognosis Prognosis for patients with laryngeal metastases is generally poor as laryngeal involvement is usually a sign of dissemination in the terminal stage of the disease. In such patients, only palliative treatment is advised; laser endoscopic resection has been reported as an excellent tool for relieving airway obstruction [323]. However, cases with isolated laryngeal metastases have been described, in which local excision and/or radiation therapy was associated with a prolonged survival.

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Ear and Temporal Bone

8

Ann Sandison

Contents

8.1 Introduction

8.1.1 Embryology of the Ear

The ear is not a single organ, but two, being the peripheral receptor site both for stimuli derived from sound waves and for changes of posture. The structures subserving both of those functions are developed from an invagination of ectoderm early in embryonic life – the otocyst – to produce the epithelia of the membranous labyrinth of the inner ear. Superimposed upon, and developing slightly later, the first and second branchial arch systems provide structures which augment the hearing function. The endodermal component of the first branchial system, the branchial pouch, gives rise to the Eustachian tube and middle ear epithelia and the corresponding ectodermal outgrowth, the first branchial cleft, to the external ear epidermis. The connective tissue part of the local branchial cranial (auditory vestibular) nerve outflow from the central nervous system, both its vestibular and cochlear branches, grows to link up with the sensory epithelia lining the otocyst-derived cochlear and vestibular endolymph-containing cavities; there is recent evidence that terminal ganglion cells, e.g. spiral ganglion cells, may also be otocyst-derived. Cartilaginous, bony and muscular configurations of the ear are developed from the mesenchyme surrounding these early epithelia. The seventh cranial (facial) nerve develops in close relation to the structures of the ear for much of its course.

8.1.2 Anatomy of the Ear

The anatomy of the ear may be considered by reference to its functions in hearing and balance (Fig. 8.1) [1].

The pinna and external canal conduct sound waves in air to the tympanic membrane, which transmits them by very

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A. Sandison, BSc(Hons), MPhil, MBCHB, FRCPath
Department of Histopathology, Imperial College Healthcare NHS Trust, Charing Cross Hospital,
Fulham Palace Road, London W68RF, UK
e-mail: ann.sandison@imperial.nhs.uk

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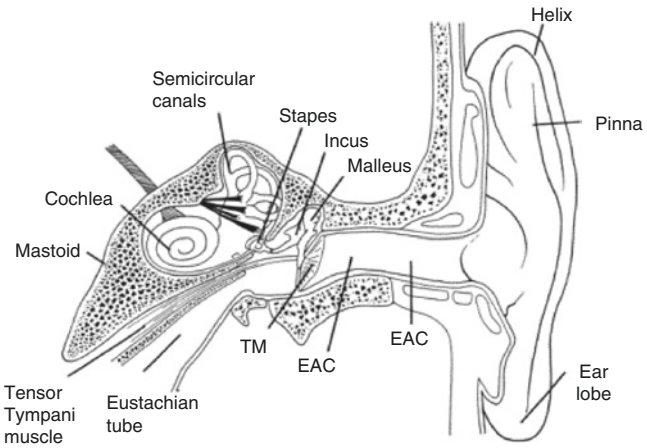

Fig. 8.1 Diagram of the anatomy of the ear. *EAC* external auditory canal, *TM* tympanic membrane (Reproduced from Michaels and Helquist [1])

delicate vibrations. The middle ear enhances this sound energy transmission by conveying vibrations from the larger area of the tympanic membrane through the ossicular chain (malleus, incus and stapes) to the much smaller area of the footplate of the stapes, which lies in the oval window of the vestibule in contact with perilymph. In this way vibrations representing sound are conducted to the fluids of the inner ear. The air space of the middle ear cavity is magnified by the mastoid air cells which are complex expansions into the mastoid bone. There is a connection of the middle ear space with the nasopharynx and so with the external air through the Eustachian tube, by which air pressure can be adjusted.

From the vestibular perilymph, vibrations derived from sound waves pass directly via the scala vestibuli into the spirally coiled perilymphatic spaces of the cochlea, where it forms an upper compartment ascending from the vestibule and oval window. A lower compartment, the scala tympani descends spirally to the round window membrane, a connective tissue disc separating the perilymph compartment from the middle ear. Between the scalae vestibuli and tympani, there is an endolymph-containing coiled middle compartment, the cochlear duct (scala media), which houses the sensory organ of sound reception, the organ of Corti. Waves of vibration are conveyed from the perilymph to the walls of the scala media, from which, through the endolymph, they affect the sensory cells of the organ of Corti.

The cochlear duct communicates with the vestibular endolymph-containing sacs through two fine canals so that the endolymphatic system of cochlea and vestibule is continuous, like the perilymphatic one. Gravitational acceleration of the head is detected in a sensory organ arranged within endolymph-containing sacs in the vestibule (the utricle and saccule), and angular acceleration is detected within tubes emanating in three dimensions from the utricle (lateral, posterior and superior semicircular canals). The sensory cells are located as a thickened portion of epithelium, the macula, in the saccule and utricle and a raised prominence of epithelium, the crista, in expansions of each semicircular canal, the ampullae. The vestibular aqueduct contains the endolymphatic duct and sac which are a blind offshoot of the endolymphatic system, probably functioning in absorption of endolymph. The cochlear aqueduct is a communication between the cerebrospinal fluid in the subarachnoid space to the perilymph of the scala tympani near the round window. Cochlea, vestibule and semicircular canals are surrounded by very dense bone, the otic capsule.

The cochlear and vestibular sensory structures are supplied by a doube nerve supply, the audiovestibular nerve or eighth cranial nerve, which enters the temporal bone through the internal auditory meatus, and the facial nerve or seventh cranial nerve, which enters the temporal bone through the same canal and, after a right-angled bend in the genu, where the geniculate ganglion is located, reaches the posterior wall of the middle ear, from which it passes down through the mastoid to emerge in the region of the parotid salivary gland, after which it provides motor nerve supply for the muscles of the face.

8.2 External Ear and Auditory Canal

8.2.1 Inflammatory and Metabolic Lesions

8.2.1.1 Diffuse External Otitis

Definition A common condition that causes inflammation of the external ear canal, the auricle or both.

Epidemiology The condition affects all ages and there is no sex predilection.

Clinical aspects The disease is usually unilateral. It is most frequently associated with *Pseudomonas aeruginosa* infection, but local trauma is also an important causative factor.

Swimmers are often affected. Patients complain of otalgia of variable severity that may be associated with otorrhoea (discharge), itching or tinnitus. The patient may complain of hearing loss and a sensation of fullness or pressure in the ear canal. The tragus may be painful to touch and characteristically it is painful when the pinna is retracted. Severe cases may be associated with fever. The disease is classified as acute diffuse, acute localised or chronic (duration longer than 6 weeks). Malignant otitis externa is the term applied when the disease extends into surrounding tissues (see below). Eczematous otitis externa is associated with dermatological conditions and mycotic otitis externa is associated with fungal infection, for example, *Candida*. Imaging studies are not

usually requested but may be useful if the infection is thought to have spread to the adjacent soft tissue or mastoid bone.

Macroscopy The ear canal may become red, swollen and narrow.

Microscopy Histological features include acute inflammation of the dermis, together with acanthosis and hyperkeratosis of the epidermis.

Treatment Treatment is symptomatic with analgesia topical application of antibiotics and anti-inflammatory drugs. Occasionally oral antibiotics are required. Surgical intervention is required for debridement of the ear canal or incision or incision and drainage of an abscess.

8.2.1.2 Perichondritis

Definition Perichondritis is a benign infective condition that most commonly affects the pinna.

Clinical aspects The condition may follow surgical trauma and recently there has been an association with the modern trend of piercing and acupuncture. As in the diffuse acute inflammation of the ear canal, *Pseudomonas aeruginosa* is the most common infecting organism.

Macroscopy The affected auricle becomes tender, red and swollen. The earlobe which does not contain cartilage is unaffected.

Microscopy Pus accumulates between the perichondrium and cartilage of the pinna. This may interfere with the blood supply of the cartilage and so lead to its necrosis (Fig. 8.2).

Treatment and prognosis Prompt treatment with antibiotics is necessary. Fluoroquinolones such as ciprofloxacin are effective against *Pseudomonas* organisms but their use is restricted to adults since there is potential toxicity to growing cartilage. Occasionally surgical debridement of the affected area is required. If left untreated, the pinna may become deformed resulting in "cauliflower ear."

8.2.1.3 Malignant Otitis Externa

Definition Malignant otitis externa (MOE) was first described as a severe infection of the external auditory canal [2].

Epidemiology MOE usually (but not always [3]) affects elderly diabetics.

Clinical aspects Patients complain of unremitting pain; there may be purulent discharge and invasion of cartilage, nerve, bone and adjacent soft tissue. The causative agent is usually *Pseudomonas aeruginosa*, but other organisms including fungi have been incriminated. The condition frequently goes on to ninth, tenth, eleventh and twelfth cranial nerve palsies and meningitis and death may result.

Macroscopy Inflammatory changes may be seen in seen in the external auditory canal and the periauricular soft tissue. The changes may not be in proportion to the perceived pain. On otoscopy granulation tissue present at the osseocartilaginous junction is diagnostic of malignant otitis externa and the bone may be exposed.

Microscopy Histopathological changes in the temporal bones of two patients who had been diagnosed clinically as

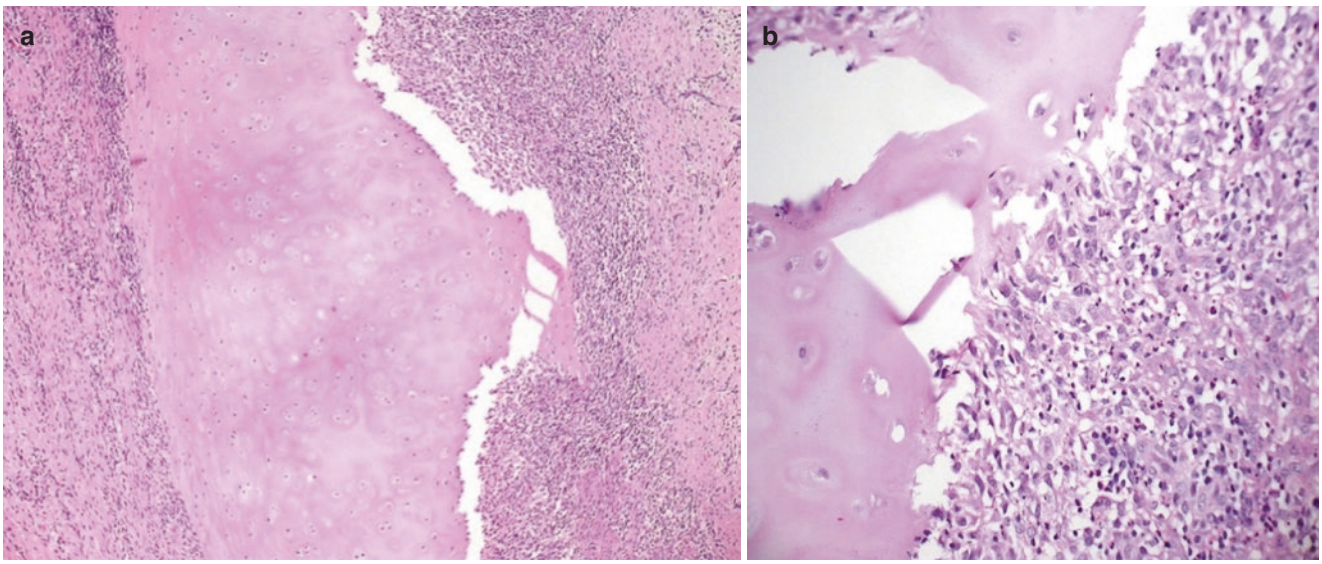

Fig. 8.2 Perichondritis. (**a**) low magnification; (**b**) higher magnification showing eroded cartilage surrounded by acute inflammatory exudate

having "malignant otitis externa" and were thought to have died of this condition [4] were those of severe otitis media and osteomyelitis of the jugular foramen secondary to it. It seems likely that the manifestations of "malignant otitis media" are due to the spread of inflammation from the tympanic cavity and mastoid air spaces to the petrous apex through bone marrow spaces by a process of osteomyelitis [5]. In recent years several patients with AIDS have been reported to have malignant otitis externa, and in one of them the presence of acute osteomyelitis of the skull base in addition supported the concept of osteomyelitis as the pathological basis for malignant otitis externa put forward above [6].

Treatment and prognosis Careful control of blood sugar is required in diabetic patients. The condition responds to long-term antibiotic therapy topical and systemic. Hyperbaric oxygen may be used for cases with complications, cases resistant to therapy and cases with recurrent disease. Surgery is reserved for local debridement and removal of necrotic bone or for drainage of an associated abscess. The disease has been reported to recur in 10–30 % of patients and recurrence is most often associated with inadequate duration of therapy. Recurrences can happen up to 1 year after the cessation of treatment so medium to long-term patient follow-up is required. Mortality has been reduced to a reported 10 % with increased awareness of the condition and improved imaging techniques. Higher mortality is associated with cranial nerve involvement, intracranial abscess and severe immunosuppression.

8.2.1.4 Relapsing Polychondritis

Definition Relapsing polychondritis is a rare disease of unknown etiology characterized by recurring bouts of inflammation affecting cartilaginous structures including in the ear, respiratory tract, joints and eye.

Clinical aspects Although the cartilage of the external ear is most frequently involved and that of the nose next in frequency, it is the inflammation with destruction of the cartilages of the respiratory tract, particularly those of the larynx, which threatens life and in most cases where death has resulted from the condition, it is from respiratory obstruction due to such cartilage damage. Rib cartilages may be swollen and tender. Episcleritis or scleritis, iritis, conjunctivitis or keratitis may also be found in relapsing polychondritis. Heart lesions are characteristically aortic, showing signs of regurgitation.

Macroscopy The cartilage of the pinna is recurrently acutely inflamed and this leads eventually to a cobblestone appearance resembling boxer's ear. Inflammation of the nasal septum leads to a sinking of this structure, producing a "saddle nose" appearance.

Microscopy The histology of this affliction is dealt with in Chap. 7.

Treatment and prognosis Disease that is localised to auricular or nasal cartilages may be treated with low-dose corticosteroids. More severe disease may require high-dose corticosteroids and immunosuppression therapy. Early diagnosis and treatment are key to avoid disfigurement and improve prognosis [7].

8.2.1.5 Gout

Definition Gout is a disease resulting from abnormal uric acid metabolism resulting in urate crystal deposition in joints and soft tissues.

Epidemiology The disease usually affects males.

Clinical aspects Gout is manifested both as an acute arthritis which is related to deposits of urates in the joint capsule, most frequently in the big toe joint, and as tophi in non-articular tissues. The external ear is one of the most frequent places for the latter and deposits may occur in the helix and antihelix.

Macroscopy The lesions are usually well circumscribed without surrounding erythema. Larger lesions may ulcerate, discharging a creamy white material within which needle-like crystals of sodium urate may be detected on microscopy.

Microscopy The characteristic needle-shaped crystals may be identified in the tissue providing it has been processed through nonaqueous solution such as alcohol (Fig. 8.3a, b). Urate is water soluble and dissolves on immersion in aqueous-based fixatives such as formalin. The crystals deposited in the tissues may induce a foreign body giant cell reaction.

Differential diagnosis The differentials of papules on the ear include chondrodermatitis nodularis chronica helicis (see below), basal cell carcinoma, squamous cell carcinoma and rheumatoid nodule clinically.

Treatment In the acute stages gouty tophi are treated with colchicine, non-steroidal anti-inflammatory drugs and corticosteroids. Drugs to lower urate levels in the blood are discontinued to avoid exacerbation of the disease. It may take years for the gouty tophus to resolve [8].

8.2.1.6 Ochronosis

Definition Ochronosis (alkaptonuria) is an inherited disease of metabolism in which a step in tyrosine metabolism is disturbed, resulting in accumulation of homogentisic acid

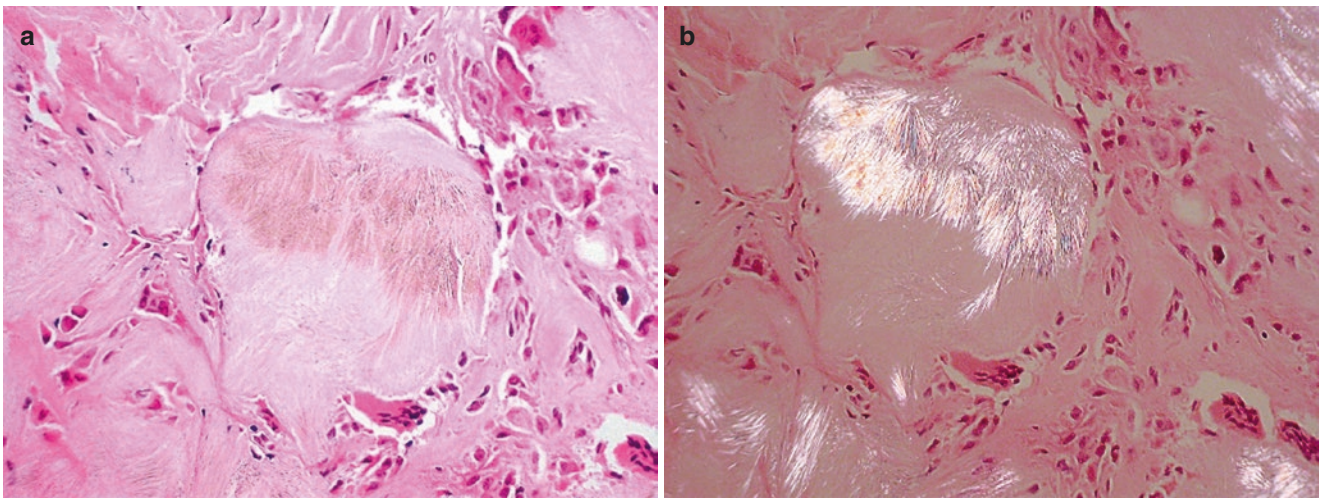

Fig. 8.3 Gout. (**a**) Islands of acellular material with foreign body giant cells at the periphery. The characteristic needle-shaped urate crystals are seen when viewed under polarised light (**b**)

(HGA) in a variety of places, but especially cartilages. The substance is colourless in the urine when first passed, but darkens to a black or brown polymer on standing.

Epidemiology The disease is inherited as an autosomal recessive. There is no sex predilection. Although alkaptonuria is a lifelong disease, symptoms may not appear in patients until middle age (fourth decade).

Macroscopy In the external ear, there may be one or both of two manifestations: (a) dark colour of the wax (when seen in a child, this may be the first manifestation of ochronosis) and (b) blue/black discolouration of the aural cartilage due to the binding of the HGA to the cartilage ground substance.

Microscopy Yellow- or ochre-coloured deposits of HGA can be seen in cartilage or connective tissue. There may be a foreign body response.

Treatment and prognosis There is as yet no effective treatment or cure for patients with alkaptonuria but they can expect a normal lifespan. If the disease is diagnosed in early life, a diet low in tyrosine and phenylalanine may be recommended. Ochronotic deposits in skin have been treated with laser.

8.2.2 Pseudocystic and Cystic Lesions

8.2.2.1 Idiopathic Pseudocystic Chondromalacia

Definition Idiopathic pseudocystic chondromalacia is a benign cystic condition affecting the auricle. Synonyms include endochondral pseudocyst, intracartilaginous cyst and cystic chondromalacia.

Epidemiology The lesion occurs mainly in young and middle-aged adults.

Etiology and pathogenesis The etiology is unknown. Cytokines including high levels of IL6 have been isolated from the cyst fluid. The association of this lesion with severe atopic eczema in four children [9] suggested that minor trauma from repeated rubbing of the auricle may play a part. It has been suggested that defective embryogenesis may result in abnormal tissue planes which are opened up after repeated minor trauma to the area.

Small pseudocysts of the elastic cartilage of the pinna may also be seen in the vicinity of inflammatory or neoplastic lesions of that region.

Clinical features Patients develop a painless swelling on the lateral or anterior auricle. There may be a history of localised trauma. The lesion has been associated with skin conditions such as dermatitis and lymphoma.

Macroscopy The gross appearance is one of a localized swelling of the auricular cartilage. Cut surface shows a well-defined cavity in the cartilage which is distended with yellowish watery fluid [7].

Microscopy The cavity shows a lining of degenerated cartilage on one surface (Fig. 8.4); on the other surface the cartilage is normal. It seems possible that the fluid is an exudate from undamaged perichondrial vessels which cannot be absorbed by the damaged perichondrial vessels.

Treatment and prognosis The aim of treatment is to preserve normal auricular architecture and prevent recurrence of

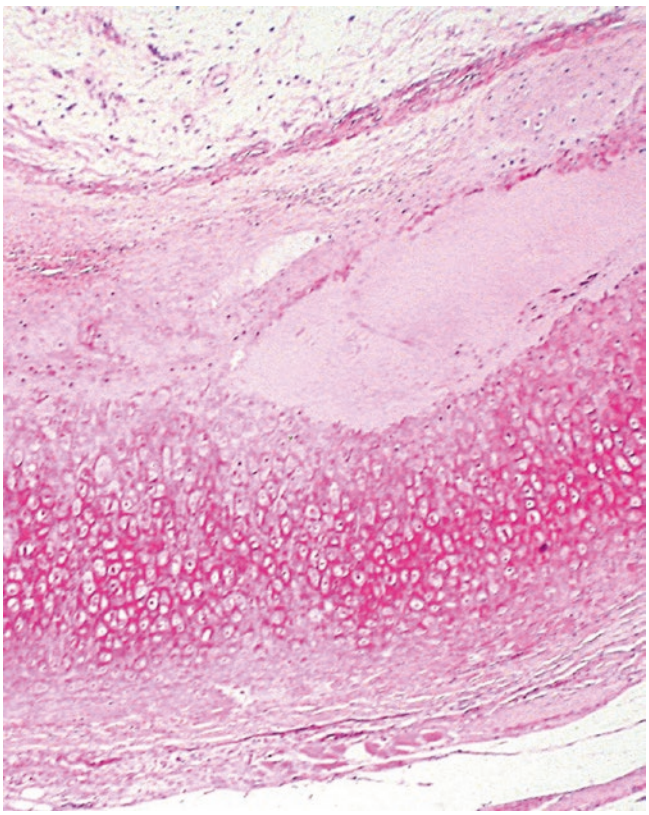

Fig. 8.4 Idiopathic pseudocystic chondromalacia. The cystic fluid-filled cavity shows a lining of degenerated cartilage on one surface and on the other surface the cartilage is normal. It seems possible that the fluid is an exudate from undamaged perichondrial vessels which cannot be absorbed by the damaged perichondrial vessels

the lesion. Multimodality treatment may be necessary for optimal effect. Treatment methods include cyst aspiration and pressure dressings, intralesional steroids, systemic medication and surgery. Recurrence is common.

8.2.2.2 First Branchial Cleft Cyst

Definition A benign congenital abnormality of the preauricular soft tissues.

Clinical aspects A developmental origin from the first branchial cleft has been assumed in some fistulas, sinuses and cysts arising in the periauricular and the parotid region and also in some fistulas arising in the neck which are located above a horizontal plane passing through the hyoid bone. The most common of these lesions is the preauricular sinus.

Macroscopy The lesion appears as a pit in skin anterior to the ear that marks the entrance to a sinus tract near to cartilage. These tracts may become inflamed or infected resulting in abscess formation.

Microscopy The cyst usually shows a stratified squamous epithelial lining, but occasionally it may be lined by respiratory epithelium, deep to which the connective tissue is chronically inflamed. There is often elastic cartilage in the deep wall of the sinus.

Treatment and prognosis Children presenting with external ear deformities are usually examined to confirm normal hearing. Infection is treated with antibiotics and incision and drainage for abscesses. Excision is undertaken when there is recurrent or persistent infection.

Branchial cleft cysts are more extensively discussed in Chap. 9.

8.2.3 Tumor-Like Lesions

8.2.3.1 Chondrodermatitis Nodularis Helicis

Definition Chondrodermatitis nodularis chronica helicis, sometimes known as Winkler's disease, is a benign painful condition affecting the pinna.

Epidemiology The disease is more common in males, middle-aged or older but it can be seen in women and younger adults.

Clinical aspects The pathogenesis of the lesions is uncertain and is probably related to cartilage ischaemia. The helix is one of the furthest points from the source of the arterial blood supply of the pinna. It seems likely that obstruction of small arteries of the perichondrium is the primary lesion leading to cartilage necrosis and that the acute inflammation and epidermal ulceration are secondary to the nearby cartilage necrosis. An association between chondrodermatitis nodularis helicis and systemic sclerosis has been described [10]. In this condition obstructive changes are frequently found in small arteries.

Development of the lesions has been attributed to sleeping on the affected side, using tight headgear or headphones. It can occur after exposure to cold or after minor trauma.

Macroscopy A small nodule forms on the auricle, usually in the superior portion of the helix (Fig. 8.5a) [11].

Microscopy Histological examination of biopsies of such lesions, in which the elastic cartilage underlying the skin of the auricle is particularly well-represented, shows ulceration of the skin of the auricle and complete necrosis of the tip of the underlying elastic cartilage of the auricle. In some cases a piece of extruded necrotic cartilage may be seen on

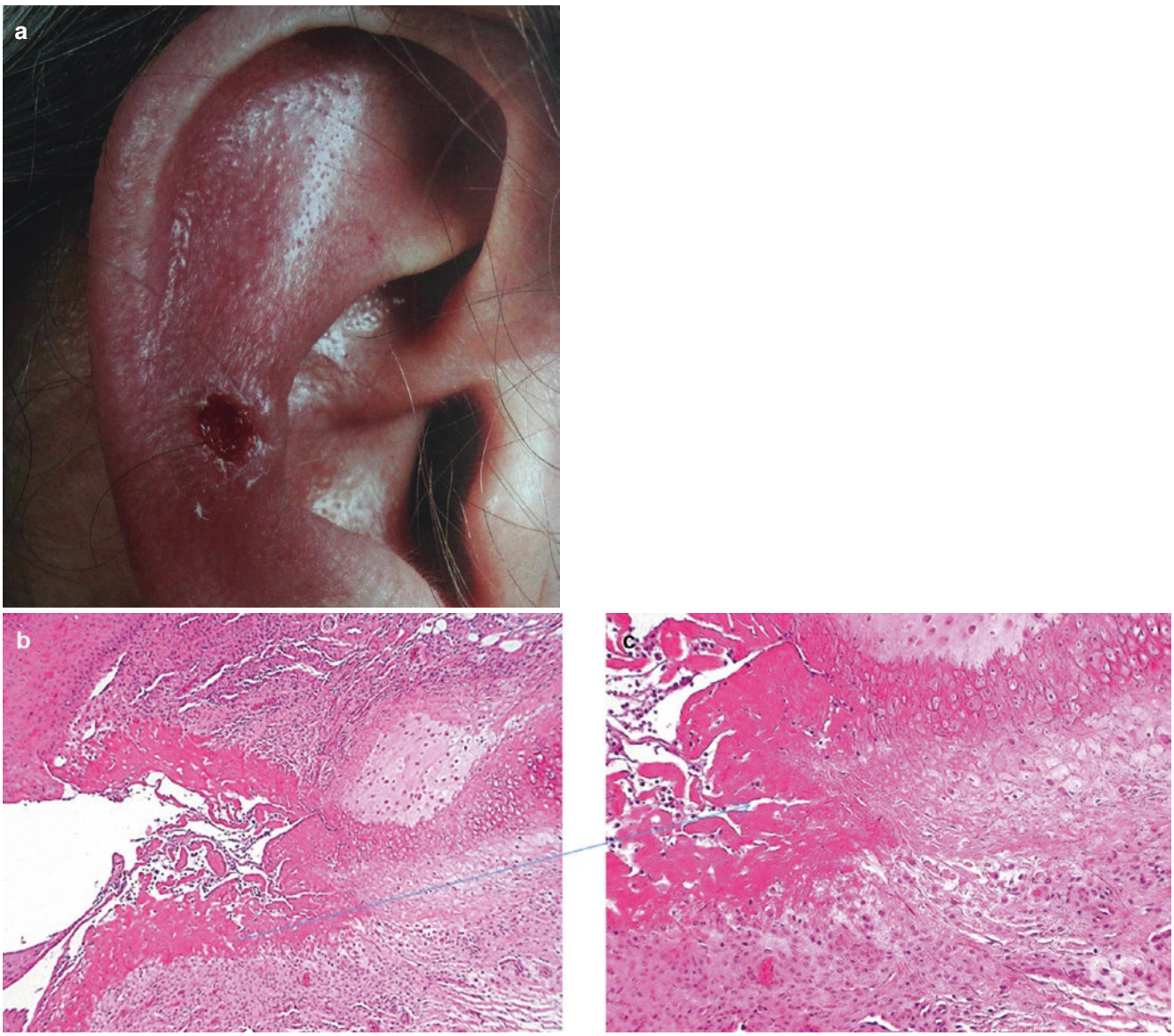

Fig. 8.5 Chondrodermatitis nodularis chronica helicis. (**a**) Macroscopic picture showing a small area of ulceration on the auricle with rolled margins (Reproduced with permission from Du Vivier- Atlas of Clinical Dermatology [11]. (**b**) and (**c**) Microscopic pictures show ulceration of the skin with surface fibrin and necrosis of the tip of the elastic cartilage (*arrow*))

the floor of the ulcer. The perichondrium of the elastic cartilage in this region shows obstructive thickening of small arteries (Fig. 8.5b, c).

Differential diagnosis Clinically the ulcerated nodule may be mistaken for squamous cell carcinoma.

Treatment and prognosis Treatment is based on protecting the affected area to allow healing. Steroid injection or topical application of liquid nitrogen or glyceryl trinitrate may be used to promote healing. Occasionally curettage or excision biopsy is required but there is a recurrence rate of 10–30 %.

8.2.3.2 Keratosis Obturans and Cholesteatoma of External Canal

Definition Keratosis obturans results from the accumulation of keratinous debris in the bony portion of the external ear canal.

Epidemiology The condition usually presents in young people. Patients complain of severe acute otalgia often associated with sinusitis and conductive hearing loss. In over 40 % of cases, the symptoms are bilateral.

Clinical features The keratin produced by exfoliation from the skin of the tympanic membrane and external canal is retained on the epithelial surface and forms a solid plug. This enlarges and may cause circumferential erosion of the bony canal. Keratosis obturans is probably the result of a defect of the normal migratory properties of the squamous epithelium of the tympanic membrane and adjacent ear canal which causes the accumulation of keratinous debris [12]. A minor degree of this process – keratosis of the tympanic membrane – in which deposits of keratin grow on the eardrum and cause tinnitus has also found to be associated with absent or defective auditory epithelial migration [13]. A condition that has been distinguished from keratosis obturans is *cholesteatoma of the external canal*. In this lesion epidermoid tissue appears to penetrate into the wall of the deep external canal causing localized osteonecrosis and bone erosion [14]. Patients with cholesteatoma of the external ear are usually elderly and the disease is most often unilateral. Otorrhoea is common in cholesteatoma and usually there is no hearing loss.

Macroscopy Large plugs of keratin are seen in the external ear canal. In keratosis obturans there are circumferential, smooth destruction and widening of the ear canal associated with expansion of the bone. In external canal cholesteatoma, the disease is localised and an ulcer in the canal wall may be seen or a defect in the bony canal lateral to the tympanic annulus usually on the posterior or inferior wall.

Microscopy Histologically there may be abundant keratin associated with hyperplasia of the underlying squamous epithelium and chronic inflammation of the stroma. There is no bone destruction in keratosis obturans while cholesteatoma of the external ear canal is often associated with bone erosion and the epithelial architecture may appear more infiltrative.

Differential diagnosis Small biopsies may be mistaken for squamous cell carcinoma particularly those from cholesteatoma of external canal.

Treatment The keratin is regularly removed by micro suctioning. Cholesteatoma of the external ear canal may require surgical debridement.

8.2.3.3 Keratin Granuloma

Definition A granulomatous process that may result in the external ear canal when keratin squames become implanted into the deeper tissues following traumatic laceration [15].

Microscopy The granuloma contains foreign body-type giant cells, histiocytes, lymphocytes, plasma cells and flakes of keratin. The latter are strongly eosinophilic and birefringent in polarized light. Aural polyps frequently contain such granulomas, but the keratin is then more likely to be derived from a middle ear cholesteatoma [see below].

8.2.3.4 Angiolymphoid Hyperplasia with Eosinophilia and Kimura's Disease

Definition A benign vascular tumor composed of immature blood vessels lined by plump epithelioid endothelial cells and associated with an inflammatory infiltrate rich in eosinophils.

Synonyms for angiolymphoid hyperplasia with eosinophilia are epithelioid hemangioma (see also Chap. 15), benign angiomatous nodules of face and scalp, atypical pyogenic granuloma and several other terms. Although this entity was first described by Kimura, "Kimura's disease" is now believed to be a different condition [see below].

Epidemiology Angiolymphoid hyperplasia with eosinophilia may occur anywhere in the skin, especially on the scalp and face, but there is a particular predilection for the external auricle and external auditory meatus. It is a lesion of young and middle-aged of both sexes and all races. Kimura's disease is more common in Orientals, Asians (endemic in China and Japan), mainly affecting young males (Fig. 8.6 a–c). It is a chronic inflammatory condition of unknown etiology.

Angiolymphoid Hyperplasia with Eosinophilia

Macroscopy Grossly there are sessile or plaque-like red or reddish-blue lesions from 2 to 10 mm in diameter, which may coalesce to form large plaques that obstruct the ear canal. On transection the lesion is seen to be present in the dermis and subcutaneous tissue.

Microscopy Microscopically there is a mixture of two proliferated elements in the dermis: blood vessels and lymphoid tissue. The blood vessels are mainly capillaries lined by plump, often protruding (hobnailed) sometimes multilayered, endothelial cells. Occasionally an artery or vein showing intimal fibrous thickening is part of the vascular component. Solid clusters of cells which are often vacuolated show features intermediate between endothelial cells and histiocytes are also observed [16]. The lymphoid tissue may possess germinal centres. Eosinophils (often extremely numerous), mast cells and macrophages may also be prominent.

Kimura's Disease

Macroscopic It presents as large, deep and often disfiguring, subcutaneous masses in the preauricular, parotid and submandibular regions. Often, there is enlargement of

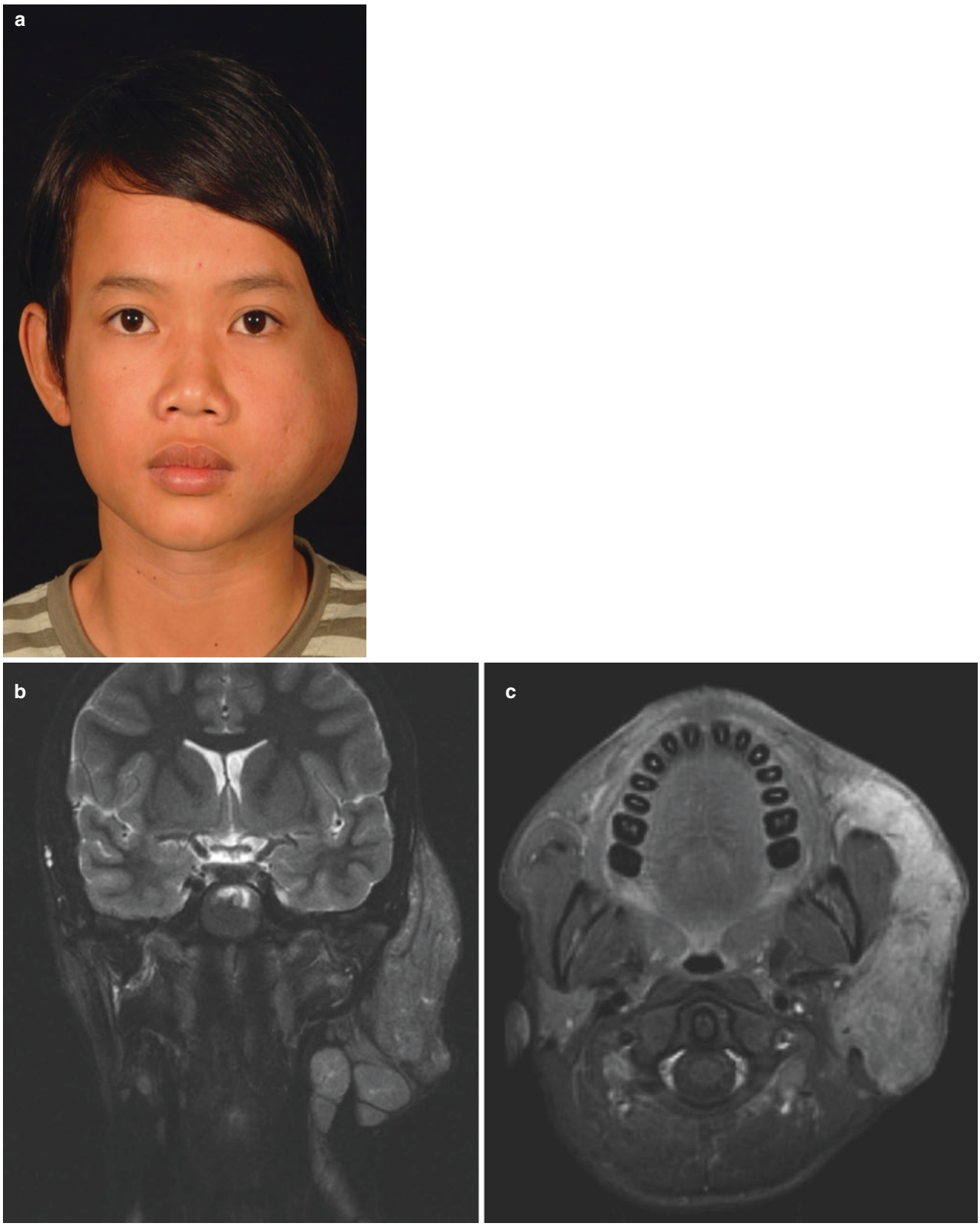

Fig. 8.6 Kimura's disease. (**a**) The lesion presents as large, deep and often disfiguring, subcutaneous masses in the preauricular, parotid and submandibular regions. Often, there is enlargement of regional lymph nodes. (**b**) The MRI images show a T2-weighted coronal slice and (**c**) a post-contrast T1 fat-saturated enhancing slice. They demonstrate a diffuse enlargement of the entire parotid gland as well as the deep cervical chain

regional lymph nodes. Occasionally, only lymph nodes are involved. There is a peripheral blood eosinophilia and raised levels of IgE.

Microscopic The subcutaneous masses are found to be composed of lymphoid follicles surrounded by oedematous connective tissue rich in eosinophils and containing numerous thin-walled blood vessels resembling high endothelial venules [16]. Infiltration of the germinal centres with eosinophils and follicle lysis is a frequent finding, as is the presence of multinucleated cells. There is deposition of IgE on the processes of the follicular dendritic cells and there are also numerous mast cells, the latter well shown by immunostaining with IgE mAb. Plasma cells may also be prominent (Fig. 8.7a–d).

Differential diagnosis Diagnostic confusion between angiolymphoid hyperplasia with eosinophilia and Kimura's disease is likely to occur if the characteristic vascular appearances in angiolymphoid hyperplasia with eosinophilia have not been recognised and if there is very prominent lymphoid hyperplasia with follicle formation and marked tissue eosinophilia in the latter. In Kimura's disease the endothelial cells in the proliferating vessels never show an epithelioid/histiocytoid appearance, nor is there an associated large vessel with intimal thickening. In angiolymphoid hyperplasia with eosinophilia, there is never lymph node enlargement or tissue deposition of IgE.

Treatment and prognosis Both epithelioid hemangioma and Kimura's disease are benign entities. Recurrence is rare

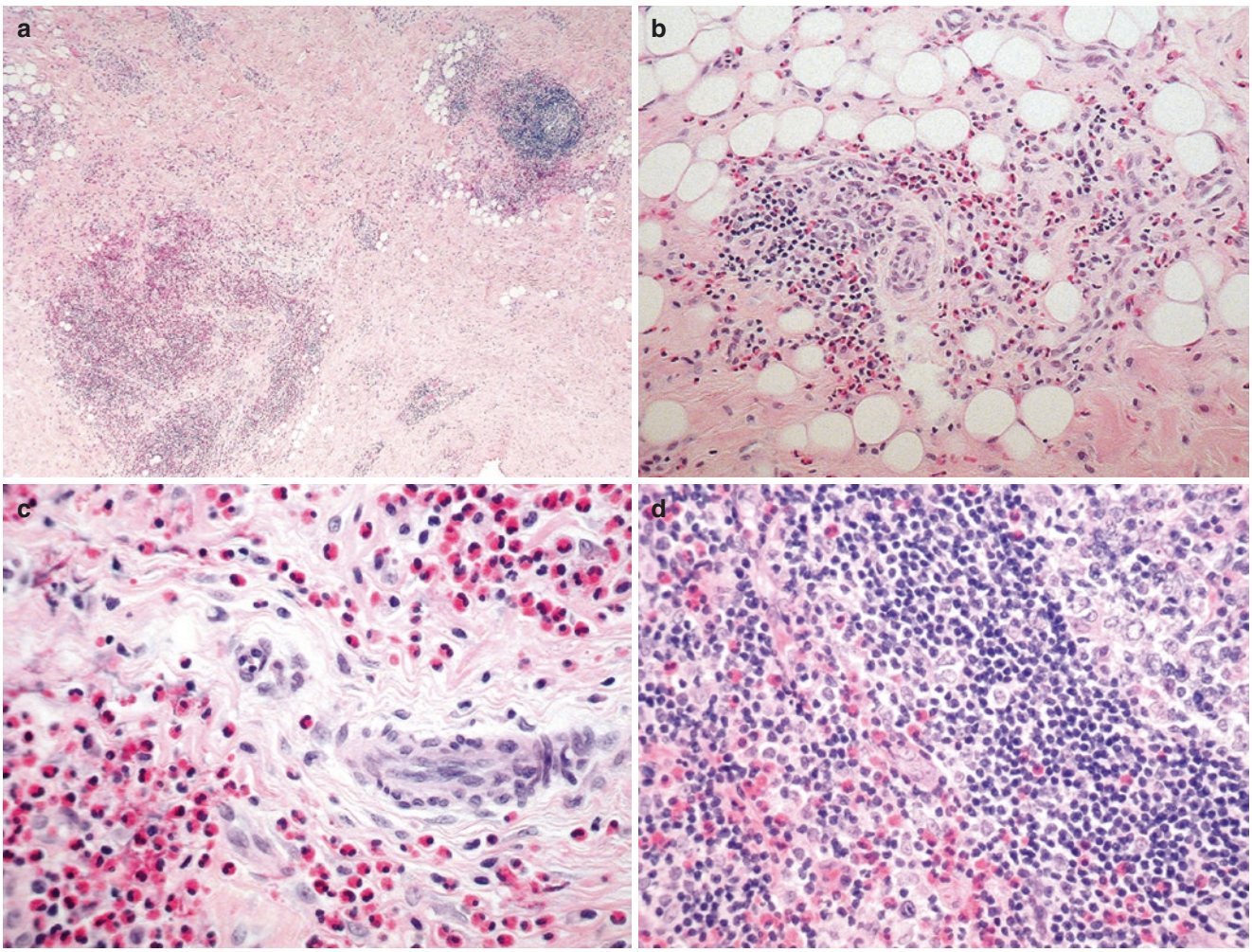

Fig. 8.7 Kimura's disease. (**a**) and (**b**) Subcutaneous masses are composed of lymphoid follicles surrounded by fibrous connective tissue rich in eosinophils and containing numerous thin-walled blood vessels resembling high endothelial venules (**c**) Infiltration of the germinal centres with eosinophils and follicle lysis is a frequent finding (**d**)

in the former if it is completely excised. It is more frequent in the latter, but eventually it becomes stationary. A nephrotic syndrome may rarely occur in Kimura's disease.

8.2.3.5 Accessory Tragus

Definition Accessory tragus is a fairly common congenital malformation of the external ear. In the majority of cases, it is an isolated developmental defect not associated with other abnormalities. It is, however, a consistent feature of the oculoauriculovertebral syndrome (Goldenhar syndrome) [17].

Epidemiology There is an equal sex incidence. The lesions are thought to develop early in embryogenesis as a result of aberration in development of the first and second branchial arch.

Macroscopy The lesion which may be bilateral is usually situated anterior to the normal tragus. In rare cases a similar structure has been found in the neck [18] or in the suprasternal region [19]. It appears as a skin-coloured nodule that may be soft or firm.

Microscopy The accessory tragus is composed of elastic cartilage with a covering of skin.

Treatment and prognosis Surgical excision is usually curative.

8.2.3.6 Keloid

Definition A benign lesion resulting from an abnormal healing response to injury to the skin of the ear, often after flame burn or piercing of the earlobes for wearing an earring. Synonym: keloid scar.

Epidemiology The condition affects both sexes equally; however female patients are more likely to present for treatment due to the disfigurement the lesions may cause. Keloids are 15 times more common in patients with pigmented skin particularly in Black and Asian populations. The lesions usually develop between the ages of 10 and 30 years. Patients present with mass lesions that may be pigmented, painful and itchy.

Clinical aspects The etiology is unclear. Keloids are associated with proliferation of dermal fibroblasts and several mechanisms have been suggested including abnormalities in growth factors, defective collagen metabolism or architectural distortion of collagen fibres as well as immune system dysfunction.

Macroscopy Grossly there is a lobulated swelling covered by normal skin.

Microscopy The dermis is enlarged by deposits of eosinophilic, poorly cellular collagen.

Treatment and prognosis Treatment is usually multimodality and includes surgery, intralesional steroid injection and occlusive dressings [20]. The latter may significantly reduce symptoms of pain itching and erythema. Treatment of keloid scars with surgery alone has resulted in recurrence rates between 45 % and 100 %.

8.2.4 Benign Neoplasms

External ear neoplasms derived from ceruminal glands are very uncommon. Only the adenoma can usually be categorized with certainty as being derived specifically from ceruminous glands since its component acinus displays an apocrine secretory structure. Syringocystadenoma papilliferum and pleomorphic adenoma arising in this region can sometimes appear to be developing from ceruminous glands.

8.2.4.1 Adenoma of Ceruminal Glands

Definition A benign tumor arising in the ceruminous or wax producing glands in the skin of the external ear.

Epidemiology There is a wide age range at presentation (12–85 years). There are very few tumors reported in the paediatric age group. The mean age is in the fifth decade. There is an equal sex incidence.

Clinical aspects They are unusual neoplasms that present with a blockage of the lateral part of the external auditory meatus, often associated with deafness and discharge. The lesions are confined to the skin over the cartilaginous part of the external ear canal (outer half). The typanic membrane is usually intact in patients with ceruminous adenoma.

Correlation with imaging is often helpful to exclude middle ear and inner ear tumors extending into external ear as well as other lesions in parotid or nasopharynx.

Macroscopy Gross appearances are those of a superficial grey mass up to 4 cm in diameter, which is covered by the skin.

Microscopy Microscopically this neoplasm lacks a definite capsule. It is composed of regular glands often with intraluminal projections (Fig. 8.8) [1]. The glandular epithelium is bilayered, the outer layer being myoepithelial, but this may not be obvious in all parts of the neoplasm. The glands are often arranged in groups surrounded by fibrous tissue. In some ceruminomas, acid-fast fluorescent pigment may be found in the tumor cells which is similar to that found in normal ceruminal glands [21, 22].

Immunohistochemistry Immunostains are of limited use in diagnosing ceruminous neoplasms. CK7 and

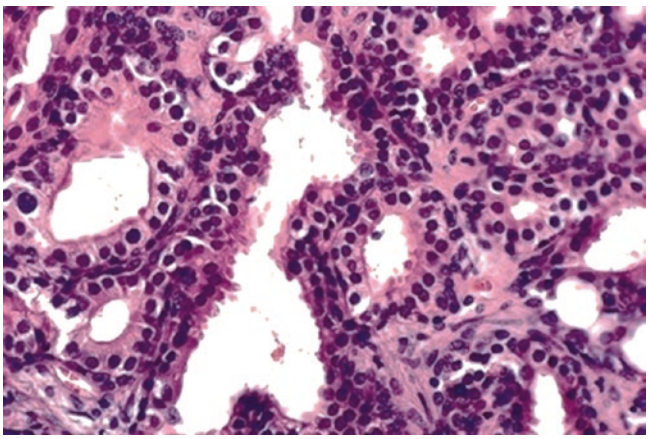

Fig. 8.8 Ceruminous gland adenoma. The lesion is composed of fairly regular glands arranged back to back and showing apocrine secretion (Reproduced from Michaels and Helquist [1])

CD117 are expressed by the luminal cells and the basal myoepithelial cell layer may be demonstrated with p63 and S100 immunostains. Neuroendocrine markers are negative.

Treatment and prognosis Complete excision is the treatment of choice. Adenoma of ceruminal glands is a benign neoplasm. Recurrence should not be expected if it is carefully excised.

8.2.4.2 Pleomorphic Adenoma of Ceruminal Glands

Definition A benign neoplasm of the skin with a structure similar to that of pleomorphic adenoma of salivary glands is also occasionally seen in the external auditory meatus.

Epidemiology The lesions arise in the lateral cartilaginous portion of the external ear canal.

Macroscopy The lesion is a well-circumscribed polypoid mass 0.4–4 cm with no ulceration.

Microscopy Histologically the lesions are indistinguishable from those arising in salivary gland. Cartilage, myoepithelial and epithelial ductal structures are features of this neoplasm.

Treatment and prognosis Complete excision is curative.

8.2.4.3 Syringocystadenoma Papilliferum of Ceruminal Glands

Definition and epidemiology A benign tumor seen in children or young adults usually on the scalp or face. Occasionally it occurs in the ear canal.

Macroscopy The lesion is a well-circumscribed polypoid mass 0.4–2 cm with no ulceration.

Microscopy The histological appearance of the neoplasm is that of an invagination from the surface epithelium forming a cyst-like structure. Projecting into the lumen are papillae lined by bilayered glandular epithelium which may show decapitation secretion typical of apocrine (ceruminal) glands.

Treatment and prognosis Complete excision is curative.

8.2.4.4 Bony Lesions

Definition and epidemiology Because of the difficulty of classifying a solitary benign neoplasm composed of woven bone and fibrous tissue into one or other of the classical groups – monostotic fibrous dysplasia or ossifying fibroma – the designation *benign fibro-osseous lesion* (*fibrous dysplasia*) may be used in most circumstances for lesions in the ear without loss of accuracy. Lesions of this type are found in the temporal bone often presenting in the bone deep to the external ear [23, 24]. At other sites, this lumping is less appropriate as discussed in Chap. 4.

Clinical aspects The main clinical features are progressive loss of hearing, conductive in most, sensorineural, which can be profound, in some and enlargement of the temporal bone with progressive bony occlusion of the external auditory meatus. Facial nerve palsy is present in some patients due to involvement of the facial nerve by the pathological process.

Macroscopy The gross appearance of benign fibro-osseous lesion is one of yellowish-white resilient tissue, which occasionally includes small cysts filled with an amber-coloured fluid. The transition to normal bone is sharp.

Microscopy Irregular trabeculae of woven bone are embedded in a connective tissue stroma. The constriction of the ear canal may cause an epidermoid cyst lateral to the tympanic membrane, referred to in some publications as "cholesteatoma" [23].

Differential diagnosis Although fibrous dysplasia has been on rare occasions associated with malignant disease such as osteogenic sarcoma, fibrosarcoma, chondrosarcoma and giant cell tumor, the temporal bone is not one of the sites where this change has been described.

Osteoma and Exostosis

Definition These are two types of benign bony enlargement of the deeper bony portion of the external auditory meatus.

Synonyms Osteochondroma and osteocartilaginous exostosis.

Clinical aspects Osteoma is a rare lesion. Symptoms are usually those of ear canal obstruction. Exostosis is a more common broad-based lesion, which is often bilateral and symmetrical. It is usually situated deeper in the ear canal than osteomas. In the bony portion of the normal external auditory meatus, there are no adnexal structures, and the subcutaneous tissue and periosteum merge to form a thin layer. The distance between the epidermal surface and underlying bone is consequently small. This explains the propensity for exostoses of the tympanic bone to develop in this region in those who swim frequently in cold water. It seems likely that the water, after dribbling into the deep external auditory canal, exerts a cooling effect on the bone surface and stimulates it to produce new bone.

Macroscopy The osteoma is a unilateral spherical mass arising from the region of the tympanosquamous or tympanomastoid suture line by a distinct bony pedicle. Exostosis results in constriction of the ear canal by new bone growth forming lumps.

Microscopy The osteoma is composed of lamellar bone and may show outer cortical and inner cancellous trabeculated areas, the latter with marrow spaces. There may be appositional new bone formation, i.e. a thin layer of woven bone on the surfaces of the lamellar bone. The osteoma is covered by the normal squamous epithelium of the ear canal (Fig. 8.9a, b). Unlike osteoma the bone formations of exostosis are said not to possess any marrow spaces.

Differential diagnosis Five cases of a benign, circumscribed, bony lesion of the external auditory canal distinct from exostosis and osteoma have recently been described [25]. They all showed a hard, round, unilateral, skin-covered mass occluding the superficial external auditory canal with no relationship to the cartilaginous tissue or to the bony structure surrounding that canal. Histologically the lesion displayed an osteoma-like bone formation with sparse osteoblastic areas; mature lamellar bone was observed some cases and also bone marrow containing adipose tissue and haematopoietic remnants. The bone showed irregular trabeculae, bordered by osteoid osteoblasts.

Treatment Osteoma and exostosis are often associated with infection of the external canal on their tympanic membrane side and surgical removal may be required to enhance drainage as well as to relieve the conductive hearing loss.

Prognosis Osteoma and exostosis are benign lesions with no malignant potential.

8.2.5 Malignant Neoplasms

8.2.5.1 Adenocarcinoma of Ceruminal Glands

Definition Adenocarcinoma not otherwise specified (NOS) of ceruminal glands is a rare malignant neoplasm presenting in the superficial part of the external ear canal.

Synonyms Adenocarcinoma of ceruminal type, ceruminous gland adenocarcinoma NOS, adenoid cystic carcinoma and mucoepidermoid carcinoma.

Epidemiology There is a wide age range at presentation. There are very few tumors reported in the paediatric age

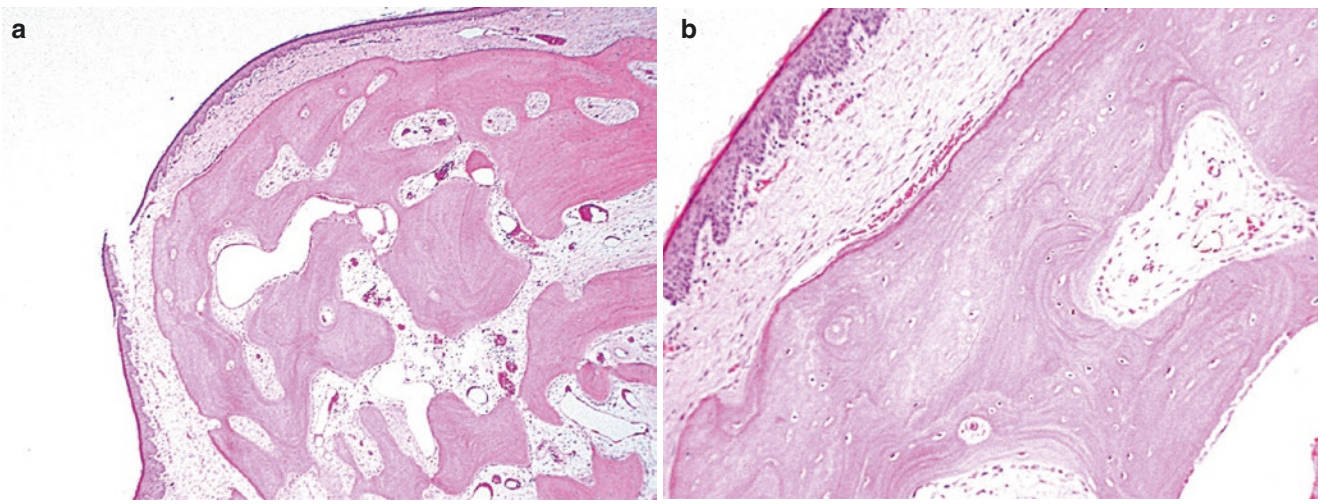

Fig. 8.9 Osteoma of external ear. (**a**) and (**b**) The lesion is composed of lamellar bone and may show outer cortical and inner cancellous trabecular bone with marrow spaces as illustrated here. The osteoma is covered by the normal squamous epithelium of the ear canal

group. The mean age is in the fifth decade. There is a slight female predominance overall with females presenting at a slightly younger age. The lesions are confined to the skin over the cartilaginous part of the external ear canal. Correlation with imaging is often helpful to exclude middle ear and inner ear tumors as well as other primaries in the parotid or nasopharynx.

Clinical features Ceruminous gland adenocarcinoma is very rare. An incidence of 0.0001 % of all surgical cases has been suggested. Patients usually present with a mass lesion in the lateral part of the external auditory meatus, often associated with deafness, and discharge. Pain may be a presenting feature and a few patients are reported to have nerve paralysis. Asymptomatic tumors have been reported.

The terminology used for these lesions is extensive and confusing. These tumors are difficult to diagnose because they are seldom seen and because of the variable clinical and histological features at presentation.

Macroscopy Ceruminous neoplasms appear as a superficial grey mass up to 3 cm in diameter, covered by skin. The site usually limits the tumor size and most are between 1.2 and 1.5 cm diameter. Malignant tumors are often associated with ulceration.

Microscopy There is always local infiltration. The neoplasm possesses a glandular structure with evidence of apocrine differentiation, but the glands show loss of a myoepithelial layer and the cells are markedly atypical with increased mitotic activity (Fig. 8.10a, b).

Immunohistochemistry It is of limited use in diagnosing ceruminous neoplasms. CK7 and CD117 are expressed by the luminal cells and the basal myoepithelial cell layer may be demonstrated with p63 and S100 immunostains. A modestly increased proliferation rate with Ki67 immunostain may be seen, particularly in those tumors of larger size.

Differential diagnosis Ceruminous adenocarcinoma should be distinguished from a lesion directly extending into the external ear from parotid gland and metastatic disease should also be excluded. Cutaneous basal cell carcinoma may be confused with ceruminous adenoid cystic carcinoma and ceruminous adenocarcinoma may resemble ceruminous adenoma or neuroendocrine adenoma of middle ear in small biopsies.

Treatment and prognosis Complete surgical excision is the optimal treatment. Due to the difficult anatomical location, the tumors are often removed piecemeal and margins are difficult to assess. Recurrence is to be expected following surgical removal. Death due to involvement of local vital structures has been reported. Rare examples of low-grade adenocarcinoma of ceruminal glands have been documented. Adjuvant radiotherapy at the time of initial therapy may prolong survival and when used to treat recurrence of tumor or for palliation.

8.2.5.2 Adenoid Cystic Carcinoma of Ceruminal Glands

Ceruminous adenoid cystic carcinoma is a rare malignant neoplasm that has the gross and microscopic features of the corresponding major or minor salivary gland neoplasm, including its tendency to invade along nerve sheaths. Ceroid pigment may not be identified in primary ceruminous adenoid cystic carcinoma.

Treatment requires extensive surgery to ensure complete resection of the tumor. Relentless, although often delayed recurrence and eventual bloodstream metastasis, particularly

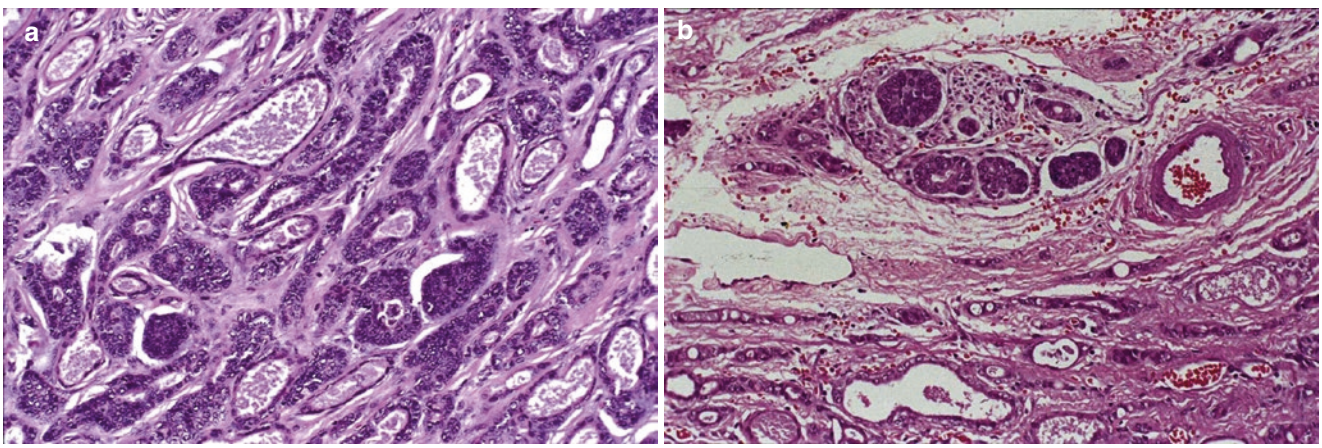

Fig. 8.10 Ceruminous gland adenocarcinoma. (**a**) Atypical glandular structures showing an infiltrative growth. There is marked nuclear atypia. No apocrine secretion is see. (**b**) The same tumor showing perineural and intraneural invasion

to the lungs, is likewise a feature of this cancer. Death usually results from extension of the tumor into the skull base and brain or from pulmonary metastases.

8.2.5.3 Basal Cell Carcinoma

Definition Basal cell carcinoma is the most common malignant epithelial tumor of the pinna arising from surface epithelium.

Epidemiology More men than women are affected which may reflect the shorter hairstyles usually worn by men and increased sun exposure.

Clinical aspects They occur in the preauricular crest, posterior auricle and helix.

The few basal cell carcinomas that occur in the ear canal arise near the external opening. Their preference for the exposed part of the external ear is in keeping with the accepted view that sunlight is in most cases the causal factor in skin insufficiently protected by melanin pigment.

Macroscopy The gross appearance of basal cell carcinoma is usually one of a pearly wax-like nodule which eventually ulcerates (Fig. 8.11a). Twenty-five per cent of basal cell carcinomas of the pinna are of the infiltrative or morphea type [see below]. The importance of this variety is that although the edge of the tumor tends to infiltrate subcutaneously, this cannot be recognized clinically or on gross pathological examination.

Microscopy The classical and most frequent form of basal cell carcinoma is composed of solid masses of cells, which are seen to be arising from the basal layers of the epidermis or the outer layers of the hair follicles. The cells are uniform with basophilic nuclei and little cytoplasm. At the periphery of the neoplastic lobules, the cells tend to be palisaded. Mitoses are frequent. Alveolar or cystic spaces are frequent. Squamous cell differentiation is also common.

The splitting up of cell groups by much hyaline fibrous tissue, so that the carcinoma appears compressed into thin strands, is referred to as the morphea type of basal cell carcinoma (Fig. 8.11b). The suggestion that tumors with this histology have a worse outlook is probably related to their tendency of insidious infiltration [see above]. There is otherwise no convincing evidence of the relationship of a particular histological appearance to prognosis in basal cell carcinoma.

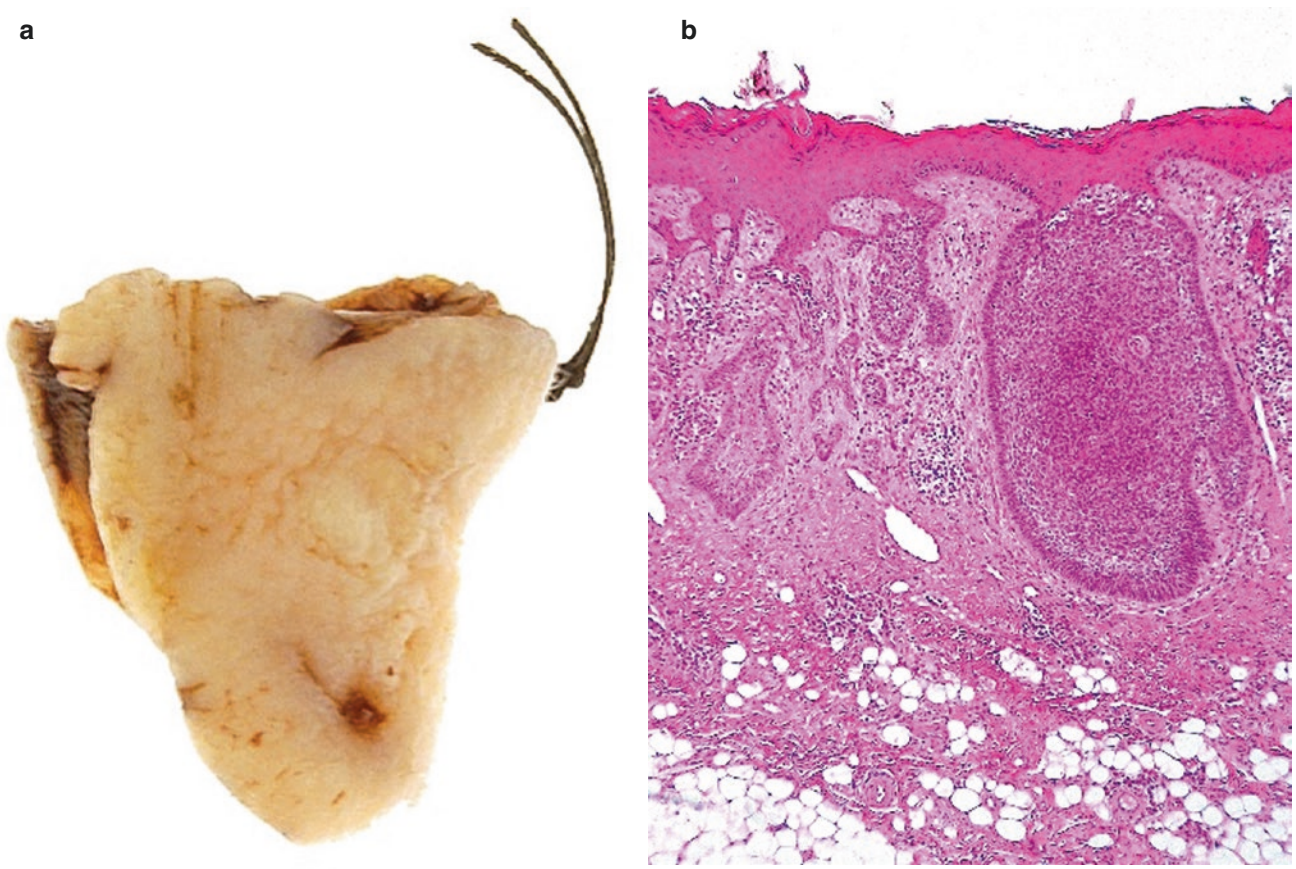

Fig. 8.11 Basal cell carcinoma of the pinna. (**a**) The macroscopic image shows a wedge resection of the pinna to remove the pale nodule seen towards the centre. (**b**) Section on the right shows nodular and infiltrative basal cell carcinoma in skin overlying the cartilage

Immunohistochemistry However, when immunohistochemical assessment for Ki-67 antigen (MIB1 in paraffin sections), a proliferation-associated antigen, is performed on basal cell carcinomas, those tumors that recur have been shown to possess a higher proportion of cells positive for that antigen than those that do not [26]. The degree of tumor angiogenesis is another histologic factor that shows promise in judging the prognosis of basal cell carcinoma [27].

Differential diagnosis In small biopsies the lesions may be mistaken for actinic keratosis, chondermatitis nodularis chronica helicis and squamous cell carcinoma.

Treatment and prognosis This is not an aggressive neoplasm and in at least 90 % of cases cure can be easily achieved by adequate surgical excision. In a few cases repeated recurrences with deep extension to the middle ear, mastoid and even cranial cavity may, however, take place. Metastasis is rare.

8.2.5.4 Squamous Cell Carcinoma

Definition A malignant tumor of stratified squamous epithelium. The majority of squamous cell carcinomas of the external ear arise in the pinna; a lesser number arise in the external canal.

Epidemiology The average age at diagnosis is 65–70 years for squamous cell carcinoma of the pinna and pinna lesions and there is a male predominance. The age at presentation is 52–55 years for squamous cell carcinoma of external canal tumors and these show a female predominance.

Clinical aspects The pinna lesions, being in a prominent position, are identified early. A serious problem with the canal lesions is the delay in diagnosis because of the minimal symptoms that may be present. Pain, hearing loss and drainage of blood or pus are the main features in that group. A plaque-like or even polypoid mass may be felt or even seen.

Macroscopy Squamous carcinomas arising on the pinna grossly resemble those seen elsewhere on the skin. They are often closely excised for best cosmetic result. This may cause difficulty at gross examination and block selection to assess margins (Fig. 8.12a, b). The larger lesions are excised by wedge excision and the margins represented are the superior medial margin and inferior medial margin of the pinna. These margins can be inked and the specimen is sampled by serial sections superior to inferior (Fig. 8.13a–d).

The appearances of the canal lesions are those of a mass, sometimes warty, occluding the lumen and invading deeply into the surrounding tissues. There may be dissolution of the tympanic membrane with invasion of the middle ear.

Microscopy Squamous cell carcinoma of the external ear usually shows significant degrees of keratinization. In the cases with a canal origin, evidence of origin from canal epidermis is usually present. In cases arising deeply within the ear canal, there is usually a concomitant origin from middle ear epithelium and dissolution of the tympanic membrane [see below]. The neoplasm may be so well-

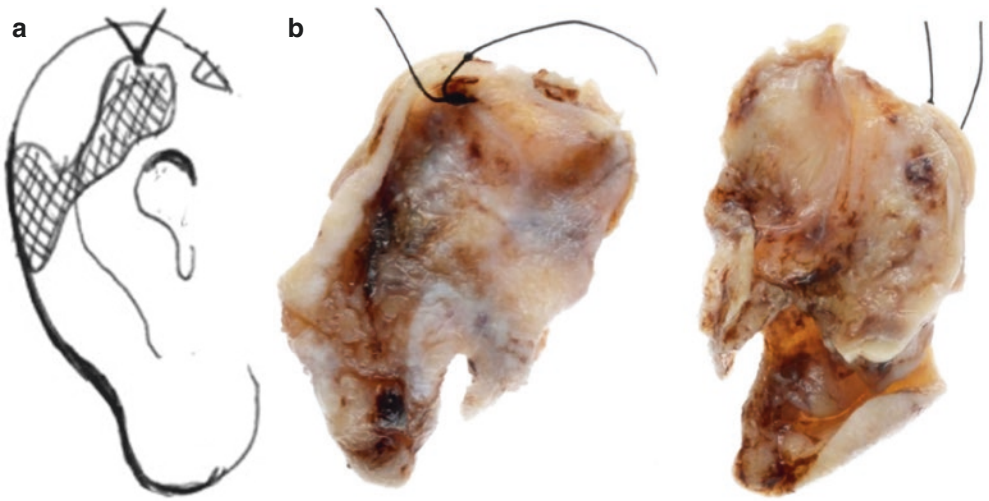

Fig. 8.12 Squamous cell carcinoma of the external ear. It is important to achieve a good cosmetic result after surgery and sometimes the resection specimen may be difficult to orientate. (**a**) A clinical diagram and (**b**) orientation sutures can assist the pathologist to assess the tumor and margins

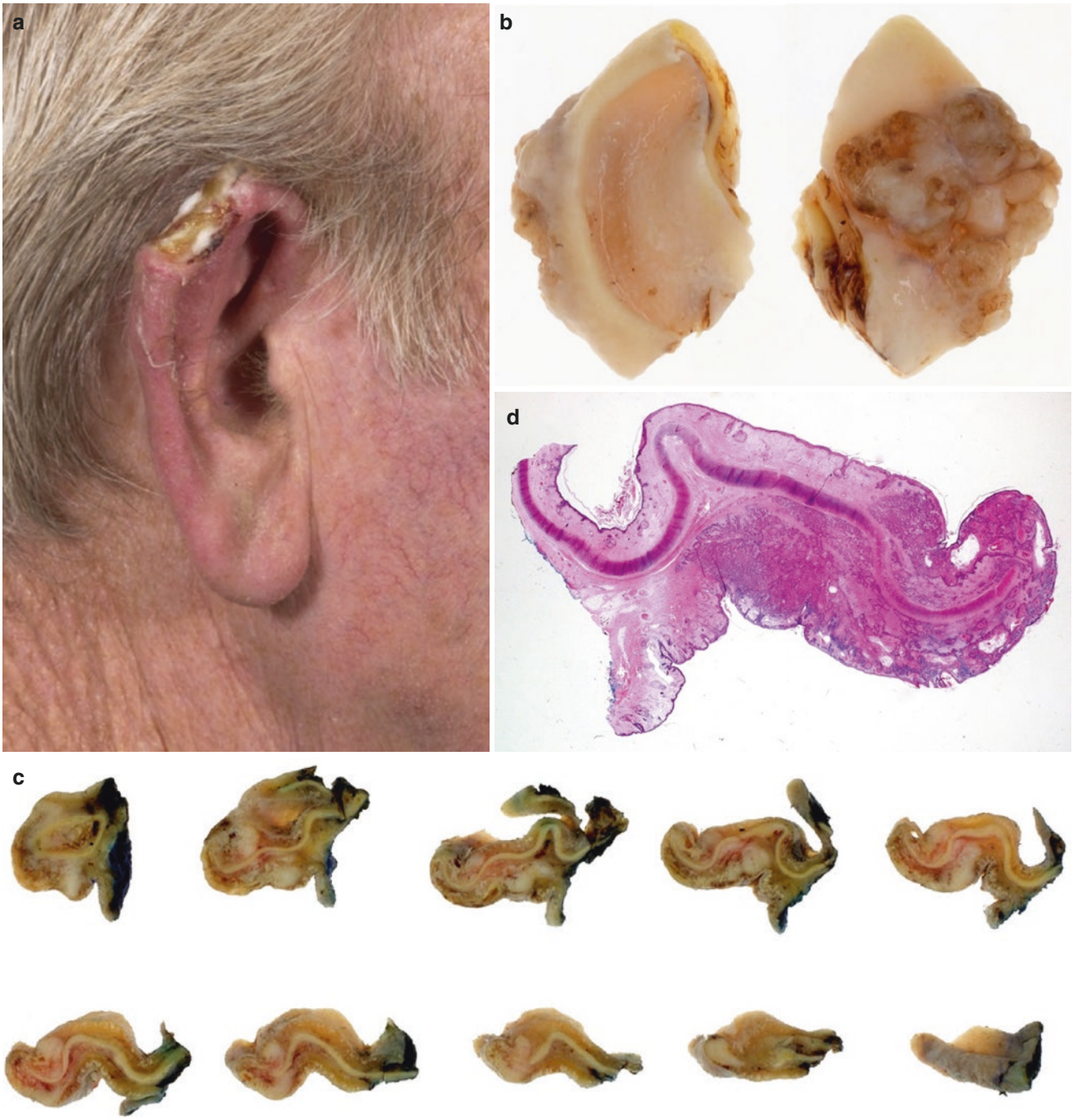

Fig. 8.13 Squamous cell carcinoma of the pinna. (**a**) A keratotic ulcerated lesion is seen on the superior helix in the clinical photograph. (**b**) Such lesions are excised by wedge excision as seen in the macroscopic specimen. (**c**) The specimen has been sliced superior to inferior and the slices are shown above. (**d**) The wholemount section shows ulcerated surface epithelium on the top right of the section. The tumor is occupying the dermis around the cartilage seen centrally and it is clear of the margin seen on the left

differentiated that it can be confused with benign papilloma. The association of a well-differentiated squamous carcinoma with marked desmoplasia may also delay the correct diagnosis (Fig. 8.14a, b). The verrucous form of squamous cell carcinoma has been seen in the external ear [28]. Metastatic spread of squamous carcinoma of the pinna and external auditory meatus to lymph nodes at presentation is unusual.

Treatment and prognosis Squamous carcinoma of the external canal is an aggressive disease with a high propensity for local recurrence. Treatment includes pinnectomy and

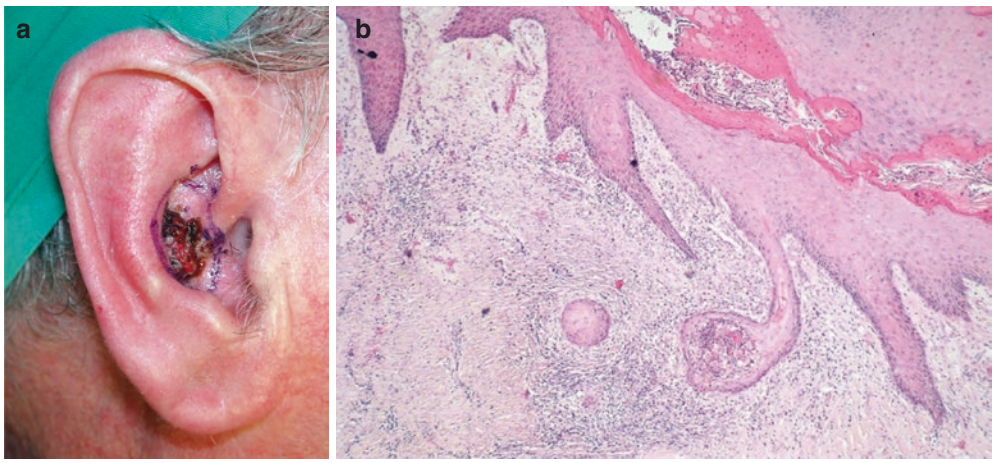

Fig. 8.14 (**a**) Squamous cell carcinoma of the external ear canal. (**b**) Biopsies may be small and misinterpreted as inflammatory changes

petrosectomy (Fig. 8.15a–c). Again these excision specimens may cause difficulty for the surgical pathologist at cut up. Part of the temporomandibular joint bone may be included on the deep aspect. The specimen should be orientated by clinicians and dissected so that all relevant margins (inferior, superior, anterior, posterior and deep) can be assessed (Fig. 8.15a–c). True margins are often sent separately and part of the temporal bone may be drilled away and not sent for histological examination so the surgical resection specimen does not reflect the true deep margin of excision.

A review of 213 cases in a Scottish series of squamous cell carcinoma of the ear showed 11 % of cases metastasised to regional lymph nodes and 66 % patients died of disease [29]. There was no correlation with histological subtype and the risk of metastasis was increased with tumors greater than 8 mm deep. The outcome of the disease following surgical excision is related to the clinical stage at presentation; the higher the stage, the worse the outcome [30].

8.2.5.5 Melanocytic Neoplasms

Definition Tumors of pigment-producing cell in the skin.

Melanotic neoplasms are unusual in the external ear. Benign nevi arise mainly in the ear canal, but are rare on the auricle.

Premalignant melanocytic lesions and malignant melanoma are usually located on the helix and antihelix. These lesions are also discussed in Chap. 15.

Lentigo Maligna

Lentigo maligna (synonym Hutchinson's freckle) is a slow non-invasive melanoma in situ.

Clinical aspects The lesion presents as an irregular black brown macule that gradually extends peripherally. It is more common in elderly patients and the conversion rate to invasive malignancy (lentigo maligna melanoma) is 30–50 %.

Treatment and prognosis Surgery with intraoperative assessment of margins such as MOHS surgery is the treatment of choice with a recommended minimum margin of 5 cm. Radiotherapy may be considered for those patients who are unable to undergo surgery.

Malignant Melanoma

Definition Malignant tumor of melanocytes.

Epidemiology There is a male predominance and all age groups are affected apart from young children. The average age at presentation is 50 years.

Clinical aspects About one fifth of melanomas affect the head and neck and of these 17–14 % affect the ear. The lesions usually arise on the auricle; origin in the external canal is extremely unusual [31]. Fair-skinned people are more at risk of developing the disease and it appears that the right ear is affected more often than the left. Malignant melanoma of the external ear is a highly malignant disease. In a review of 16 patients with this condition as many as nine cases showed invasion to Clark level IV or more [32]. It is likely that cervical and parotid gland lymph nodes will be involved when malignant melanoma of the external ear is first diagnosed [33]. A third of patients are reported to present with nodal metastasis.

Macroscopy Patients notice a flat irregular variably pigmented area or a nodule that has changed colour or has increased in size. Non-pigmented or amelanotic lesions also occur.

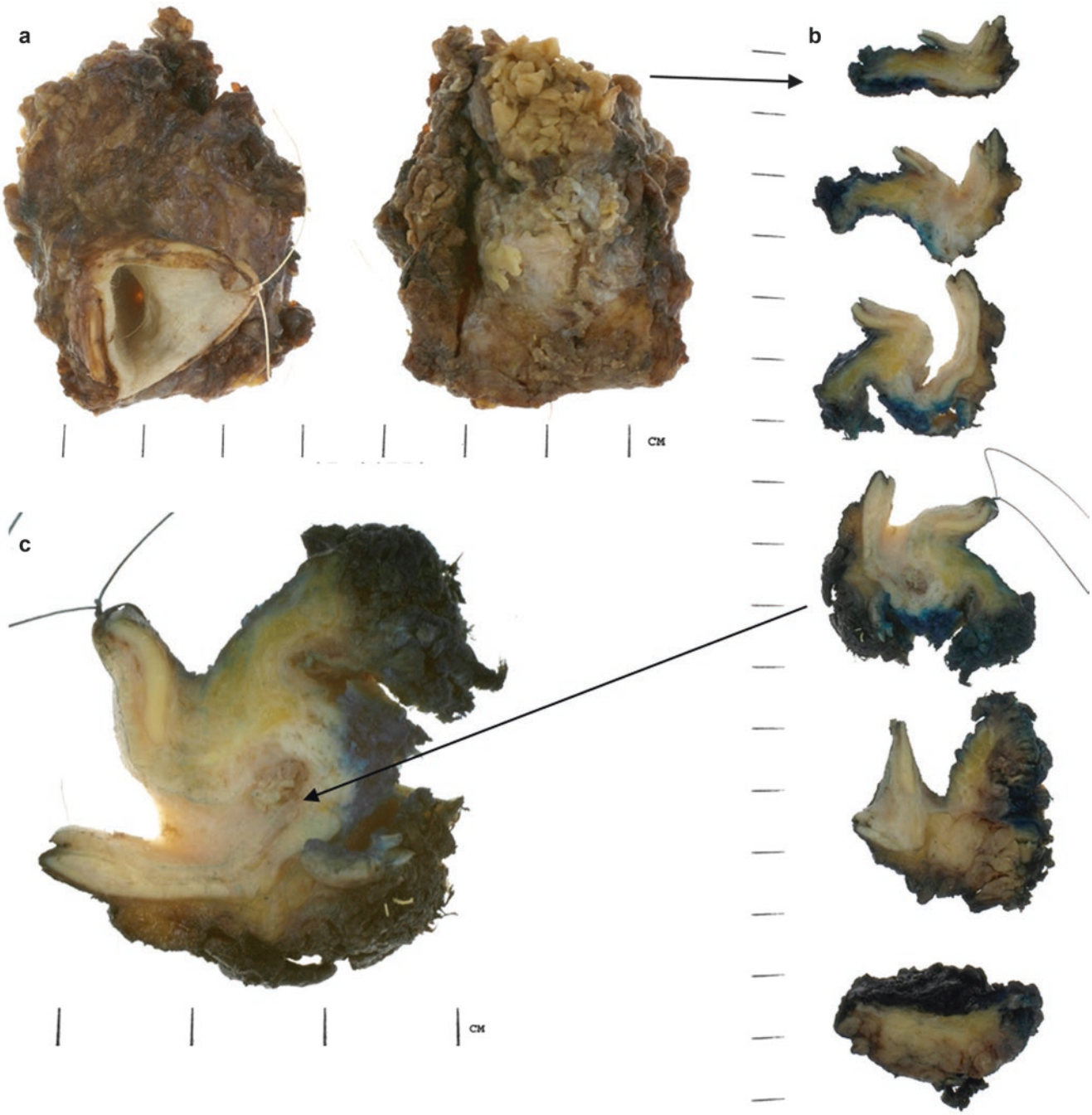

Fig. 8.15 Squamous cell carcinoma of external ear canal. (**a**) The macroscopic specimen is an excision of the ear canal and petrosectomy. (**b**) The specimen has been sliced superior to inferior and (**c**) the exophytic tumor can be seen in the canal in the macroslice (*arrow*)

Microscopy Lentigo maligna melanoma, superficial spreading melanoma and nodular melanoma are the main subtypes. Nodular melanoma is the most deeply invasive and most aggressive. Prognostic indicators include the histological subtype, tumor thickness and depth of invasion together with the presence of ulceration.

Differential diagnosis Clinically the differential diagnosis includes pyogenic granuloma, benign compound nevus actinic keratosis and pigmented basal cell carcinoma.

Treatment and prognosis Age at presentation and margin status are factors that influence local recurrence. The main

predictors of survival for melanoma of the ear are the tumor thickness and Clark's level of invasion. An excision margin of >10 mm is associated with a decreased risk of recurrence; however wide excision margins do not appear to affect overall survival and surgery is becoming less aggressive.

Surgical excision is the mainstay of therapy. A margin of 20 mm is recommended for tumors >2.1 mm deep. Sentinel lymph node biopsy is undertaken after lymphoscintigraphy to accurately identify the nodes since the site of the melanoma on the ear does not correlate consistently with lymph node drainage. Adjuvant treatment includes radiotherapy, chemotherapy and immunotherapy [34].

8.3 Middle Ear and Mastoid

8.3.1 Inflammatory Lesions

8.3.1.1 Acute and Chronic Otitis Media

Definition Inflammation of the middle ear.

Epidemiology Otitis media is one of the most common of all diseases, particularly in young children. The majority of cases occur in children under 6 years old with an equal sex incidence.

Clinical aspects The disease is usually caused by bacterial infection, *Haemophilus influenzae* and Gram-positive cocci usually being incriminated in the acute form and Gram-negative bacilli in the chronic form. The clinical forms of the acute and chronic conditions correspond to the pathological changes, but intermediate or mixed states are frequent. Perforation of the tympanic membrane may occur at any phase of otitis media, but an effusion, accompanied by all of the other manifestations of chronic otitis media, is often present behind an intact tympanic membrane, a condition known as serous otitis media. The pathogenesis is uncertain. Recently inflammatory mediators including PAF, IL-1 and TNF-alpha have been implicated in the initiation and maintenance of the inflammatory response to infection and injury. Such inflammatory mediators may play a role in the progression from acute to chronic otitis media and the development of cholesteatoma [35].

Macroscopy The tympanic membrane in children with acute otitis media is described as cloudy, red and bulging. Otitis media with effusion or glue ear results from an accumulation of fluid in the middle ear space. It is not associated with a bulging tympanic membrane and antibiotics are not indicated.

Microscopy The appearances of the middle ear mucosa in acute otitis media may be seen in the bone chips removed at mastoidectomy. There are congestion and oedema of the mucosa of the mastoid air cells. Haemorrhage may be severe and the mucosa and air cells are filled with neutrophils. Pus destroys bone, the actual dissolution being carried out by osteoclasts. At the same time new bone formation takes place, commencing as osteoid, later becoming woven and finally lamellar. Fibrosis may also be active even in the acute stage. Acute inflammatory changes are also prominent in other parts of the middle ear. The tympanic membrane shows marked congestion, the dilated vessels distending the connective tissue layer. Pus cells fill the middle ear cavity. The acute inflammation may spread deep into the temporal bone as osteomyelitis.

The chronic form of otitis media is associated with necrosis, caused by the bacterial infection. There is, as in the acute form, marked congestion. The latter results in haemorrhage in many cases. Because of the poor lymph drainage in the middle ear, old haematoma becomes converted into cholesterol granuloma, with cholesterol clefts surrounded by foreign body-type giant cells and haemosiderin.

Associated with these changes and representing an important part of the pathological picture is proliferative activity of middle ear tissue. The columnar epithelium of the middle ear has, in the presence of inflammation or other pathological changes in the middle ear, the remarkable property of invaginating itself to produce glands, which often develop luminal secretion. The glandular transformation of the middle ear mucosa, known as glandular metaplasia, may be seen in any part of the cleft, including the mastoid ear cells. The secretion of the glands contributes to the exudate in otitis media with effusion. Fibrous tissue proliferation may also occur in combination with glandular transformation – a process which, in the advanced state, has been called "fibrocystic sclerosis" [36].

A specific form of reparative reaction following inflammation is the development of granulation tissue. In this process, the endothelium of blood vessels and fibroblasts are the newly formed cells. Mononuclear inflammatory cells usually accompany the latter. The granulation tissue is usually particularly prominent in the middle ear under the mucosa covering the promontory from which it frequently protrudes into the external canal through a perforation of the tympanic membrane, forming an aural polyp which is covered by pseudostratified columnar, ciliated respiratory or stratified squamous epithelium. Fibroblasts and collagen are abundant in the terminal phase of the reparative stage.

A normal degree of fibroblast cellularity in the fibrous reaction is seen in *adhesive otitis media*. A peculiar form of scar tissue production occurs in the middle ear, in which the collagen is poorly cellular and hyalinised. This condition, known as *tympanosclerosis*, is characterized also by deposi-

tion of calcium salts in the hyaline fibrous tissue. The bony walls of the middle ear also frequently react to the inflammatory process by a new formation of bone. This is woven in the early stages and lamellar later.

Treatment and prognosis The condition is treated with systemic antibiotics. Mortality rates are very low but there is significant morbidity. Possible complications that may arise are classified as (a) intra-temporal, including hearing loss cholesteatoma and mastoiditis, and (b) intra-cerebral including meningitis, subdural empyema, brain abscesses and lateral sinus thrombosis.

8.3.1.2 Cholesteatoma

Definition Cholesteatoma is the presence of stratified squamous epithelium in considerable quantities in the middle ear. The lesion may be congenital or acquired. The common acquired form of cholesteatoma is associated with severe otitis media.

Congenital cholesteatoma and acquired cholesteatoma will be considered separately below.

In order to discuss congenital cholesteatoma, it is necessary to revise the embryology of the ear.

Stratified squamous epithelium is present in the normal fetal middle ear: Small colonies of cells being epidermoid in nature as confirmed by immunohistochemistry are found near the tympanic membrane on the lateral anterior superior surface of the middle ear in every temporal bone after 15 weeks gestation. These epidermal colonies, which are known as "epidermoid formations", increase significantly in size with increasing age and pari passu undergo increasing epidermoid differentiation [37]. During the first post-partum year, these epidermoid formations disappear. It is possible that the entry and growth of epidermoid formations in the fetal middle ear may lead to a local cellular immunity as a defence mechanism against the entry of keratinocytes into the middle ear. This could cause the eventual dissolution of all epidermoid formations. However, if immunity is delayed or defective epidermis could continue to grow and lead to congenital cholesteatoma.

Stratified squamous epithelium in the middle ear of a young child is termed *congenital cholesteatoma*:

Macroscopy Congenital cholesteatoma is seen, in most cases, as a spherical whitish object in the anterosuperior part of the tympanic cavity behind an intact tympanic membrane (Fig. 8.16). In some cases the lesion may fill most of the tympanic cavity. At operation the cholesteatoma is reported usually to be a cyst in the anterosuperior part of the middle ear. Bone erosion is not present when the cholesteatoma is small. In larger lesions some degree of this change is present [38] and eventually it may enlarge to involve the mastoid,

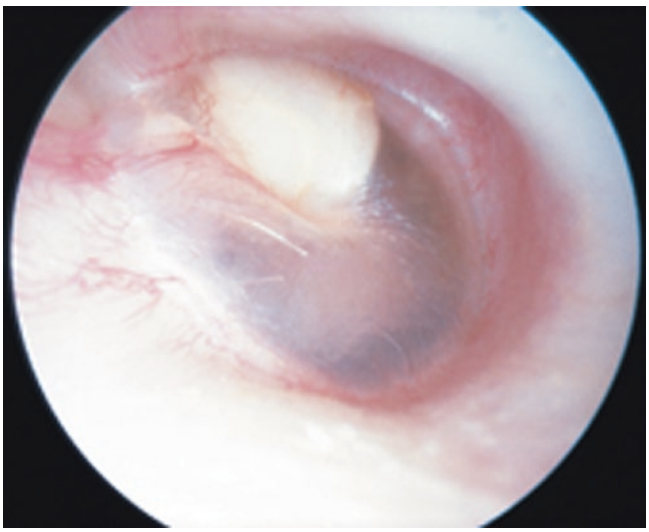

Fig. 8.16 Congenital cholesteatoma, otoscopy. Congenital cholesteatoma is seen, in most cases as a spherical whitish object in the anterosuperior part of the tympanic cavity behind an intact tympanic membrane (Reproduced from Michaels and Hellquist [1])

cause a perforation of the tympanic membrane and even grow into the middle cranial fossa [39] so that it becomes indistinguishable from acquired cholesteatoma [see below]. Indeed it is possible that many cases of acquired cholesteatoma have originated as congenital cholesteatoma.

In approximately 10 % of cases, the cholesteatoma is not cystic, but open, and shows layers of squames and a matrix (living basal and Malpighian layers of epidermis) which is plastered to the wall of the tympanic cavity [40].

Microscopy The microscopic appearances of the matrix of congenital cholesteatoma are those of skin epidermis, comprising a single row of basal cells, several rows of Malpighian cells and a thin granular layer. The surface of dead, keratinous squames merges with the keratinous contents of the cyst or lamellae in the case of the open type. When the histological appearances of these cases are compared with those of acquired cholesteatoma, little difference can be seen.

Stratified squamous epithelium in the middle ear of an older child or adult is termed *acquired cholesteatoma*:

Clinical aspects Typically in acquired cholesteatoma a lesion far more common than that of congenital cholesteatoma, the patient presents with a foul-smelling aural discharge and conductive hearing loss. Just as congenital cholesteatoma probably arises by the continued growth of the epidermoid formation, a structure derived from external ear epidermis, it seems likely that acquired cholesteatoma is also derived from entry of external ear canal epidermis into the middle ear. This is clearly shown in those cases of acquired cholesteatoma which follow blast injury with perfo-

ration of the tympanic membrane at the time of the injury [41]. Acquired cholesteatoma also is known to follow retraction pocket of the tympanic membrane. This is not due to obstruction of the mouth of a retraction pocket, but rather, it seems to the ingrowth of a band of stratified squamous epithelium from the fundus of the retraction pocket deeply into the middle ear (Fig. 8.17) [42]. A similar entry of stratified squamous epithelium from the external ear epidermis through the tympanic membrane may be observed sometimes in human temporal bone sections in cases of severe otitis media. The placement of irritants or bacteria into the middle ear cavity of animals has been known to provoke an otitis media that is associated with epidermal invasion through the tympanic membrane with the subsequent development of cholesteatoma. In chinchillas, destruction of the epithelium of both middle ear and lateral tympanic membrane surfaces takes place in the early stages of such an artificial acute otitis media, induced by insertion of propylene glycol into the middle ear. This is followed by re-epithelialization with hyperplastic epidermal cells and then penetration of the thickened fibrous layer of the tympanic membrane by the epidermal cells to reach the middle ear cavity and the formation of keratinous masses in the middle ear typical of cholesteatoma [43, 44].

Macroscopy On examination of the tympanic membrane, there is, in most cases, a perforation of the superior or posterosuperior margin (Fig. 8.18). The cholesteatoma appears as a pearly grey structure in the middle ear cavity. The wall of the cyst may often be seen as a thin membrane.

The cholesteatoma is usually situated in the upper posterior part of the middle ear cleft and discharges usually through a perforation of the pars flaccida of the tympanic membrane, sometimes through a perforation located at the edge of the tympanic membrane near the annulus. The cholesteatoma may extend through the aditus into the mastoid antrum and mastoid air cells. Frequently the outline of the cholesteatomatous sac is adapted to that of normal structures such as ossicles. Chronic inflammatory changes are always present. In most cases at least one ossicle is seriously damaged, so interrupting the continuity of the ossicular chain. The scutum, the upper part of the bony ring of the tympanic opening, is eroded in most cholesteatomas.

Microscopy Under the microscope acquired cholesteatoma is usually "open" rather than "closed" or cystic. The pearly material of the cholesteatoma consists of dead, fully differentiated anucleate keratin squames. This is the corneal layer of the squamous cell epithelium. As in any normal stratified epithelium, there is one to three basal layers of cells above which is a prickle (Malpighian or spinous) layer composed of five or six rows of cells with intercellular bridges.

The deeper layers of the epithelium of the cholesteatoma matrix frequently show evidence of activity in the form of down growths into the underlying connective tissue (Fig. 8.19). Such excessive activity has been confirmed by (a) the strong expression of cytokeratin 16, a marker for hyperproliferative keratinocytes, by cholesteatoma, but its absence in middle ear and external ear epithelium, except in the annulus region of the external tympanic membrane epithelium [45]; (b) the strong expression of MIB-1, an antigen related to Ki-67, which also indicates hyperproliferative activity [46]; (c) counts of silver-stained argyrophil nucleolar organizer regions, a technique which likewise displays

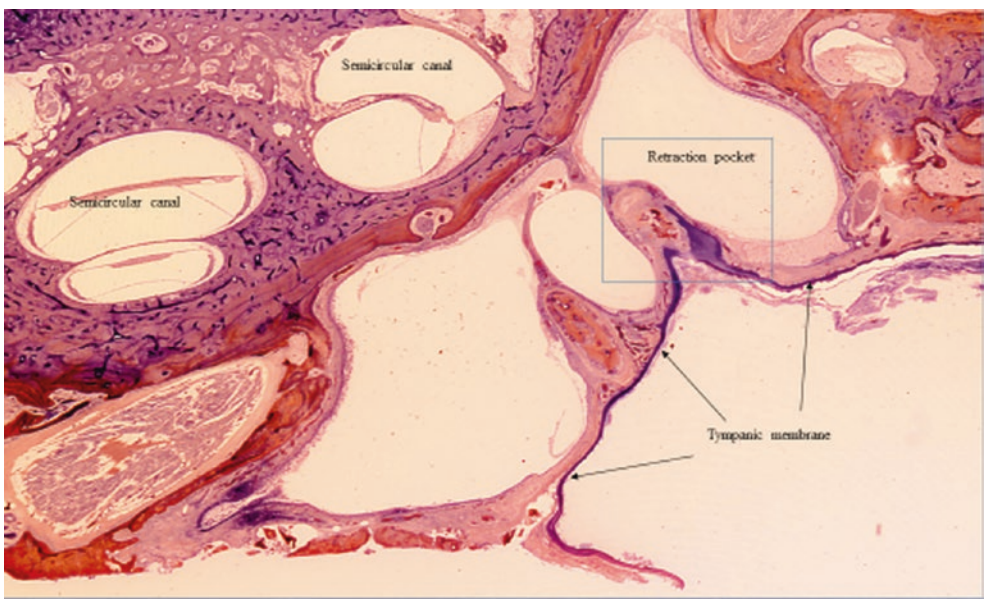

Fig. 8.17 Pathogenesis of acquired cholesteatoma. Retraction pocket of tympanic membrane. Section of temporal bone. Acquired cholesteatoma is derived from entry of external ear canal epidermis into the middle ear. It is known to follow retraction pocket of the tympanic membrane that develops after recurrent bouts of inflammation

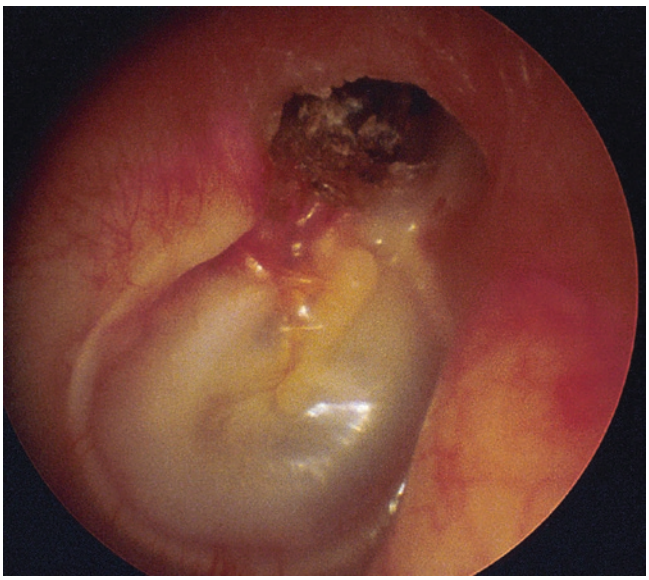

Fig. 8.18 Acquired cholesteatoma, otoscopy. On examination of the tympanic membrane, there is, in most cases, a perforation of the superior or posterosuperior margin

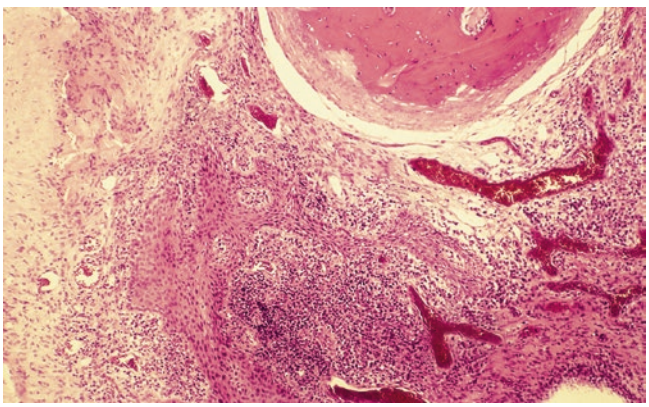

Fig. 8.19 Acquired cholesteatoma. The deeper layers of the epithelium of the cholesteatoma matrix frequently show evidence of activity in the form of down growths into the underlying connective tissue

proliferative activity, that showed significantly larger numbers of these structures in the nuclei of acquired cholesteatoma as compared with those of the epidermis of the deep external auditory meatal skin [47]; (d) acquired cholesteatomatous epithelium that shows an abnormally high concentration of IL-1, TGF-alpha, EGF-R and 4F2, all being growth factors [48] indicating greater growth and differentiating activity than is present in normal epidermis.

Treatment and prognosis Cholesteatoma is usually excised and the procedure requires removal of all the mastoid air cells and any other affected structures including the ossicles if they are involved. The surgical technique may be open or closed with respect to the ear canal. Both techniques have advantages and disadvantages. The open technique is more effective to eradicate the cholesteatoma. The risk of recurrence is high with the closed technique but structure is preserved as well as normal appearance. A "second look" surgical procedure is usually undertaken 6 months to 1 year following the initial surgery if the closed technique is used to detect and remove recurrent disease. Recently it has been suggested that diffusion-weighted MRI may be equally effective in detecting disease recurrence [49]. Post-operatively the cavity needs to be regularly cleaned and inspected. Long-term follow-up is required as recurrence can occur a long time after the initial surgery. The most significant complications of surgery are complete neurosensory hearing loss in the affected ear and damage to the facial nerve, but with experienced surgeons, the risk is less than 1 %.

8.3.1.3 Tuberculous Otitis Media

Definition This is an unusual form of chronic otitis media, which is generally associated with active pulmonary tuberculosis.

Clinical aspects The diagnosis is usually made by histopathological examination of biopsy material from middle ear contents. This is often delayed because surgeons are reluctant to take biopsies from cases of chronic otitis media that seem fairly typical. Culture of middle ear inflammatory tissue may produce tubercle bacilli.

Macroscopy In the initial stages multiple perforations of the tympanic membrane develop. Granulations in the middle ear may appear pale and often are profuse. Complications, especially involvement of the facial nerve, are more frequent than in the more common form of chronic otitis media.

Microscopy Histological examination shows tuberculoid granulation tissue composed of epithelioid cells, Langhans giant cells and areas of caseation situated in the middle ear mucosa (Fig. 8.20a, b). There is much bone destruction. Acid-fast bacilli are found with difficulty in the granulomatous material.

8.3.2 Neoplasms and Lesions Resembling Neoplasms

8.3.2.1 Choristoma (Salivary Gland, Glial and Sebaceous Types)

Definition A hamartoma is a focal overgrowth, in improper proportions, of tissues normally present in that part of the body. A choristoma is similar to hamartoma, except that the tissues of which it is composed are not normally present in the part of the body where it is found.

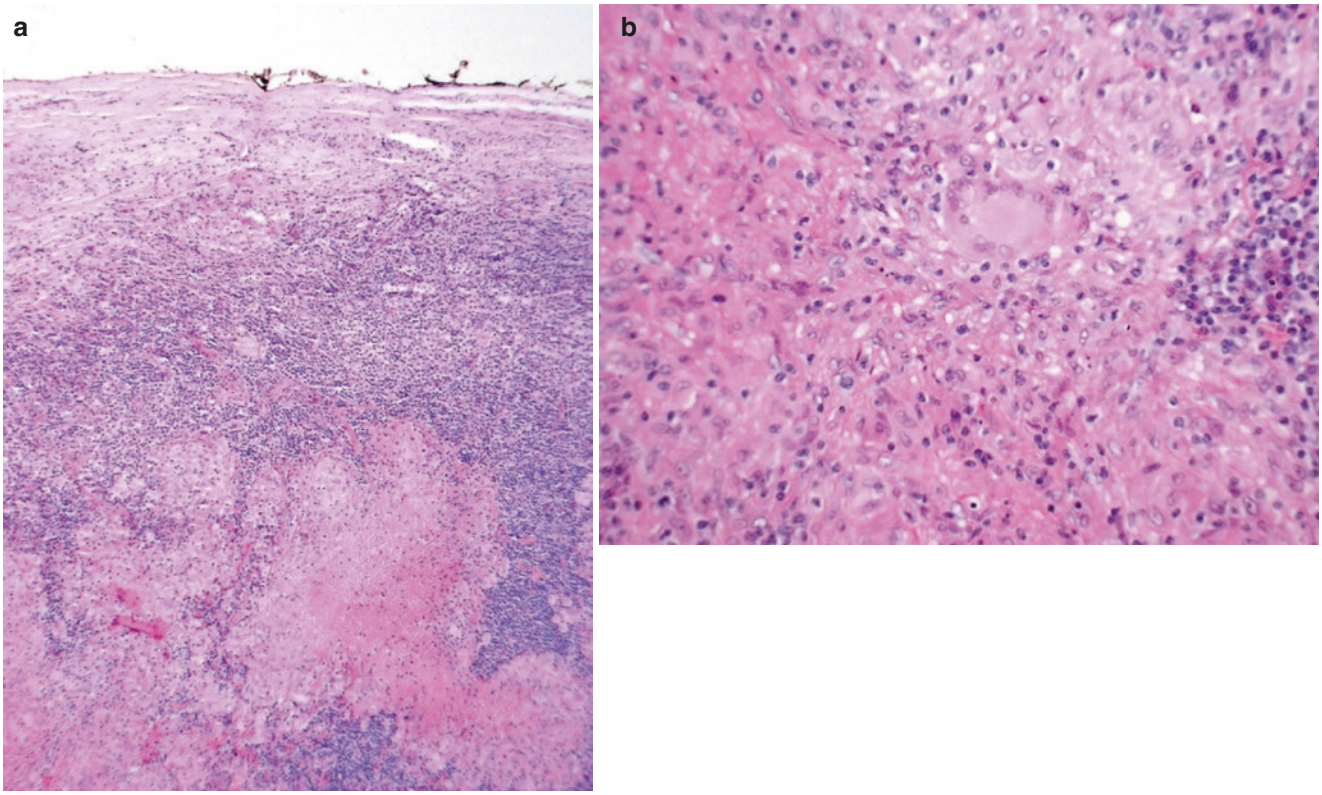

Fig. 8.20 Tuberculous otitis media. (**a**) The characteristic necrotising granulomatous inflammation composed of epithelioid cells, (**b**) Langhans giant cells and areas of caseation situated in the middle ear mucosa. Acid-fast bacilli are not easily is identified

Clinical aspects Choristomas are occasionally seen in the middle ear. They are composed of one or other of three types of tissue: salivary gland, glial or sebaceous glandular tissue.

When glial tissue is identified in biopsy material from such masses in the middle ear, a bony deficit with consequent herniation of brain tissue into the middle ear should be ruled out [50]. Three cases of heterotopic brain tissue in the middle ear associated with cholesteatoma have been reported [51]. It is possible that in all three, brain herniation occurred as a result of inflammatory damage to the tegmen tympani. Spontaneous herniations of brain (encephaloceles) may occur into the middle ear through a congenital deficiency of the tegmen or other sites [52].

Microscopy *Salivary gland choristomas* consist as a rule of mixed mucous and serous elements like the normal submandibular or sublingual gland, but unlike the parotid gland. The lesion typically consists of a lobulated mass of histologically normal salivary gland tissue in the middle ear attached posteriorly in the region of the oval window. Frequently the mass is intimately associated with the facial nerve. There are usually absent or malformed ossicles [53].

Glial choristomas are composed largely of astrocytic cells with large amounts of glial fibrils, the identity of which may be confirmed by immunohistochemical staining for glial acidic fibrillary protein. A case of *sebaceous choristoma* of the middle ear has been described [54].

Differential diagnosis: Clinically the differential diagnosis of middle ear choristoma includes inflammatory lesions such as granuloma or cholesteatoma as well as benign and malignant neoplasms including paraganglioma, schwannoma, adenoma, endolymphatic sac tumor and metastatic disease. Histologically, hamartoma, teratoma and dermoid cyst may be considered in the differential.

Treatment and prognosis Excision is performed when removal will not damage the facial nerve. If the lesion is closely related to the facial nerve, then biopsy and observation only are recommended. The lesions are benign. No metastases have been recorded even when excision is incomplete.

8.3.2.2 Adenoma

Definition Adenoma of the middle ear is the most common neoplasm. The epithelium of the middle ear has a propensity for gland formation in otitis media (see above) and

adenoma would seem to represent a benign neoplastic transformation of the epithelium along the same lines. Synonyms including carcinoid tumor and ceruminous gland adenoma have been applied to tumors arising in the middle ear. Since ceruminous glands are not present in the middle ear mucosa and the lesion follows a benign course the term neuroendocrine adenoma of middle ear (NAME) may be more appropriate [55].

Epidemiology Patients usually present in the fourth decade (mean age 37 years) and there is an equal sex incidence.

Clinical aspects Patients complain of hearing loss, a sensation of fullness and tinnitus. Benign glandular tumors of the middle ear were not described until 1976 [56, 57]. It was soon reported that a glandular tumor of the middle ear, otherwise apparently identical to an adenoma, was Grimelius positive and on electron microscopy showed numerous membrane-bound granules [58]. The use of immunohistochemistry from 1987 further confirmed the presence of neuroendocrine features in some of these neoplasms [59]. In an investigation of five cases of adenoma of the middle ear by light microscopic methods, immunohistochemistry and transmission electron microscopy, the glandular areas of the tumor in each patient showed a bidirectional mucinous and neuroendocrine differentiation. This was demonstrated by the presence of two cell types. Apically situated dark cells contained mucous granules; these cells were negative for neuroendocrine markers. Basally situated cells contained neuroendocrine granules; these cells were positive for neuroendocrine markers – vasoactive intestinal polypeptides or neuron-specific enolase [60].

Macroscopy The neoplasm has been described as being white, yellow, grey or reddish brown at operation and unlike paraganglioma is usually not vascular. It is usually situated in the middle ear cavity, sometimes extending into the mastoid. It seems to peel away from the walls of the surrounding middle ear with ease, although ossicles may sometimes be entrapped in the tumor mass and may even show destruction.

Microscopy Adenoma is formed by closely apposed small glands with a "back to back" appearance (Fig. 8.21a). In some places a solid or trabecular arrangement is present. Sheet-like, disorganized areas are seen in which the glandular pattern appears to be lost. This may be artefactual and related to the effects of trauma used in taking the biopsy specimen on the delicate structure of the cells, but the appearance may erroneously lead one to suspect malignancy. The cells are regular, cuboidal or columnar and may enclose luminal secretion. A distinct and predominant "plasmacytoid" appearance of the epithelial cells of the neoplasm may be displayed [61].

No myoepithelial layer is seen. Periodic acid-Schiff and Alcian blue stains may be positive for mucoprotein secretion in the gland lumina and in the cytoplasm of the tumor cells. Ceroid pigment is not identified.

Immunohistochemistry Neuroendocrine and epithelial mucinous differentiation is present. The tumor expresses cytokeratins, particularly cytokeratin 7 which is present in luminal cells of the glands. Neuroendocrine markers are always positive particularly in the solid and trabecular areas (Fig. 8.21b).

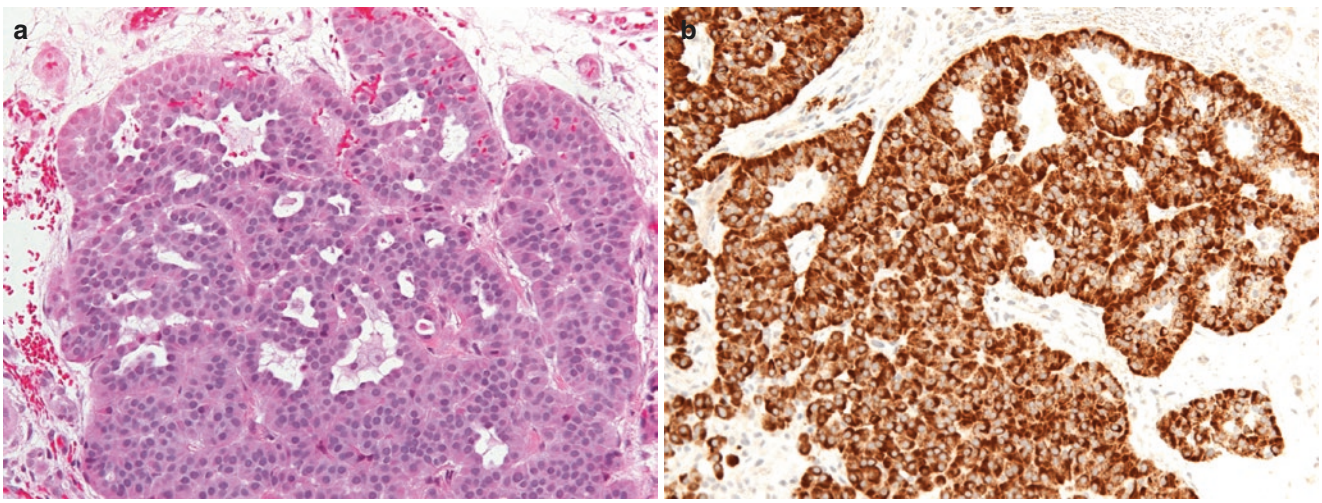

Fig. 8.21 Neuroendocrine adenoma of middle ear. (**a**) The tumor is often composed of closely apposed small glands with a "back to back"appearance. The cells are regular, cuboidal or columnar. No myoepithelial layer is seen. (**b**) Neuroendocrine immunomarkers, such as chromogranin demonstrated here, are always positive

Treatment and prognosis Complete excision is the treatment of choice and should include the ossicular chain if this is involved. One study has suggested a risk of recurrence of 18 %. All recurrences occurred when the ossicular chain was spared [62]. Adjuvant radiotherapy is not required and one study has suggested there may be a risk of malignant transformation post-irradiation [62]. Contrary to what has been suggested by some authors, there is no evidence that the presence of neuroendocrine differentiation reflects a more aggressive potential in adenomas, which are benign tumors.

8.3.2.3 Papillary Tumors

Definition A locally aggressive non-stratified epithelial neoplasm with papillary architecture.

Synonyms include papillary adenocarcinoma of middle ear, endolymphatic sac tumor and low-grade adenocarcinoma of probable endolymphatic sac origin.

Aggressive papillary tumor is characterized by a papillary, non-stratified epithelial histological pattern which shows aggressive, often invasive behaviour. Forty-six cases in which the temporal bone was affected by this neoplasm were collected from the literature in 1994 [63]. Some of these had been reported as low-grade adenocarcinoma of probable endolymphatic sac origin (see below) [64]. Each of the case reports cited in these two studies together with cases reported in the literature more recently was reviewed and this produced a total of 25 cases in which the middle ear was definitely involved by the neoplasm. Some of the literature sources reported more than one case [63–78]. The 25 literature cases with this middle ear neoplasm comprised 18 females and 7 males. In most cases with this neoplasm, clinical and audiological features point to a middle ear lesion. Suspicion of a neoplasm of the middle ear is enhanced by the otoscopic features.

Epidemiology The age range at the time of diagnosis was between 16 and 55 years with a median age of 33 and a mean age of 34 years. In many of the cases, however, the patient had already suffered symptoms subsequently ascribable to the tumor for some years when the diagnosis was made, so that the age of onset may be considerably younger than is suggested.

Clinical aspects The tumor is found in any area of the middle ear, including the mastoid process and air cells, and may fill the tympanic cavity. In all of the described cases except three [68, 76, 78], there was extensive invasion outside the middle ear, involving the apical portion of the petrous bone in most and in a few cases the tumor reached the cerebellopontine angle and the cerebellum.

It has been suggested that cases of aggressive papillary middle ear tumor with widespread involvement of the temporal bone may arise from a primary papillary adenocarcinoma of the endolymphatic sac (*endolymphatic sac tumor, low-grade adenocarcinoma of probable endolymphatic sac origin*) [64]. The frequent association of papillary tumors in the middle ear with apical petrous bone neoplasia of the same type, the similarity of the histological appearances of the neoplasm in the two regions and the association of some cases of papillary tumors in both regions with von Hippel-Lindau disease would seem to favour of this concept. Such an origin has not yet been confirmed by autopsy study. Indeed in the single description of the pathological changes of aggressive papillary tumor of the middle ear in an autopsy-acquired temporal bone, widespread deposits of tumor at inner ear sites are depicted, but no mention is made of involvement of the endolymphatic sac or duct [79]. Thus a middle ear origin for some cases of this neoplasm at least has not been definitely excluded. There have been recent case reports of tumors histologically identical to low-grade adenocarcinoma of endolymphatic sac origin confined to the hypo- and epi-tympanum of the middle ear suggesting the current classification may be oversimplified [80]. Whatever the site or sites of origin of this tumor, it should be recognized that papillary epithelial tumor of the middle ear is an aggressive neoplasm, in contrast to the non-papillary adenoma of the middle ear which is quite benign [81].

Macroscopy The middle ear cleft, including the mastoid air cells, is usually filled with the papillary tumor. Bone invasion is often seen (Fig. 8.22a).

Microscopy A papillary-glandular pattern is present with complex interdigitating papillae lying loosely or infiltrating fibrous connective tissue. The papillae are lined by a single layer of low cuboidal to columnar epithelial cells with uniform nuclei, eosinophilic cytoplasm and indistinct cell borders (Fig. 8.22b, c). Thyroid follicle-like areas may be present similar to those seen in endolymphatic sac carcinoma [see below].

Immunohistochemistry Markers for cytokeratin, epithelial membrane antigen and S100 are positive. The absence of thyroglobulin must be determined to exclude metastatic papillary carcinoma of the thyroid. Markers for CK7, CK20 and carcinoembryonic antigen may also useful to exclude metastatic deposits from lung and colon.

Genetics In view of the association of some cases of von Hippel-Lindau disease with aggressive papillary middle ear tumors, it is suggested that the clinical assessment of each case with the latter neoplasm should include an investigation for the gene mutations of von Hippel-Lindau disease.

Treatment and prognosis Complete surgical excision is the treatment of choice and may be curative. Surgery carries

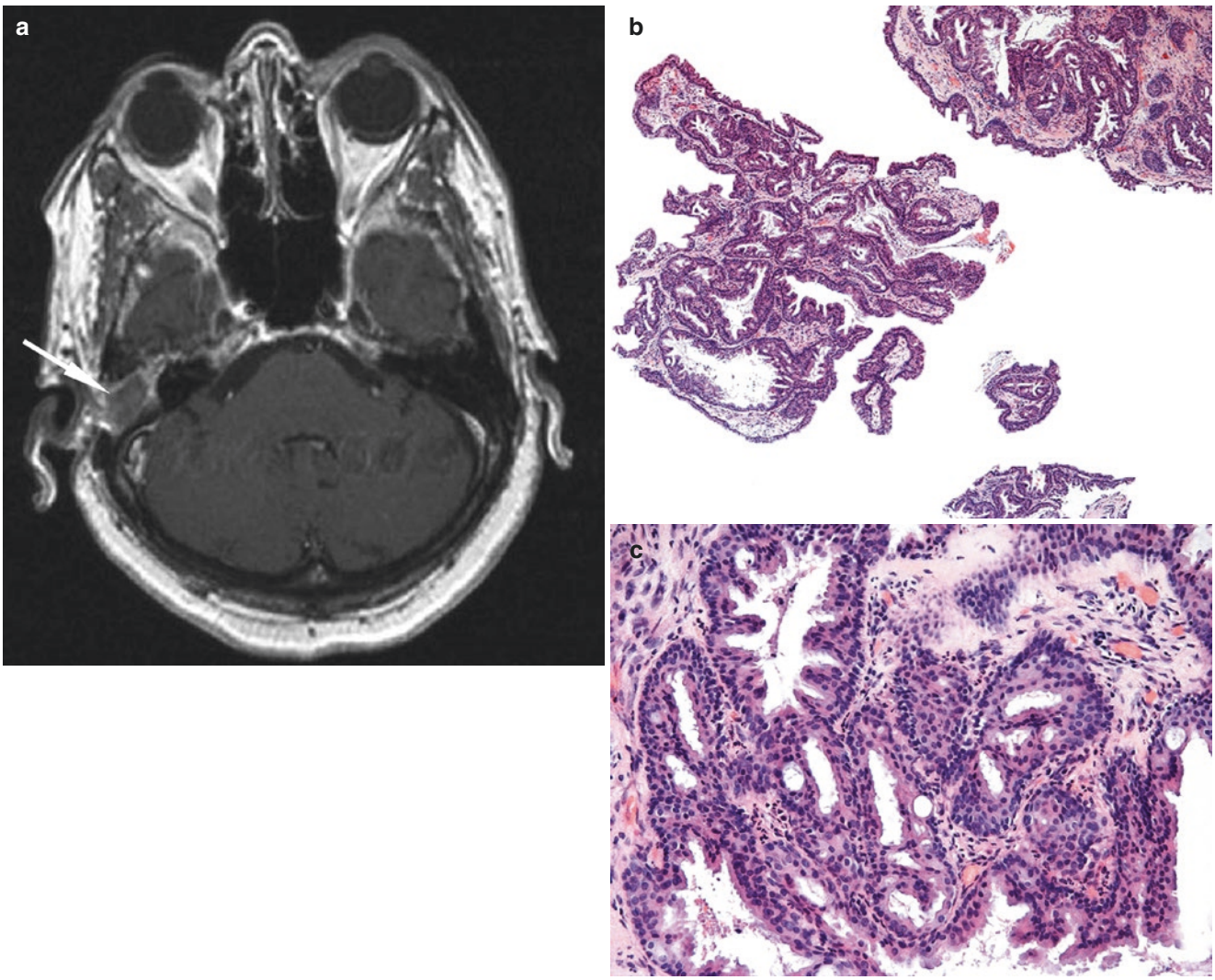

Fig. 8.22 Aggressive papillary tumor of middle ear. (**a**) In the axial slice MRI scan, the *arrow* indicates a tumor confined to the middle ear that is not extending into the middle or posterior cranial fossae. (**b**) Tumor with papillary-glandular pattern and complex interdigitating papillae lying loosely and infiltrating fibrous connective tissue. (**c**) The papillae are lined by a single layer of low cuboidal to columnar epithelial cells with uniform nuclei, eosinophilic cytoplasm and indistinct cell borders

a risk of high morbidity since resection may necessitate the sacrifice of cranial nerves. Various treatment modalities may be employed depending on the stage at presentation including radiotherapy alone or mixed, surgery and post-operative radiotherapy.

8.3.2.4 Jugulotympanic Paraganglioma

Definition A benign neuroendocrine tumor arising from the paraganglion situated in the wall of the jugular bulb or from the paraganglion situated near the middle ear surface of the promontory. See also Chapter 11 for further discussion.

Synonyms Tumors arising from the paraganglion situated in the wall of the jugular bulb have been referred to as jugular paragangliomas or glomus jugulare tumors. Those arising from the paraganglion situated near the middle ear surface of the promontory have been referred to as tympanic paragangliomas or glomus tympanicum tumors. The distinction between jugular and tympanic paragangliomas can easily be made in the patient by modern imaging methods at which the jugular neoplasm is identified as arising from the jugular bulb region and shows evidence of invasion of the petrous bone, while the tympanic neoplasm is confined to the middle.

The gross and histological appearances of the two types of neoplasm in the *middle ear* are, however, identical.

Epidemiology Solitary jugulotympanic paragangliomas arise predominantly in females. The neoplasm has been seen

at ages between 13 and 85 years with a mean age of about 50 years.

Clinical aspects Most patients present with conductive hearing loss. Pain in the ear, facial palsy, haemorrhage and tinnitus are also described as symptoms of this lesion. Jugulotympanic paragangliomas may also be multicentric or coexist with tumors of other types. They may be bilateral in the same patient and coexist with carotid body paragangliomas which may be bilateral [82]. They may also coexist with adrenal gland pheochromocytomas which can produce hypertension. A familial tendency to grow paragangliomas has been noted particularly in cases with multiple tumors of this type.

The incidence of clinically functioning paraganglioma with symptoms and signs of norepinephrine excess, particularly hypertension, is only 1–3 % [83].

Macroscopy Solitary jugulotympanic paragangliomas are present as a red vascular mass, seen either behind the intact tympanic membrane or sprouting through the latter into the external canal. Surgical approach to the mass at biopsy often results in severe bleeding.

The neoplasm associated with familial paraganglioma is a reddish sprouting mass at its external canal surface. In the jugular variety, the petrous temporal bone is largely replaced by red, firm material and the middle ear space is occupied by soft neoplasm as far as the tympanic membrane. The otic capsule is rarely invaded by paraganglioma. Investigation of a paraganglioma in an autopsy temporal bone by the microslicing method showed origin of the tumor from the jugular bulb region and its spread through the petrous bone and middle ear to the tympanic membrane (Fig. 8.23).

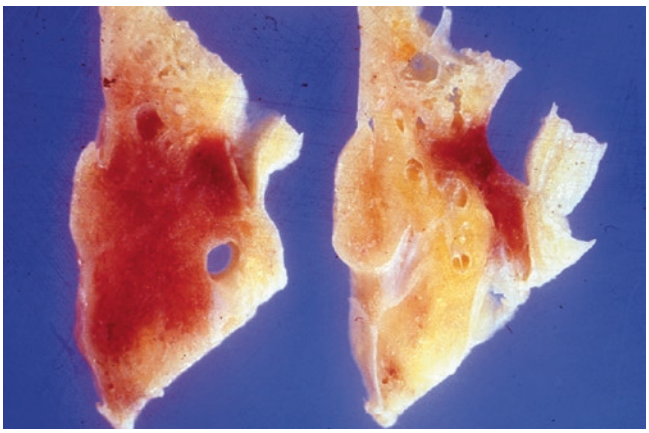

Fig. 8.23 Microsliced specimen of jugular paraganglioma removed at autopsy. Two slices of the temporal bone in the region of the neoplasm are seen. The one on the left shows invasion of the temporal bone by the reddish paraganglioma from its apical region as far as the tympanic membrane. The slice on the right is taken at a higher level and shows sparing of the cochlea and bony labyrinth by the tumor

Microscopy The tumor is composed of the characteristic nests of epithelioid cells (zellballen) with spindled sustentacular cells at the periphery of the nests (Fig. 8.24a–d). The surrounding stroma is vascular. There may be cytological atypia with large and pleomorphic nuclei but this is of no prognostic significance. Rarely mitoses and necrosis may be identified. The histological appearance does not predict biological behaviour (Fig. 8.25d). There is often crush artefact and reticulin stain may be of use to highlight the characteristic nested morphology. The zellballen express neuroendocrine markers including chromogranin and synaptophysin and the sustentacular cells are highlighted by S100 immunostain (Fig. 8.24b, d and 8.25a–c) [84].

Genetics In families containing patients with head and neck paragangliomas, including jugulotympanic paragangliomas, there is, unlike the solitary jugulotympanic paraganglioma, a preponderance for the male sex and inheritance is autosomal dominant, with increased penetrance with age [85]. There is evidence from molecular genetic studies that the gene underlying familial paragangliomas is located on chromosome 11q proximal to the tyrosinase gene locus [86].

Treatment and prognosis Jugulotympanic paraganglioma is a neoplasm of slow growth. The jugular variety infiltrates the petrous bone, but distant metastasis is rare (Fig. 8.25d). Complete excision may be difficult and may be used in conjunction with preoperative embolization and radiation therapy. The local recurrence rate is 50 %.

8.3.2.5 Meningioma

Definition Meningioma is a benign tumor which usually grows intracerebrally, but sometimes is seen involving bony structures around the brain including the middle ear. It arises from the pia-arachnoid cells of the meninges. These structures may be formed at a number of sites in the temporal bone, including the internal auditory meatus, the jugular foramen, the geniculate ganglion region and the roof of the Eustachian tube. Thus meningiomas, which arise from them, may thus be found in a wide area within the temporal bone itself [87].

Epidemiology Meningioma of the middle ear affects females more than males and shows an age range of between 10 and 80 years with a mean age of 49.6 years, with female patients presenting at an older age (mean 52.0 years) than male patients (mean, 44.8 years) [88]. In a series of 146 extracranial meningiomas reported, [89] Rushing et al. showed similar demographics; 38 tumors (26 %) affected the ear and temporal bone. There were 25 females (mean age 45.5 years) and 13 males (mean age 39.6).

Clinical aspects The most common temporal bone site for primary meningioma is in the middle ear cleft. In a recent

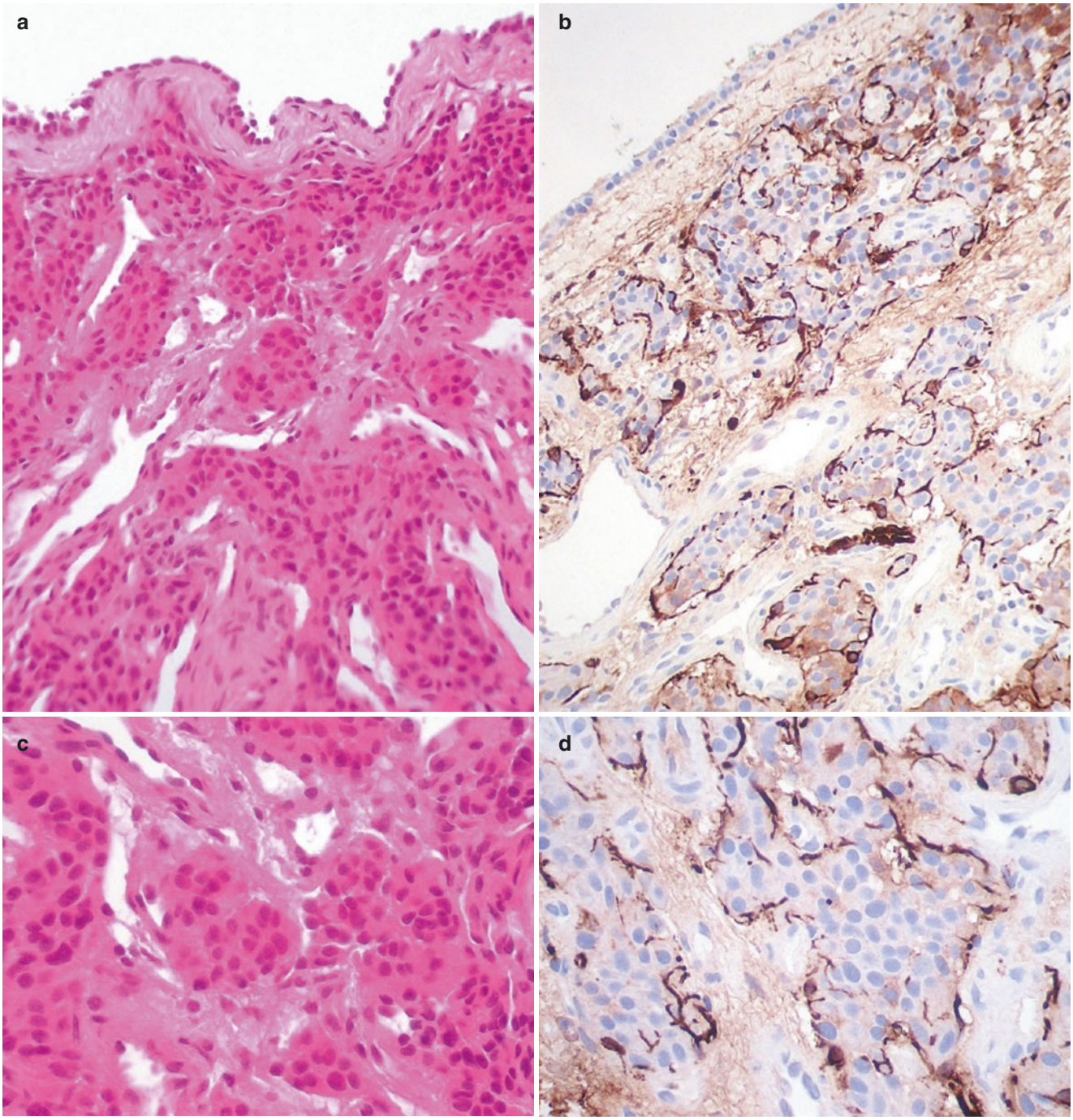

Fig. 8.24 Jugulotympanic paraganglioma. (**a**, **c**) Characteristic clusters of epithelioid cells "zellballen" in vascular stroma. The S100-immunostained sections on the right (**b**, **d**) demonstrate the sustentacular cells at the periphery of the zellballen

study of 36 cases most of which involved the middle ear, but a few involved adjacent structures such as the external canal or temporal bone, only two showed CNS connection on radiography [88].

Patients present clinically with hearing change, otitis media, pain and/or dizziness/vertigo.

Macroscopy Gross appearances are those of a granular or even gritty mass.

Microscopy The neoplasm takes the same forms as any of the well-described intracranial types of meningioma. The most common variety seen in the middle ear is the

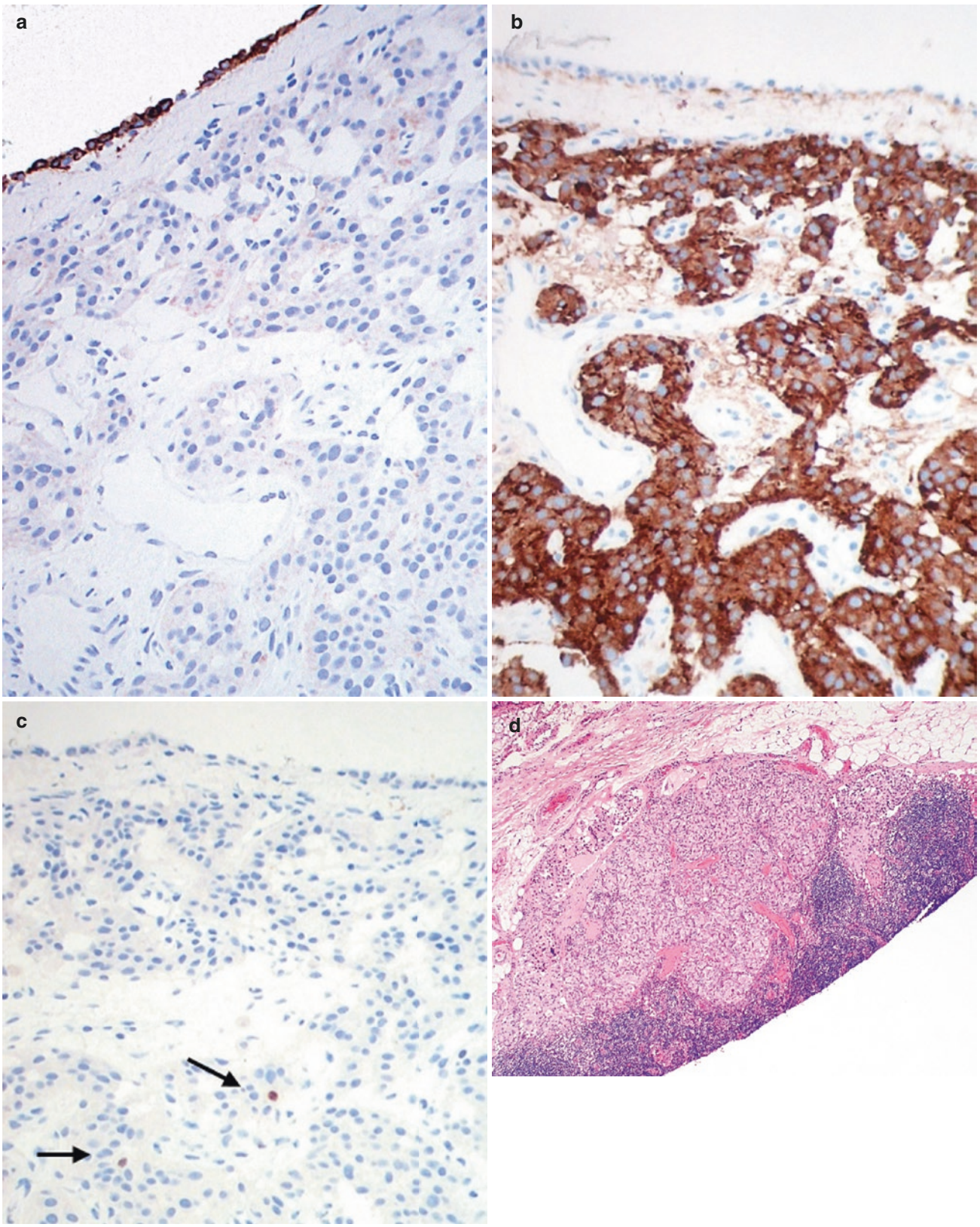

Fig. 8.25 Jugulotympanic paraganglioma. (**a**) Immunostained sections show the tumor is negative for pan cytokeratin while the surface epithelium is positive. (**b**) The tumor is diffusely positive for neuroendocrine markers including synaptophysin. (**c**) The proliferation index on Ki67 is very low, brown positive tumor nuclei indicated by *arrows*. (**d**) Metastatic paraganglioma in a lymph node

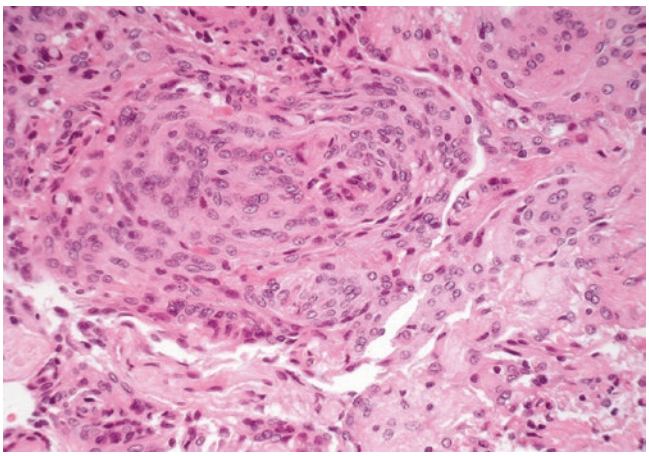

Fig. 8.26 Meningioma of the middle ear. The most common type seen in the middle ear is the meningothelial type, in which the tumor cells form masses of epithelioid, regular cells often disposed into whorls

meningothelial type, in which the tumor cells form masses of epithelioid, regular cells often disposed into whorls which may be large or small (Fig. 8.26). In the series described by Rushing et al., 100 % of the ear and temporal bone meningiomas were grade 1 histologically and 95 % were meningothelial [89]. Fibroblastic and psammomatous varieties are also sometimes seen in the middle ear.
Histological diagnosis of meningioma may be difficult because the above features are indistinct.

Immunohistochemistry Under these circumstances immunocytochemistry may be of some diagnostic value. Most markers are negative, including those for cytokeratins. Vimentin and epithelial membrane antigen are, however, positive in the majority of meningiomas.

Treatment and prognosis Meningiomas of the middle ear behave as slow-growing neoplasms with a good overall prognosis (raw 5-year survival, 83 %). Extent of surgical excision is probably the most important factor in determining outlook because recurrences develop in 28 % of cases.

Nager's review of temporal bone meningiomas indicated that only 2 out of 30 patients survived a 5-year period [87]. More recent experience of middle ear meningiomas signals a better outlook after careful local excision. In a recent study of 36 patients in which the tumor was sited mainly in the middle ear [88], surgical excision was used in all patients. Ten patients developed a recurrence from 5 months to 2 years later and 5 patients died with recurrent disease (mean, 3.5 years); the remaining 30 patients were alive ($n = 25$, mean: 19.0 years) or had died ($n = 5$, mean: 9.5 years) of unrelated causes without evidence of disease.

8.3.2.6 Squamous Cell Carcinoma

Definition A malignant tumor of stratified squamous epithelium that is usually metaplastic in the middle ear.

Clinical aspects Squamous cell carcinoma is uncommon in the middle ear. It sometimes accompanies squamous cell carcinoma of the external canal or may arise solely from the middle ear epithelium. The mean age at presentation is 60 years (range 35–80 years). The patient always has an aural discharge and conductive hearing loss. Pain in the ear, bleeding and facial palsy are common.

Macroscopy A plaque-like or polypoid mass may be seen or palpated in the ear canal. Tumor may be seen filling the middle ear and extending into mastoid air spaces.

Microscopy In microscopic sections the tumor may be seen arising from surface stratified squamous epithelium, itself metaplastic from the normal cubical epithelium. In certain areas an origin directly from basal layers of cubical or columnar epithelium may be seen. There is no evidence that the epidermoid formation, a cell rest which occurs normally in the middle ear during development [see above], may be a source of squamous cell carcinoma. The neoplasm is squamous cell carcinoma with variable degree of differentiation. Atypical change and even carcinoma in situ may be seen in some parts of the middle ear epithelium adjacent to the growth. The mode of spread of the neoplasm from the middle ear epithelium has been ascertained in temporal bone autopsy sections [90] and this pattern has been confirmed by imaging in living patients. The carcinoma tends to grow into and erode the thin bony plate which separates the medial wall of the middle ear, at its junction with the Eustachian tube, from the carotid canal. This bony wall is normally up to 1 mm in thickness and may be recognized radiologically. Having reached the carotid canal, the growth will extend rapidly along the sympathetic nerves and the tumor is then impossible to eradicate surgically. Another important method of spread is through the bony walls of the posterior mastoid air cells to the dura of the posterior surface of the temporal bone. From there it spreads medially, enters the internal auditory meatus and may then invade the cochlea and vestibule. Spread into the lamellar bone in both of these situations is along vascular channels between bone trabeculae. A similar type of bone invasion may also occur from other parts of the middle ear surface such as in the region of the facial nerve. The special bone of the otic capsule is, on the other hand, peculiarly resistant to direct spread of growth from tumor within the middle ear; and even the round window membrane is not invaded. When invasion does occur, it takes place after entry of the tumor into the internal auditory meatus and penetration of the bone by way of the filaments of the vestibular and cochlear

divisions of the eighth nerve. In the later stages tumor grows extensively in the middle cranial fossa; it may also invade the condyle of the mandible.

Treatment and prognosis The disease is often advanced at presentation and the prognosis is generally poor. Diagnosis may be delayed and the disease may not be recognised due to the non-specific symptoms resembling chronic otitis media. Five-year survival has been reported between 25 % and 50 % following treatment with surgery and radiotherapy. Death is usually due to direct intracranial extension. Lymph node metastasis is unusual and spread by the bloodstream even more so [90].

8.3.2.7 Rhabdomyosarcoma

Definition A primitive malignant tumor with phenotypic and biological features of embryonic smooth muscle. Synonyms include myosarcoma, embryonal sarcoma and botryoid sarcoma. See also Chapter 12 for further discussion.

Epidemiology The middle ear and mastoid are the third most common sites for rhabdomyosarcoma of the head and neck after the orbit and nasopharynx. The tumor occurs almost exclusively in young children (78 % of cases are under 12 years old; 43 % are under 5 years old at presentation). On rare occasions it is found in the middle ear of adults [91].

Clinical aspects It presents as a persistent unilateral painless otitis media associated with serosanguinous discharge. Approximately a third of patients have facial nerve deficit at presentation. Clinically the lesion resembles an aural polyp.

Macroscopy The tympanic membrane is usually eroded by the growth which extends into the external canal. Grossly the tumor is lobulated and dark red with a haemorrhagic cut surface.

Microscopy Almost all temporal bone rhabdomyosarcomas are of the embryonal type which includes spindle and botryoid subtypes. The tumors show variable cellularity displaying mainly spindle or round primitive skeletal muscle cells, some of which have clear cytoplasm staining positively for glycogen and others have eosinophilic areas in the cytoplasm together with more differentiated large eosinophilic cells. Cross striations can be seen in 50–60 % cases and are more apparent in the spindle cell component. Mitoses are numerous in the primitive cells and there is usually necrosis. Heterologous elements such as cartilage and bone are more typical in rhabdomyosarcoma at other sites such as retroperitoneum and genitourinary tract.

The botryoid variant confers a better prognosis. Grossly the tumors consist of polypoid nodules. Histologically there is a characteristic dense aggregation of tumor cells in the subepithelial stroma (cambium layer).

Immunohistochemistry Immunohistochemical markers for desmin, muscle-specific actin and antibodies against MyoD1 and myogenin confirm this diagnosis.

Genetics Cytogenetic analysis distinguishes between the different subtypes of rhabdomyosarcoma. Embryonal rhabdomyosarcoma shows abnormalities of the short arm of chromosome 11 and there is a consistent loss of heterozygosity at 11p15.5 that may reflect inactivation of a tumor suppressor gene. Botryoid rhabdomyosarcoma contains a deletion on the short arm of chromosome 1 and trisomies of chromosomes 13 and 18. The majority of alveolar rhabdomyosarcomas show a translocation between chromosomes 2 and 13 (t (2; 13) (q36; q14)) resulting in the juxtaposition of the *PAX3* gene on chromosome 2 with the *FKHR* gene on chromosome 13.

Differential diagnosis Diagnosis may be delayed because the symptoms at presentation may mimic chronic otitis media particularly in the paediatric population. Associated facial nerve palsy should raise the suspicion of malignancy and referral to a specialist centre for investigation treatment [92]. Histological samples may be small and not representative since inflammatory tissue or polyp may be overlying the tumor.

Treatment and prognosis Rhabdomyosarcoma of the temporal bone is highly malignant and spreads extensively into the cranial cavity, externally or to the pharyngeal region. Lymph node and bloodstream metastases frequently develop in these patients. Treatment with combined modalities including surgery chemotherapy and radiation therapy has dramatically improved the outlook for patients with this disease. There is an 80 % 5 year survival for patients with localised disease, but patients with metastatic disease have a poor prognosis (5 year survival less than 30 %).

8.3.2.8 Metastatic Carcinoma

Metastasis of malignant neoplasms to the temporal bone including the middle ear is not uncommon. The breast and cutaneous malignant melanoma are the most common primary of metastatic tumors, followed by the lung, kidney, stomach and larynx [93, 94] Two distinct modes of spread may be involved in bringing the neoplasms from their primary sites to the middle ear: (a) along the vascular channels in the petrous bone. These convey tumor deposits to the temporal bone from distant sites and (b) along the nerves emanating from the internal auditory meatus into the labyrinthine structures and bone. In this way tumors reaching the menin-

ges may spread into the temporal bone. In addition direct spread may bring tumors into the ear from primary sites in areas adjacent to the temporal bone.

8.4 Inner Ear

8.4.1 Bony Labyrinth

8.4.1.1 Otosclerosis

Definition Otosclerosis is a bony lesion associated with hearing loss. Fine-slice computerised tomography scan demonstrates abnormal bone deposition in the temporal bone.

Epidemiology The condition usually presents between the ages of 15 and 35 years. There is an equal sex incidence though the condition is more often diagnosed in females.

Clinical aspects Otosclerosis is a disease of the bony labyrinth, which, by involvement and fixation of the stapes footplate, leads to severe conductive hearing loss. The lesion always commences in the otic capsule tissue anterior to the footplate of the stapes. In this position it does not produce symptoms. These take place when the otosclerosis invades the adjacent stapes footplate and produces fixation of that structure and thus conductive hearing loss. It later spreads widely in the otic capsule and may involve the round window ligament. Blood vessels are prominent and evenly distributed. X-rays of temporal bone specimens show the well-defined lesion as a patch of mottled translucency (Fig. 8.27) [1]. The disease usually affects both ears symmetrically. A unique bony plaque develops in the otic capsule predominantly in the region posterior to the cochlea which then involves the stapes footplate resulting in conductive hearing loss. The posterior plaque in otosclerosis characteristically has a well-defined margin at the level of posterior edge of the processus cochleariformis extending to the level of one third of the distance from the processus along the tensor tympani muscle. The lesion then expands in all directions, with a broad pushing margin into the otic capsule often completely obscuring the fissula ante fenestram. It passes through the stapedovestibular joint and along the stapes footplate. Inferiorly and laterally the plaque invades the vestibule and parasaccular region where it may involve branches of the audiovestibular nerve to the saccule. Anterior cochlea plaques of otosclerosis may also be present. These have a wide area of contact with the periosteum bordering the canal for the internal carotid artery. Occasional plaques have been described in other locations within the otic capsule.

Macroscopy Otosclerosis usually affects both ears symmetrically. The disease process is probably confined to the temporal bone. The pink swelling of otosclerosis may sometimes even be detected clinically through a particularly transparent tympanic membrane as a well-demarcated and pink focus near the promontory. A characteristic translucency of bone adjacent to the cochlea and anterior to the footplate is identified on CT scan.

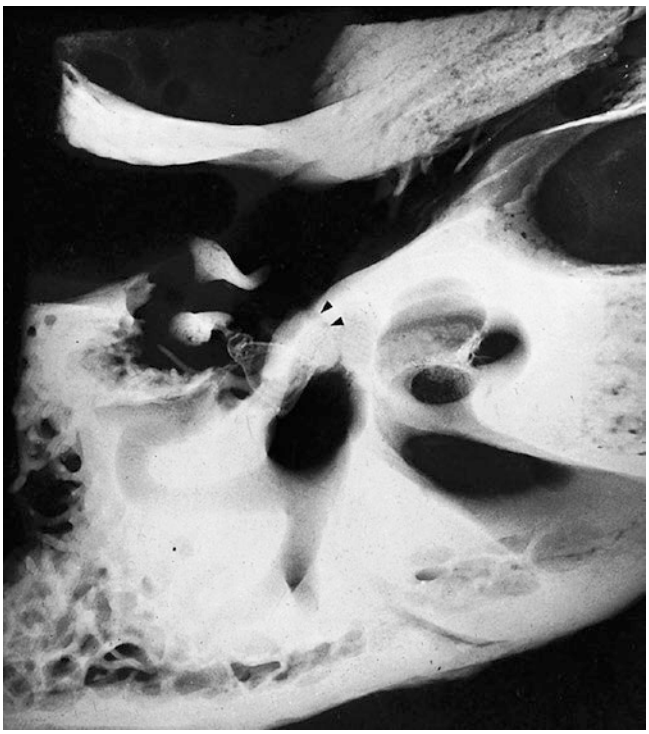

Fig. 8.27 Radiograph of microslice of autopsy temporal bone with focus of otosclerosis. The focus is an area of mottled translucency in the region of the fissula ante fenestram, which is indicated by *arrowheads*

Microscopy Biopsy material is seldom sent for pathology review and the morphology of the lesions is based on an analysis of temporal bones taken at post-mortem. Stapedectomy specimens may contain otosclerosis plaque tissue usually associated with the anterior part of the footplate If the specimen of stapes is composed of the head and crura only, microscopical examination will not show the changes of otosclerosis. On the other hand, if the whole stapes is removed, as is usually the case, otosclerotic bone will possibly be observed in sections of the anterior part of the footplate.

The histological characteristic of otosclerosis is the presence of trabeculae of new bone, mostly of the woven type with marked vascularity (Fig. 8.28). This contrasts with the well-developed lamellar bone under the outer periosteum, the endochondral middle layer and the endosteal layer of the otic capsule, a sharply demarcated edge between normal and otosclerotic bone being a prominent feature. In most places osteocytes are very abundant within the woven bone. Otosclerotic bone sometimes reaches the endosteum of the

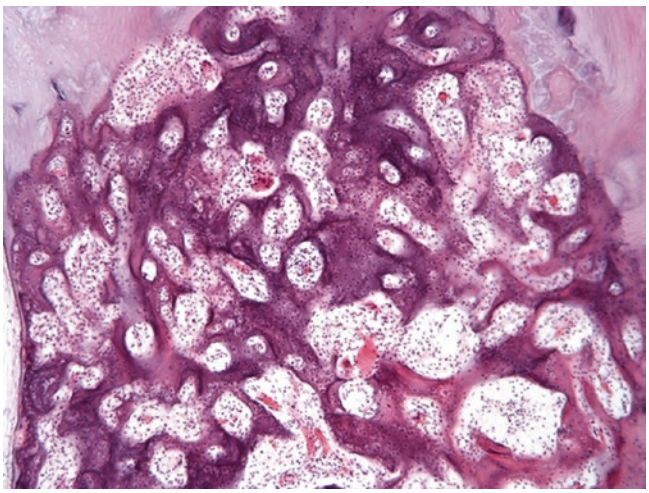

Fig. 8.28 Otosclerosis. The osteosclerotic plaque is composed of tumor-like masses of compressed otic capsule tissue comprising vascular spaces as illustrated here

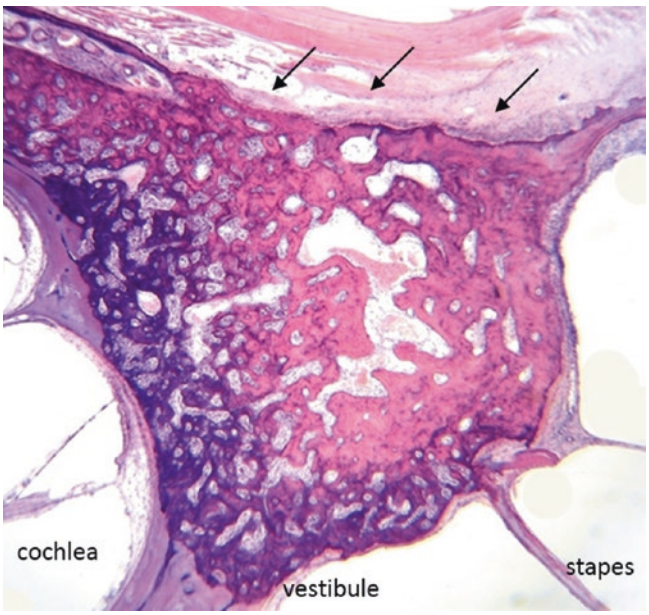

Fig. 8.29 Otosclerotic plaques are oriented as shown in the cochlear otic capsule. It appears to arise from periosteum (*arrows*). Invasion into cochlea, vestibular region and joint with stapes is taking place

cochlear capsule. In some cases it may lead to a fibrous reaction deep to the spiral ligament. These changes are probably the basis of the sensorineural hearing loss that is also occasionally found in cases of otosclerosis.

Immunohistochemistry has no role to play in the diagnosis of otosclerosis.

Treatment and prognosis Stapedectomy with insertion of prosthesis to replace the fixed stapes and so reinstate the mobility of the ossicular chain is frequently performed as a treatment for otosclerosis.

The prognosis is good. Untreated otosclerosis leads to very significant hearing loss (50–60 dB) but total deafness is rare. It is suggested that patients remain asymptomatic until the stapes is affected when there is conductive hearing loss. Cochlear invasion would lead to sensorineural hearing loss and involvement of vestibular structures resulting in symptoms of loss of balance would develop in later stages.

Until now otosclerosis has been regarded as a disorder of bone remodelling of the otic capsule. However, recent studies have demonstrated that the otosclerotic plaque has histological features suggestive of a low-grade neoplasm in that pre-existing normal structures in the cochlear and vestibular otic capsules are invaded and replaced (Fig. 8.29) [95]. Also autopsy studies have revealed the lesions continue to grow and expand throughout life as would be expected in a neoplastic process. The recognition and acceptance of otosclerosis as a low-grade neoplasm may result in a new understanding of the disease. The neoplastic nature of constituent cells in the osteosclerotic plaque has yet to be confirmed. Access to the tissue is difficult and most studies have been done on post-mortem samples.

8.4.1.2 Paget's Disease

Definition Paget's disease of the bone is a disorder of bone remodelling with disorganised bone resorption and bone formation.

Synonym Osteitis deformans.

Clinical features Paget's disease is a common condition affecting particularly the skull, pelvis, vertebral column and femur in people over 40 years of age. The cause is not yet certain, but the presence in many cases of paramyxovirus-like structures seen within osteoclasts has prompted the suggestion that Paget's disease may be of viral etiology and the measles virus and canine distemper viruses have been under scrutiny as candidates.

Macroscopy The pathological change is one of active bone formation proceeding alongside active bone destruction. The affected bones are enlarged, porous and deformed. It is associated with a mixed hearing loss [96]. In the temporal bone the petrous apex, the mastoid and the bony part of the Eustachian tube are most frequently affected [97, 98] The periosteal part of the bony labyrinth is the first to undergo pagetoid changes and the pagetoid changes spread through the bone towards the membranous labyrinth, usually with a sharp line of demarcation between the pagetoid area and the normal bony labyrinth.

Microscopy Active remodelling is seen in trabeculae of bone that may have a lining of numerous osteoblasts. A

mosaic appearance is formed in the bone by the frequent successive episodes of remodelling, cessation of bone deposition resulting in thin, blue "cement lines", followed again by resumption of bone resorption and redeposition and its cessation with production of further cement lines. Bone destruction is shown by the presence of numerous, large osteoclastic giant cells in Howship's lacunae. Areas of chronic inflammatory exudate intermixed with the bone are common.

Genetics Familial cases show autosomal pattern of inheritance with incomplete penetrance and a mutation has been identified in the sequestosome 1 gene on the long arm of chromosome 5 (5q35-qter).

Differential diagnosis Paget's disease can resemble fibrous dysplasia on imaging (Pagetoid sclerotic and cystic forms) but this is more often associated with younger patients and may affect craniofacial bones. In older patients the early lytic phases of Paget's disease may mimic malignancy. The temporal bone is rarely biopsied. Histologically the appearances of Paget's disease may be mistaken for a reactive process or even a primary bone neoplasm. Clinicopathological correlation including the imaging is essential.

Treatment and prognosis The sensorineural hearing loss associated with Paget's disease of temporal bone is thought to be associated with decreased mineral density in the cochlear capsule. Treatment is centred on early diagnosis and treatment with bisphosphonates to reduce bone loss.

8.4.1.3 Osteogenesis Imperfecta

Definition Osteogenesis imperfecta is a general bone disease with a triad of clinical features: multiple fractures, blue sclerae and conductive hearing loss.

Epidemiology There is a congenital recessive form in newborn which is often rapidly fatal and a tardive one in adults that is inherited as a Mendelian dominant and is more benign.

Clinical aspects Mutations of type I collagen genes have been established as the underlying cause leading to a general disturbance in the development of collagen, hence the thin sclerae appearing blue as well as poorly formed bone tissue. Hearing loss is variable in the different clinical types of osteogenesis imperfecta and no association has been found between the gene mutations and the severity and the nature of the hearing loss [99].

Microscopy In the long bones the resorption of cartilage in the development of bone is normal, but the bony trabeculae themselves are poorly formed and the same may be seen in the temporal bone [100]. The ossicles in the tardive form are very thin and subject to fractures. The stapes footplate is also frequently fixed. The disturbance in lamellar bone formation can lead to extreme thinness, dehiscence and nonunion of the stapedial superstructure with the footplate or thickening with fixation of the footplate. The nature of the bony tissue causing this fixation is problematical. It has been suggested that osteogenesis imperfecta can be associated with otosclerosis so that the fixation is indeed otosclerotic [101]. Otosclerosis, like osteogenesis imperfecta, may indeed be part of a general connective tissue disturbance [102]. Indeed, some cases of clinical otosclerosis may be related to mutations within the *COL1A1* gene that are similar to those found in mild forms of osteogenesis imperfecta [103]. However biochemical studies of enzymes in otosclerosis and osteogenesis imperfecta have demonstrated divergent pathways and histological studies suggest otosclerosis is localised to the temporal bone [99].

Genetics Ninety-five per cent of cases are due to dominantly inherited mutations in *COL1A1* and *COL1A2* genes that form the polypeptide chains of the type 1 collagen triple helix. In 3–5 % of cases, a lethal form of the disease is associated with recessively inherited mutations that affect the intercellular processing of type 1 collagen.

Treatment and prognosis The hearing loss in osteogenesis imperfect may be conductive (involving ossicles, ear drum and middle ear) or sensorineural (involving cochlea auditory nerve and brain) or a mixture of these. The percentage of patients in whom conductive hearing loss cannot be corrected is higher in those affected by osteogenesis imperfect than in those unaffected [99].

8.4.1.4 Osteopetrosis

Definition Osteopetrosis (often known as marble bone disease) is a rare disease of bone, in which there is a failure to absorb calcified cartilage and primitive bone due to deficient activity of osteoclasts.

Epidemiology A relatively benign form, inherited as a dominant, presents in adults and a malignant and a malignant form, inherited as a recessive, in infants and young children. The patients with the benign form often survive to old age and present prominent otological symptoms.

Macroscopy The intermediate, endochondral portion of the otic capsule is swollen and appears as an exaggerated thickened form of the normal state.

Microscopy Globuli ossei composed of groups of calcified cartilage cells are normally present in this region, and in osteopetrosis they are greatly increased in number and are

arranged into a markedly thickened zone. The periosteal bone is normal. The ossicles are of fetal shape and filled with unabsorbed, calcified cartilage. The canals for the seventh and eighth cranial nerves are greatly narrowed by the expanded cartilaginous and bony tissue and these changes are probably responsible for the characteristic symptoms of facial palsy and hearing loss respectively [104–106].

Treatment and prognosis Treatment is based on symptoms that depend on the severity of the disease and include auditory canal stenosis, otitis media, sensory and conductive hearing loss and facial nerve paralysis.

8.4.2 Membranous Labyrinth and Cranial Nerves

8.4.2.1 Viral, Bacterial and Mycotic Ear Infections

Definition Inflammation of the inner ear caused by organisms.

Cytomegaloviruses (CMV)

CMV are DNA-containing members of the herpesvirus group.

Epidemiology General infection is frequent, an intrauterine source often being incriminated. The developing human ear has been thought to be particularly susceptible to CMV infection [107] and the virus has been incriminated on clinical and virological grounds as the most common cause of congenital hearing loss [108–112]. CMV infection is commonly seen in patients with AIDS.

Clinical aspects In infant inner ears, the endolabyrinth is mainly involved. Thirty-nine per cent of patients with AIDS were found to have a hearing loss of sensorineural type [113].

Microscopy In a study of the temporal bones at autopsy of 25 patients, CMV infection was identified in the inner ears of 5 patients by the presence of the characteristic inclusions. The inclusions were found in the vestibular nerve in the internal canal (Fig. 8.30), in the stria and in the saccule, utricle and lateral semicircular duct [114].
It is likely, therefore, that the hearing loss in patients with AIDS is due to cochlear CMV infection.

Rubella

Epidemiology Maternal *rubella* is an important factor in the genesis of congenital sensorineural hearing loss. The virus is an RNA one.

Microscopy In two cases the temporal bones showed inflammatory collections at the upper end near the junction with Reissner's membrane and adherent to it [115]. The organ of Corti was mainly normal.

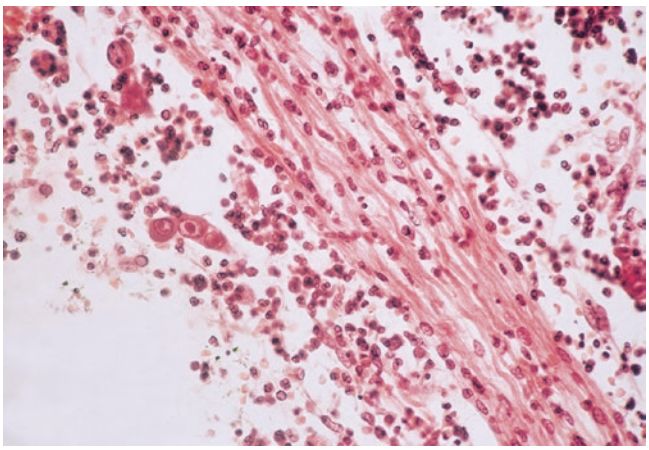

Fig. 8.30 Cytomegalovirus infection of vestibular nerve in the region of Scarpa's ganglion. The nerve is inflamed with the infiltration of neutrophils, leucocytes and plasma cells. Four enlarged cells, three to left of centre, the fourth on the far right with purplish inclusions, each surrounded by a pale halo, features characteristic of cytomegalovirus infection and can be identified. Autopsy findings in a patient with AIDS

Herpes Zoster Auris (Ramsay Hunt Syndrome)

Epidemiology Herpes zoster infection is seen as a disease of older people >60 years but all ages can be affected including the paediatric population.

Clinical aspects The virus (the *DNA herpes varicella virus*) enters the inner ear along the seventh and eighth cranial nerves, presumably from nerve ganglia where it lies dormant until the immunological status of the patient deteriorates. The usual presenting complaint is pain deep within the ear that may radiate to the pinna. The patient may complain of vertigo or tinnitus and there may be facial nerve palsy. Blisters may be present on auricle or external ear canal.

Microscopy In histopathological studies previously described, there were extensive inflammatory changes mainly in those two cranial nerves serving in the transmission of the virus. Varicella zoster has also been detected in the cytoplasm and nuclei of inflammatory cells of the middle ear in two cases of the Ramsay Hunt syndrome by an immunofluorescence method [116]. Herpes varicella-zoster viral DNA has been identified, using the polymerase chain reaction, in archival celloidin-embedded temporal bone sections from two patients who clinically had Ramsay Hunt syndrome (herpes zoster oticus) [117].

Bell's Palsy

Definition A condition possibly due to viral infection in the inner ear, which is manifested clinically as the sudden onset of a peripheral facial paralysis.

Clinical aspects It is a fairly common disorder and patients present with an acute unilateral weakness of facial muscles and/or alteration in taste. The suggestion has been made, with some virological support, that this condition is the result of infection with herpes simplex virus, type 1. Anatomical, vascular and neurological causes have to be excluded clinically.

Microscopic There have been a very small number of reports of temporal bone studies from patients with Bell's palsy. In two cases of Bell's palsy studied by Prof Leslie Michaels, serial sections of the temporal bones both showed the following histological findings. In the genu region there appeared to be constriction of the facial nerve by inflammatory tissue, which formed a sheath around it and encroached on its interior. The adjacent bone showed foci of resorption with abundant osteoclasts (Fig. 8.31). The geniculate ganglion was infiltrated by lymphocytes. In some places the affected facial nerve appeared severely oedematous and nerve cells were shrunken and showed an eosinophilic cytoplasm. The descending part of the facial nerve presented swelling and vacuolation of myelin sheaths with some loss of axis cylinders. These findings are compatible with geniculate ganglionitis. In one of these cases, herpes simplex viral type 1 was demonstrated in archival paraffin-embedded sections of the affected geniculate ganglion by carrying out PCR, followed by electrophoresis on agarose gel [118].

Genetics Most cases are sporadic but about 25 % are familial and associated with an inherited defect of the facial canal. An autosomal dominant pattern of inheritance has been suggested with low or variable penetrance.

Treatment and prognosis Over 70 % of cases resolve spontaneously. Antiviral therapy has not shown to be effective in increasing the number of patients who make a complete recovery [119].

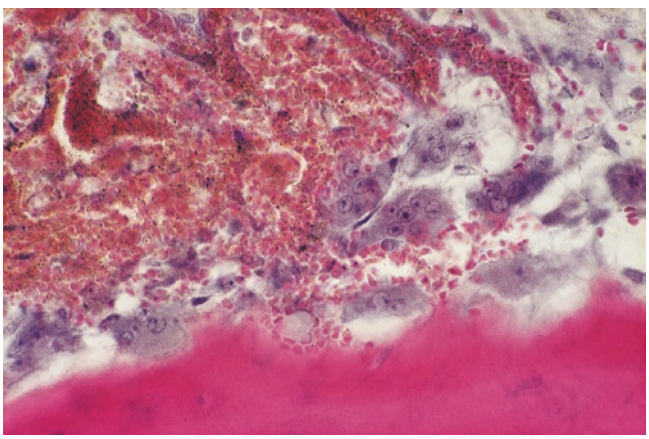

Fig. 8.31 Interface between geniculate ganglion and adjacent bone in a case of Bell's palsy, showing numerous osteoclasts with Howship's lacunae

Petrositis

Definition Bacterial infections of the inner ear that may involve both the petrous bone itself and the labyrinthine structures within it.

Clinical aspects Bacterial infection of the petrous bone is frequently derived by extension from middle ear infection. There are four possible routes by which infection may extend from the middle ear into the petrous bone [1].

(a) Via air cells. Mastoid air cells frequently extend in the temporal bone as far as the apical region. It is possible, therefore, that infection to the petrous apex may extend from the middle ear by the medium of infection of air cells.
(b) As direct spread of the inflammatory process by bone necrosis (osteitis).
(c) By extension through the bone marrow of the petrous bone (osteomyelitis).
(d) Along vessels and nerves.

Microscopy In addition to inflammatory infiltration, the pathological process of petrositis comprises three main changes in the bone tissue, all of which may be seen simultaneously: (a) bone necrosis, (b) bone erosion and (c) new bone formation.

Prognosis Petrositis is of great importance because involvement of the labyrinth, nerves, artery, veins, meninges and cerebral tissue embedded in and surrounding the petrous bone may each cause serious symptoms and perhaps death.

Extension to the labyrinth may lead to labyrinthitis with destruction of the organs of hearing and balance. Important nerves may be damaged. The facial nerve is at risk early. Involvement of the trigeminal ganglion and the sixth cranial nerve leads to "Gradenigo's syndrome". Extension to the jugular foramen region by the inflammatory process may cause palsy of the ninth, tenth and eleventh cranial nerves ("jugular foramen syndrome").

The wall of the internal carotid artery may become inflamed and this may lead to thrombosis of the vessel with possible cerebral complications. Similarly the lateral sinus may become thrombosed and this and/or extension of the thrombus to the superior sagittal sinus may be associated with the somewhat arcane syndrome of otitic hydrocephalus. Spread of the infection to the immediately adjacent cranial structures will lead to meningitis and cerebral abscess.

Labyrinthitis

Definition Inflammation of the labyrinth of the inner ear.

Clinical aspects The source of labyrinthitis is, in many instances, otitis media, as with petrositis. Infection may enter the labyrinth by penetrating the oval or the round window. An infected air cell may rupture into the labyrinthine system at some point of its complex periphery. Occasionally damage to bone by the inflammation may produce a fistula between the middle ear and the labyrinth, usually in the lateral semicircular canal because this is the nearest vulnerable point to the middle ear. The latter complication takes place in most cases when a cholesteatoma is present, which has the effect of stimulating the inflammatory process.

Infection may also be conveyed from meningitis through the cochlear aqueduct and the internal auditory meatus into the labyrinth. Sensorineural hearing loss is an important sequel of acute bacterial meningitis [120].

Microscopy In suppurative labyrinthitis the perilymph spaces display usually a massive exudate of neutrophils. If the process extends to the endolymphatic spaces, there is concomitant destruction of membranous structures and irreparable damage to sensory epithelia.

Prognosis Healing is at first by fibrosis, but later osseous repair is frequent, leading to a condition of "labyrinthitis ossificans". In this condition the spaces of the bony labyrinth are filled in by a newer bone, which appears in striking contrast with the normal bone surrounding the bony labyrinth.

Cryptococcosis

Definition A fungus infection which usually infects the meninges.

Clinical aspects There may be extension by the organism *Cryptococcus neoformans*, from the meninges along the internal auditory meatus and then into the cochlea via the modiolus. Such a progression was clearly present in two cases of AIDS with cryptococcal meningitis that had spread to the labyrinth [114].

8.4.2.2 Lesions of the Vestibular System

The pathology of the vestibular labyrinth has not been as well studied as that of the cochlea and other parts of the labyrinth. This is the result of the paucity of operative procedures with biopsy carried out in this area and also of the rapidity of autolysis which takes place after death so that histological study of this area is difficult. The non-neoplastic lesions listed below are described in detail in [1].

Ototoxicity

Definition Damage to the hearing or balance functions of the ear usually by drugs or chemicals.

Clinical aspects Many drugs damage the sensory epithelia of the inner ear. The most obvious clinical effect is when the cochlear sensory cells are involved so that hearing loss results. Part of the damage produced by aminoglycoside antibiotics such as gentamycin, however, may be to the sensory epithelium of the cristae and maculae, producing symptoms of imbalance.

Virus infection In rubella and cytomegalovirus infection, changes have been observed in the utricle and saccule (see above).

Bacterial infection Bacterial infection may involve the vestibular system as part of labyrinthitis. In most bacterial infections, spread occurs from the middle ear via the oval window. A direct fistula resulting from the bone erosion of otitis media may take place leading into the lateral semicircular canal, particularly in the presence of cholesteatoma.

Bone Diseases

Paget's disease frequently involves the bony vestibule and semicircular canals to a severe degree and as a result clinical symptoms referable to this system are likely to occur. Otosclerosis, although frequently present in relation to the bony wall of the vestibule, rarely involves the membranous structures of the vestibular system so that vestibular symptoms are rare in this condition.

Hydrops of the Saccule

Definition Increased hydraulic pressure in the endolymphatic system of the inner ear resulting in distortion of the membranous labyrinth.

Clinical aspects *Hydrops* of the saccule, which sometimes extends to the utricle, is the major pathological feature of Ménière's disease and is responsible for the characteristic symptom of that disease – vertigo.

Microscopy The scala media of the cochlea is usually distended in Ménière's disease (Fig. 8.32), and this is the pathological basis of the hearing loss and tinnitus that are the other disturbing symptoms in attacks of the disease. Saccular hydrops may also be a manifestation of syphilitic and bacterial inflammation involving the labyrinth.

Positional Vertigo

Definition A very common condition in which vertigo is induced in the patient by alteration in the position of the head.

Microscopic In 1969 Schuknecht described the temporal bone findings in two cases of positional vertigo [121]. Attached to the posterior surface of the cupula of the left

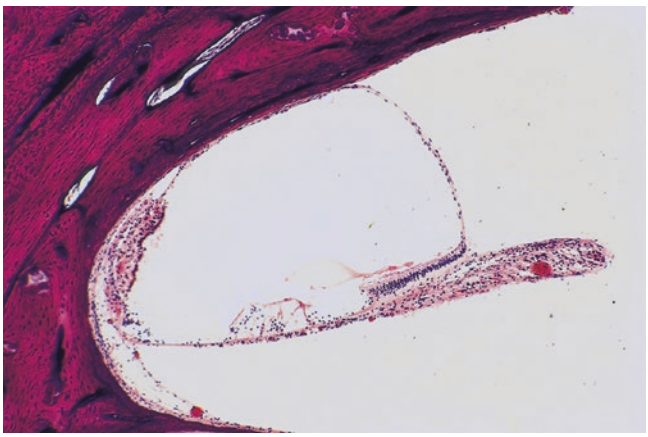

Fig. 8.32 Hydrops of scala media of cochlea. Reissner's membrane is distended to such a degree that it touches the top of the scala vestibuli

posterior semicircular canal in each of the cases was a basophilically stained homogeneous deposit.

Clinical aspects On the basis of these cases, Schuknecht has built up an explanation of the symptomatology of positional vertigo postulating that the calcific material derived from otoconia in a degenerated utricular macula will descend by gravity along the endolymph and form on the crista of the posterior semicircular canal, the lowest region of the labyrinthine sensory epithelium. This ingenious theory has attracted much interest and the term "cupulolithiasis" is nowadays frequently used as a synonym for positional vertigo.

8.4.2.3 Tumors and Tumor-Like Lesions

The most important neoplasm of the vestibular system is *schwannoma* (*acoustic neuroma*); this does not usually invade the vestibule, but may do so in cases of neurofibromatosis 2 [see below]. Another much rarer neoplasm is the low-grade adenocarcinoma of the endolymphatic sac (endolymphatic sac tumor) [see below]. Metastatic deposits are unusual; invasion of the vestibular system from the internal auditory meatus by way of the vestibular nerve may occur in metastatic neoplasm or in carcinoma of the middle ear [see above].

Vestibular Schwannoma

Definition A benign nerve sheath tumor arising in the internal auditory canal from the vestibular division of the eighth cranial nerve. See also Chapter 12 for further discussion on Schwannoma.

Epidemiology The age at presentation is the fifth and sixth decade. Younger patients may be affected in association with neurofibromatosis type 2 vestibular schwannoma is the most common temporal bone neoplasm and accounts for most cerebellopontine angle tumors. Unilateral sporadic tumors account for 5–10 % of intracranial tumors.

Clinical aspects Vestibular schwannoma is stated to arise most commonly at the glial-neurilemmal junction of the eighth nerve, which is usually within the internal auditory meatus. In one study of five temporal bones with small vestibular schwannomas, the tumor arose more peripherally, however [122]. When seen at surgery or autopsy, vestibular schwannoma in most cases is found to occupy a much greater part of the nerve. Usually it is the vestibular division of the nerve which is affected; in a few the cochlear division is the source of the neoplasm.

Growth takes place from origin, both centrally onto the cerebellopontine angle and distally along the canal (Fig. 8.33a, b). Vestibular schwannoma is usually unilateral but may be bilateral [see below].

The neoplasm may grow slowly for years without causing symptoms and may be first diagnosed only at post-mortem where it has been found in about 1 in 220 consecutive adults [123]. Although it arises on the vestibular branch of the eighth cranial nerve, hearing loss and tinnitus are early symptoms produced by involvement of the cochlear division of the nerve; in the later stages vertigo and abnormal caloric and electronystagmographic responses develop from damage to the vestibular division itself.

Macroscopy Vestibular schwannoma seen grossly is of variable size and of round or oval shape. Small tumors either do not widen the canal at all or produce only a small indentation in the bone (Fig. 8.34). The larger tumors often have a mushroom shape with two components, the stalk – an elongated part in the canal – and an expanded part in the region of the cerebellopontine angle. The bone of the internal auditory canal is widened funnel wise as the neoplasm grows. The tumor surface is smooth and lobulated. The cut surface is yellowish, often with areas of haemorrhage and cysts. The vestibular division of the eighth nerve may be identified on the surface of the tumor.

Granular or homogeneous fluid exudate is usually present in the perilymphatic spaces of the cochlea and vestibule. This may arise as a result of pressure by the neoplasm on veins draining the cochlea and vestibule in the internal auditory meatus. Hydrops of the endolymphatic system may occur (see above) and in larger tumors there is atrophy of spiral ganglion cells and nerve fibres in the basilar membrane.

Microscopy The tumor has the features of a neoplasm of Schwann cells showing Antoni A and Antoni B areas. Antoni A areas display spindle cells closely packed together with palisading of nuclei. Verocay bodies, which may be present in the Antoni A areas, are whorled formations of

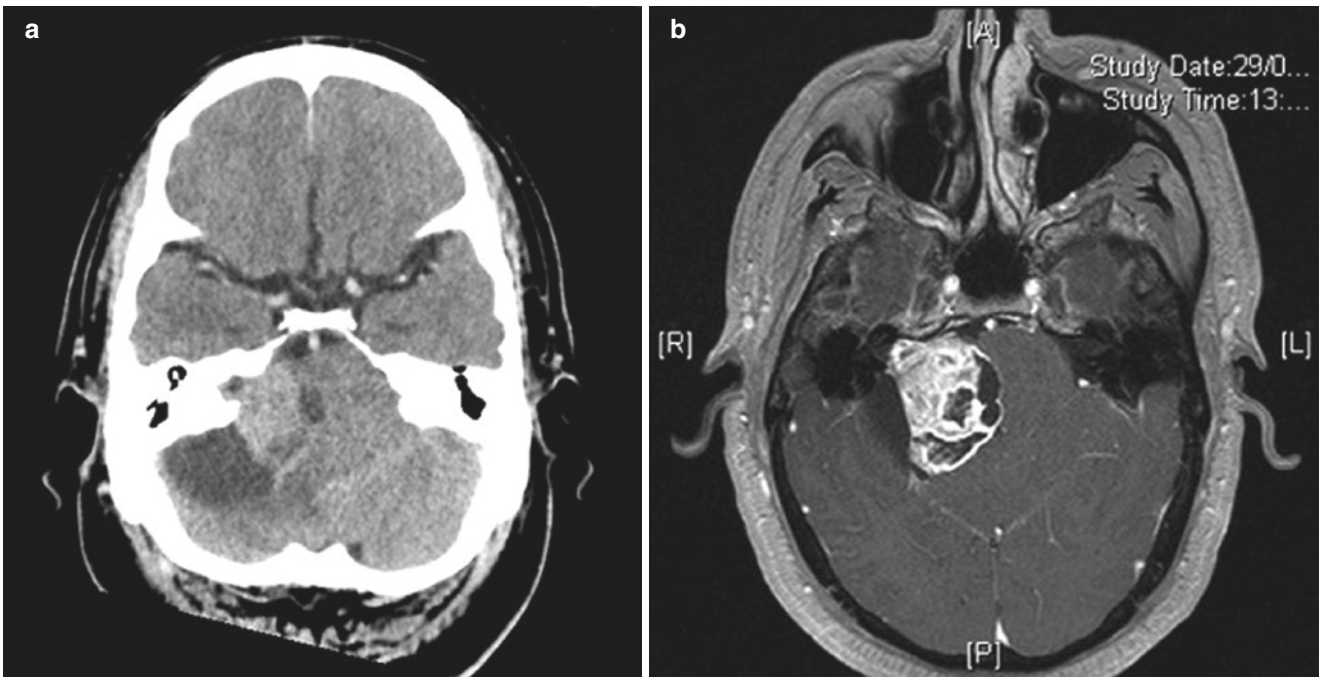

Fig. 8.33 Vestibular schwannoma. (**a**) Axial slice from post-contrast CT and (**b**) axial slice from post-contrast MRI. The well-circumscribed lesion in the right cerebellar pontine angle is more clearly seen in the MRI scan. The tumor is bright in comparison to the adjacent brain tissue and cystic hypo-intense areas are visible within it

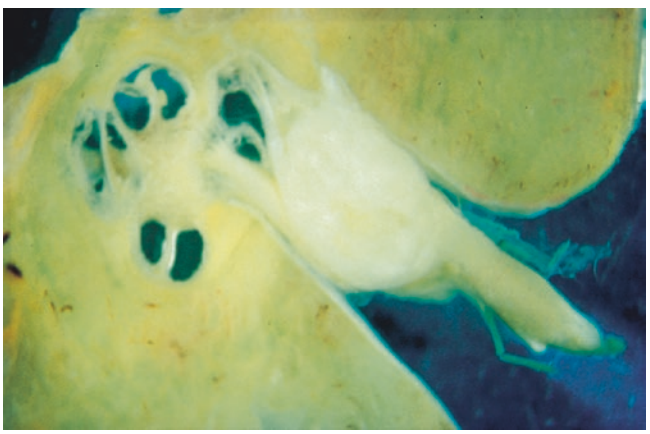

Fig. 8.34 Vestibular schwannoma in a microsliced temporal bone. The neoplasm is arising from the vestibular division of the eighth nerve and compressing the cochlear division. Note the granular deposit lining the cochlea

palisaded tumor cells. The degree of cellularity of the neoplasm can be high or low. The spindle cells frequently are moderately pleomorphic, but mitotic figures are unusual. The presence of pleomorphism does not denote a malignant tendency. Antoni B areas, probably a degenerated form of the Antoni A pattern, show a loose reticular pattern, sometimes with histiocytic proliferation. Thrombosis and necrosis may be present in some parts of the neoplasm. A mild degree of invasion of modiolus or vestibule along cochlear or vestibular nerve branches may be present even in solitary vestibular schwannoma*s*. The NF2-associated tumors are histologically similar to those of the single tumors except that the former have more Verocay bodies and more foci of high cellularity. The NF2 tumors are more invasive, however, tending to infiltrate the cochlea and vestibule more deeply.

Immunohistochemistry As with all schwannomas, there is diffuse strong expression of S100 protein. Vimentin is also usually positive. These findings are common to both unilateral vestibular schwannoma and the schwannomas of NF2. Glial fibrillary acidic protein and neurone-specific enolase markers are also sometimes positive; the tumors are consistently negative for CD34, a marker widely used for the diagnosis of solitary fibrous tumors, unless the vestibular schwannoma is widely degenerated [124].

An antibody against Ki67 (MIB1 in paraffin sections) has been utilized in a number of investigations to determine whether the degree of positivity with this proliferation marker can be related to the clinical activity of the tumor. It has been demonstrated that tumors 18 mm or smaller in diameter have lower proliferation indices and growth rates, compared with tumors larger than 18 mm [125]. The degree of labelling with the proliferation marker is higher in cases of NF2 [126] than in those of solitary vestibular schwannoma [127].

Genetics Bilateral vestibular schwannoma occurs in association with NF2. Unlike neurofibromatosis 1 (von Recklinghausen's disease), NF2 is not associated with large numbers of cutaneous neurofibromas and cafe-au-lait spots, but the temporal bone locality of the neural tumor and its bilaterality are inherited as an autosomal dominant trait. This condition has been related to a gene localized near the centre of the long arm of chromosome 22 (22q12). The gene for NF2 is a suppressor gene that codes for a protein which has been called by two names: *MERLIN* which stands for moesin-ezrin radixin like protein because it resembles the family of cytoskeletal-associated proteins and *SCHWANNOMIN* because of its role in preventing schwannoma formation. At autopsy of cases of neurofibromatosis 2, neural neoplasms are present in both eighth nerves and other central nerves. There are often many small schwannomas and collections of cells of neurofibromatous and meningiomatous appearance growing on cranial nerves and on the meninges in the vicinity of the vestibular schwannomas and sometimes even intermixed with them.

Treatment and prognosis Vestibular schwannoma is benign and usually grows slowly so management is conservative where possible. Serious symptoms and even death may occur, however, due to damage to cerebral structures if the neoplasm grows to a large size. The NF2-associated tumors are more invasive, however, tending to infiltrate the cochlea and vestibule more deeply.

Surgical removal may be carried out by drilling from the external canal through the temporal bone, by craniotomy and the middle fossa approach to the internal auditory meatus or by stereotactically guided gamma knife surgery.

Meningioma

Definition Meningiomas are benign tumors that usually form intracranial masses.

Epidemiology Primary meningiomas of ear and temporal bone are more common in females. Meningiomas as well as acoustic neuromas may appear in the inner ear in the NF2 syndrome.

Clinical aspects They arise from arachnoid villi, which are small protrusions of the arachnoid membranes into the venous sinuses. Arachnoid villi may be found in parts of the temporal bone, including the inner ear, and on occasion meningiomas may arise from these structures as primary neoplasms of the inner ear region. The most likely position for a primary inner ear meningioma is in the wall of the internal auditory meatus, where arachnoid villi are normally frequent.

Microscopy The histological appearances of a meningioma are those of a tumor with a whorled arrangement of cells: meningotheliomatous if the tumor cells appear epithelioid, psammomatous if calcification of the whorled masses is prominent and fibroblastic if the tumor cells resemble fibroblasts.

Treatment and prognosis The meningioma is a slowly growing tumor of the temporal bone which has had a reputation for complete benignity. In the temporal bone, however, middle ear meningioma sometimes has a strong propensity for local recurrence and invasion [see above].

Lipomas

Definition A benign tumor of adipocytes.

Epidemiology Lipomas of the internal auditory canal and cerebellopontine angle are rare tumors that may be confused clinically with the much more common vestibular schwannoma. The lesions occur in the third to fifth decade of life predominantly in Caucasian males.

Clinical aspects Patients complain of hearing loss dizziness or unilateral tinnitus. Symptoms may involve the facial or trigeminal nerve. On magnetic resonance using fat-suppressed T1-weighted images after gadolinium enhancement, this tumor displays characteristics of adipose tissue rather than those of schwannoma (Fig. 8.35).

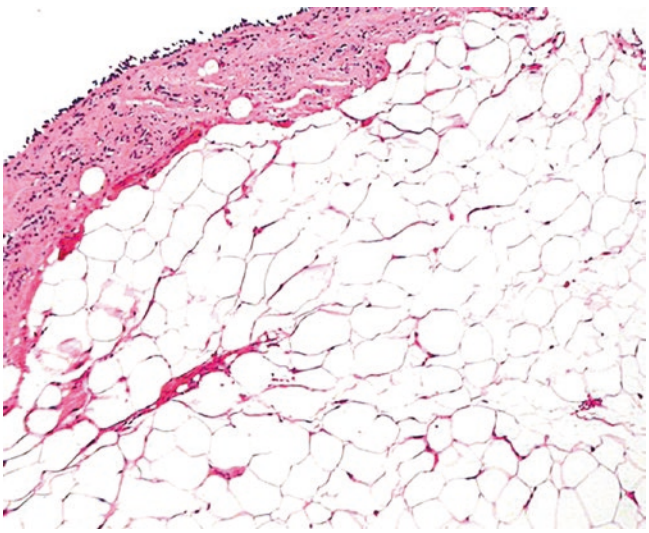

Fig. 8.35 Vestibular lipoma. The lesion is composed of mature adipocytes and it is histologically identical to lipomas occurring at other sites

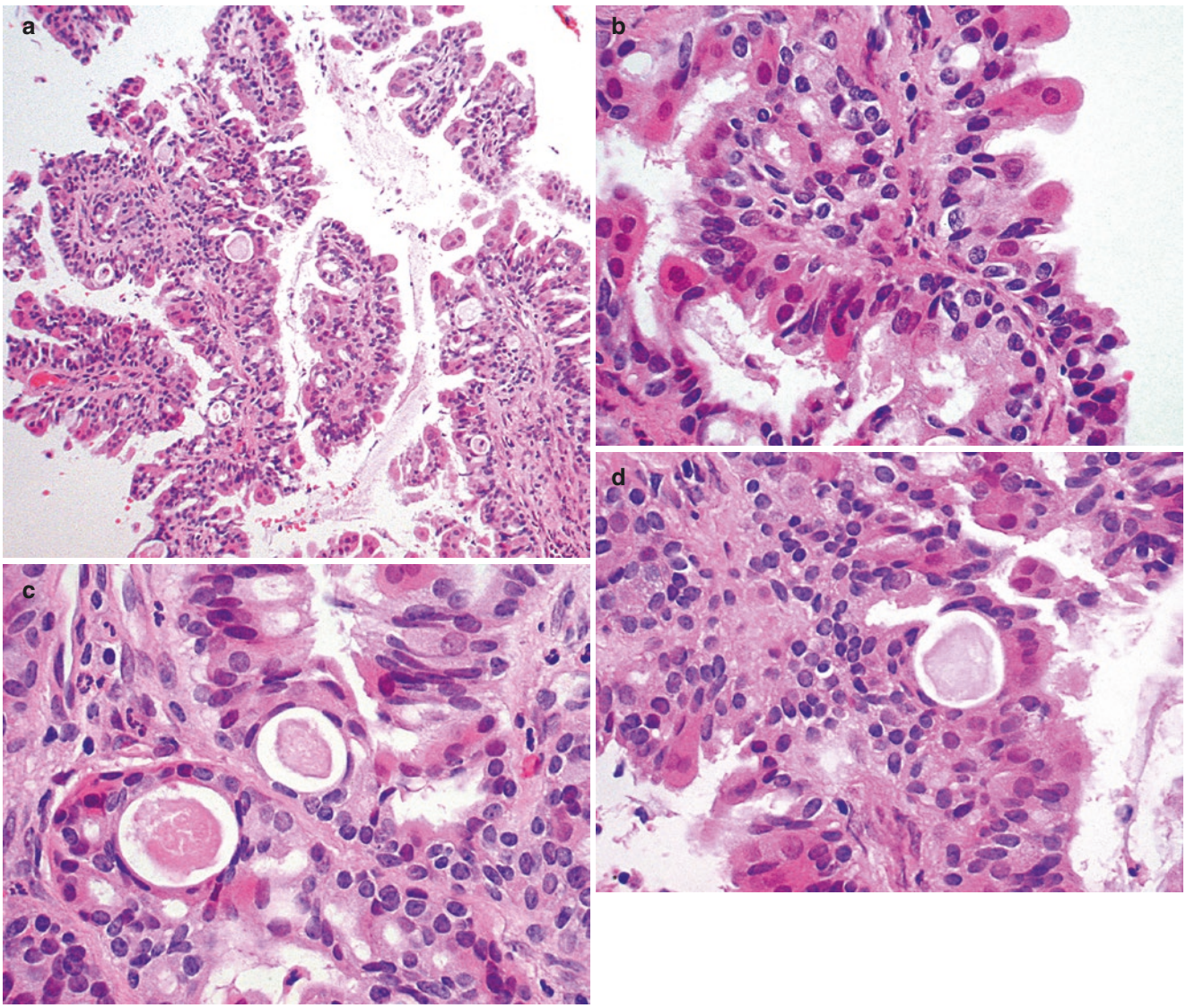

Fig. 8.36 (**a**) and (**b**) Endolymphatic sac tumor showing a papillary configuration. The lining cells are cuboidal and columnar with pleomorphic hyperchromatic nuclei. (**c**, **d**) There are small follicular structures containing eosinophilic material resembling thyroid tissue

Macroscopy There may be erosion of the walls of the internal auditory canal as with vestibular schwannoma, and lipoma may appear similar to the latter at operation. Since the seventh and eighth cranial nerves or their branches may pass through the lesion and their integrity be damaged by removal of the tumor, it is recommended that diagnosis be made whenever the possibility of this neoplasm is suspected at operation by examination of frozen sections. If a diagnosis of lipoma is made in this way, the tumor should not be resected since its further growth does not constitute a threat to vital structures [128].

Microscopy The lesion is composed of mature adipocytes and it is histologically identical to lipomas occurring at other sites (Fig. 8.35).

Low-Grade Adenocarcinoma of Probable Endolymphatic Sac Origin (Endolymphatic Sac Tumor)

Definition Low-grade malignant neoplasm believed to originate in the inner ear from epithelium of endolymphatic sac [64, 71, 72]. Other terms such as endolymphatic sac tumor (ELST) and Heffner's tumor are also in use.

The diagnosis depends on careful clinicopathological correlation with the radiological imaging.

Epidemiology ELST occurs over a wide age range from second to eighth decades. The tumor has been described in a 4-year-old child. There is an equal sex incidence in ELST although some studies have suggested a female predominance.

Clinical aspects The patients usually present with unilateral hearing loss that is sensorineural rather than conductive and that has persisted for more than 6 months. Other presenting symptoms include tinnitus, vertigo and less commonly cranial nerve deficits.

The course of the tumor growth may extend over many years. Tinnitus or vertigo, similar or identical to the symptoms of Ménière's disease, are present in about one third of patients. It is presumed that early obstruction of the endolymphatic sac leads to hydrops of the endolymphatic system of the labyrinth and so to the Ménière's symptoms.

ELST is rare and the diagnosis is challenging for clinicians, radiologists and pathologists. The tumors may be sporadic, occurring in about 1 in 30,000 patients, or they may be associated with the autosomal dominant von Hippel-Lindau (VHL) disease [129] when the tumors may be bilateral. About 30 % of VHL-associated ELSTs are bilateral. Sporadic tumors appear to be larger at presentation and more aggressive than those associated with VHL.

These tumors have a unique site predilection in the posterior temporal bone. They arise from the vestibular aqueduct on the posteromedial aspect of temporal bone. The tumor is slow-growing and may be widely infiltrative. Typically the imaging shows a retrolabyrinthine mass eroding bone. There is usually a lytic lesion that can range in size between 3 and 10 cm and appearing to originate from the region between the internal auditory canal and sigmoid sinus (which is the approximate position of the endolymphatic sac).

The tumor may expand posteromedially into the cerebellar pontine angle, anteromedially towards the cavernous and sphenoid sinuses, laterally into the middle ear and superiorly into the middle cranial fossa.

Recently a grading system has been proposed: Grade 1 lesions being confined to temporal bone middle ear and external auditory meatus, Grade 2 extending into posterior fossa, Grade 3 involving posterior and middle cranial fossae and Grade 4 lesions extending into clivus or sphenoid wing.

Papillary tumors histologically indistinguishable from ELST arising in temporal bone have been described confined to the middle ear. These lesions have not been associated with the VHL mutation and are probably best considered distinct from ELST at the characteristic site.

Some cases have presented bilateral neoplasms of the same type and some have been associated also with von Hippel-Lindau disease [129].

Macroscopy The tumor is red/brown and fibrous.

Microscopy The histological appearances of low-grade adenocarcinomas of probable endolymphatic sac origin are indeed in keeping with the normal histological structure of the endolymphatic sac, which is lined by a papillary columnar epithelial layer.

In most cases the tumor has a papillary-glandular appearance, the papillary proliferation being lined by a single row of low cuboidal cells. The vascular nature of the papillae in some cases has given the tumor a histological resemblance to choroid plexus papilloma (Fig. 8.36a, b). In some cases the tumor shows also areas of dilated glands containing secretion which has some resemblance to colloid and under these circumstances the lesion may resemble papillary adenocarcinoma of the thyroid (Fig. 8.36c, d). Such thyroid-like areas may even dominate the histological pattern. A few cases show a clear cell predominance resembling carcinoma of the kidney.

Immunohistochemistry It shows the tumor cells express pan cytokeratin as well as cytokeratins CK5, CK7 and CK19 but CK20 is negative. There is variable expression of EMA, Ber EP4, GFAP and neuroendocrine markers including synaptophysin, neurone-specific enolase and chromogranin. There is no expression of thyroglobulin or thyroglobulin transcription factor 1 (TTF1). The proliferation rate with Ki67 immunostain is low.

Genetics Patients with VHL carry a mutation on the short arm of chromosome 3 (3p26-p25) and 11 % of those affected develop ELST.

Differential diagnosis It seems possible that many cases of the so-called aggressive papillary middle ear tumors may be low-grade adenocarcinomas of endolymphatic sac with extension of neoplasm to the middle ear [63] Not all of such tumors may arise in the endolymphatic sac [130].

Treatment and prognosis Complete surgical excision is the treatment of choice and may be curative [126]. Surgery carries a high morbidity since it may require resection of the petrous temporal bone and mastoid, necessitating the sacrifice of cranial nerves. Advanced tumors may be treated by radiotherapy alone or the treatment may be mixed, surgery and post-operative radiotherapy.

The prognosis depends on the size of the lesion at presentation and the adequacy of the surgical excision. Distant metastases have been rarely described.

Cholesteatoma (Epidermoid Cyst)

Definition A benign sac formed by connective tissue and epidermal structures,

Clinical aspects The lesion usually presents with symptoms relating to its involvement of the seventh and eighth cranial nerves in the cerebellopontine angle.

Microscopic The histological appearance is similar to that of middle ear cholesteatoma [see above]. It is probably of

congenital origin, but no cell rest has been discovered from which it might arise.

Cholesterol Granuloma

Definition A lesion of the petrous apex with the typical features of cholesterol granuloma as seen in the middle ear and mastoid in chronic otitis media is being identified in recent years with increasing frequency.

Macroscopic At operation it appears cystic, the contents being altered blood, and cholesterol clefts with a foreign body giant cell reaction.

Microscopy Histological examination shows non-specific granulation tissue and hemosiderin deposits in the cyst wall.

Pathogenesis Cholesterol granuloma is believed to result from an inflammatory response to an obstruction of the pneumatized air cells at the apex of the temporal bone, and low cuboidal epithelium, the epithelium of the air cell, is sometimes identified near the cholesterol granuloma. As the process develops, bone is eroded by this expansile lesion, often involving the petrous apex, the cerebellopontine angle and the middle ear.

Differential diagnosis The lesion may be mistaken for an invading neoplasm such as the adenocarcinoma of the endolymphatic sac [131].

8.4.3 Presbycusis

Definition Presbycusis is age-related hearing loss.

Epidemiology Sensorineural hearing loss is an affliction that affects all people to a greater or lesser degree over the age of 60 years.

Clinical aspects Characteristically both ears are affected. The greatest losses are sustained in the higher frequencies, but there is a moderate degree of loss of hearing throughout the whole range of audible frequencies.

Microscopic It has been shown that the pathological basis of this condition is a complete degeneration of the organ of Corti and associated nerve supply at the end of the basal coil of the cochlea and moderate outer hair cell loss throughout the whole of the rest of the cochlea. An illustrated account of this condition will be found in reference [1].

Genetics Presbycusis has been found to be associated with genetic mutations in mitochondrial DNA. Such mutations may arise as a result of the effects of ageing on the cochlear. Damaged mitochondrial DNA may result in abnormal oxidative phosphorylation resulting in damaged neural function and possibly anatomical abnormalities.

8.4.4 Malformations

Modern methods of imaging have identified a wide range of malformations of the inner ear. In addition, there is a common form of congenital sensorineural hearing loss in which no change can be identified by CT or MRI scan. In such cases it is inferred that the loss is caused by microscopic lesions within the organ of Corti, many of which have been described. Some inner ear malformations are associated with gene mutations. An account of this subject is beyond the range of this chapter. A detailed and illustrated review will be found in reference [1].

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Cysts and Unknown Primary and Secondary Tumors of the Neck and Neck Dissection

9

Maria P. Foschini, Nina Zidar, Keyla Pineda-Daboin, and Mario A. Luna†

Contents

9.1 Introduction

The neck connects the organs of the head with those of the thorax. It contains important anatomic structures, including blood and lymphatic vessels, nerves and paraganglia, muscles and vertebrae and numerous lymph nodes, in addition to salivary, thyroid and parathyroid glands. The neck also contains organs of the upper aerodigestive tract: larynx, hypopharynx and segments of the oesophagus and trachea.

The fact that a neck mass can originate in any of the cervical structures means that a host of disorders challenges the diagnostic ability of the surgical pathologist. The differential diagnosis of a neck mass includes developmental, inflammatory, benign and malignant neoplastic lesions. The purpose of this chapter is to review the pathology and diagnosis of cervical cysts. Occult primary tumors of the neck and neck dissection also are discussed.

The present chapter is the updated version of the previous edition that was written in his unique style by the late Mario A. Luna.

†Author was deceased at the time of publication.

M.P. Foschini, MD (✉)
Section of Anatomic Pathology "M. Malpighi", Department of Biomedical and Neuromotor Sciences, University of Bologna, Bellaria Hospital, Via Altura 3, Bologna 40139, Italy
e-mail: mariapia.foschini@unibo.it

N. Zidar, MD, PhD
Institute of Pathology, Faculty of Medicine, University of Ljubljana, Korytkova 2, Ljubljana 1000, Slovenia
e-mail: nina.zidar@mf.uni-lj.si

K. Pineda-Daboin, MD
Department of Pathology, Military Hospital "Carlos Arvelo" and Instituto Anatomopatologico, Universidad Central de Venezuela, Caracas, Venezuela
e-mail: keylapineda@gmail.com

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9.2 Anatomy

Exact knowledge of the neck anatomy is essential for performing a correct preoperative diagnosis. To this purpose, several descriptions based on a CT scan or MRI imaging are available [1].

9.2.1 Triangles of the Neck

It is customary to divide the neck into two large triangles, the anterior cervical triangle and the posterior cervical triangle. The anterior triangle is bounded by the midline of the neck, the anterior border of the sternocleidomastoid muscle and the inferior border of the mandible. The posterior triangle is bounded by the anterior margin of the trapezius muscle, the posterior border of the sternocleidomastoid muscle and the clavicle.

The anterior cervical triangle can be further subdivided into four lesser triangles (submental, submandibular, superior carotid and inferior carotid) and the posterior triangle into two (occipital and supraclavicular). The mylohyoid muscle is important in separating the sublingual space from the submandibular space [1]. The boundaries of the neck triangles are described in greater detail in other sources [2].

9.2.2 Lymph Node Regions of the Neck

The cervical lymph nodes can be divided into superficial and deep nodes and each of these groups into lateral and medial. The deep lateral nodes are distributed among several large groups: (1) the anterior group (submental and submandibular), (2) the internal jugular chain (superior, middle and inferior), (3) the spinal accessory nerves chain and (4) the supraclavicular node chain. The deep medial cervical group consists of the prelaryngeal, prethyroidal, pretracheal and paratracheal lymph nodes. The superficial medial lymph nodes are distributed around the anterior jugular vein. The superficial lateral cervical nodes are located along the external jugular vein.

Figure 9.1 shows the system for describing the location of lymph nodes in the neck, which uses the levels recommended by the Committee for Head and Neck Surgery and Oncology of the American Academy for Otolaryngology – Head and Neck Surgery [3].

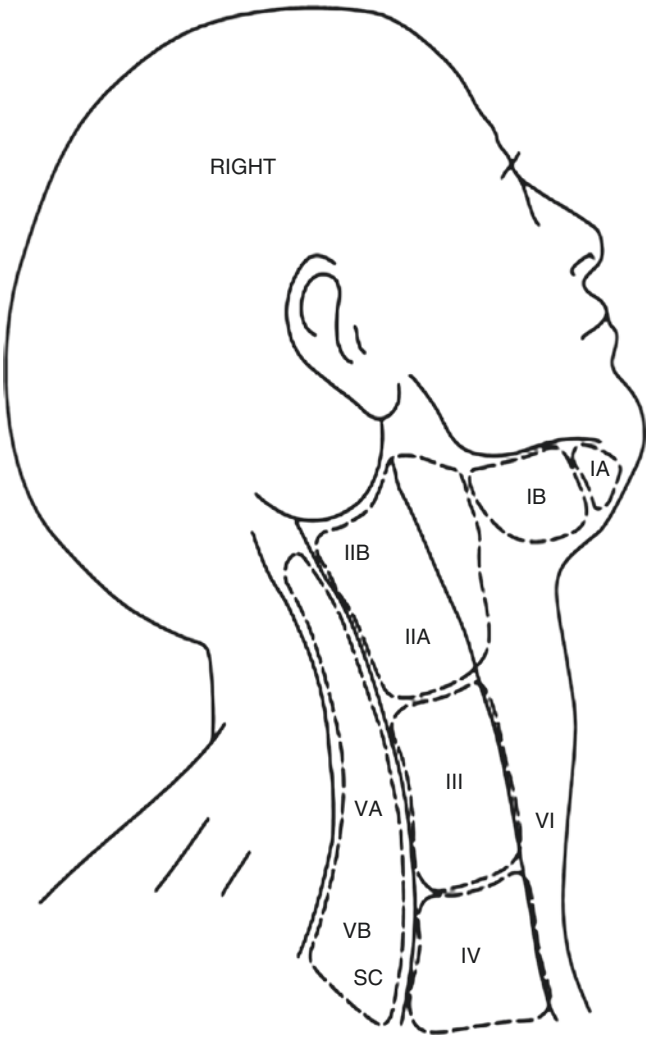

Fig. 9.1 Cervical lymph nodes by levels and sublevels: IA submental and IB submandibular, II upper jugular, IIA jugulodigastric, IIB supraspinal accessory, III middle jugular, IV lower jugular, V posterior triangle, VA spinal accessory nerve, VB transverse cervical, SC supraclavicular, VI anterior group

9.3 Cysts of the Neck

Definition Cysts are pathological cavities lined by the epithelium. The type of epithelium varies and the cavity may contain fluid, keratin, mucus or other products. Cervical cysts can be divided into two large groups: developmental and non-developmental. Establishing the precise nature of these cysts is important because there are considerable differences in their biological and clinical behaviour [4].

Clinical aspects Because of the frequent similarities in the morphological aspects of various cysts, a definitive diagnosis is dependent on clinical data. These include the exact location of the lesion and the age of the patient. The clinical manifestations of cysts depend largely on their size. Most cysts at the early stages are asymptomatic and are found on routine physical or radiographic examination. Rupture and drainage lead to infection, abscess and sinus formation, which are frequently accompanied by pain and swelling. In certain instances, a computed tomography scan can be of

Table 9.1 Frequency of cervical cystic lesions according to age

Infants and children	Adolescents	Adults
Thyroglossal duct cyst	Thyroglossal duct cyst	Metastatic cystic carcinoma
Branchial cleft cyst	Branchial cleft cyst	Thyroglossal duct cyst
Lymphangioma	Bronchogenic cyst	Cervical ranula
Hemangioma	Thymic cyst	Branchial cleft cyst
Teratoma and dermoid	Teratoma and dermoid	Laryngocele
Bronchogenic cyst	Metastatic thyroid carcinoma	Parathyroid cyst
Thymic cyst		Thymic cyst
Laryngocele		
Metastatic thyroid carcinoma		

Extracted from Refs. [6–9]

benefit in establishing the diagnosis and/or extension into adjacent structures [5]. Aspiration needle biopsy can also be useful in distinguishing between cysts and other lesions that present a similar radiological appearance (Table 9.1) [10].
In adults, an asymptomatic neck mass should be considered a malignancy until proven otherwise. With the exception of thyroid nodules and salivary gland tumors, neck masses in adults have the following characteristics: 80 % of the masses are neoplastic, 80 % of neoplastic masses are malignant, 80 % of malignancies are metastatic and, in 80 %, the primary tumor is located above the level of the clavicle [11]. In contrast, 90 % of neck masses in children represent benign conditions. In a review of 445 children with neck masses, 55 % of the masses were congenital cysts, 27 % were inflammatory lesions, 11 % were malignant and 7 % were miscellaneous conditions [12, 13]. Table 9.1 lists the causes of neck masses in order of frequency with which they occur, according to the age of the patient.

9.3.1 Developmental Cysts

9.3.1.1 Branchial Cleft Cysts, Sinuses and Fistulae

Definition Branchial apparatus anomalies are lateral cervical lesions that result from congenital developmental defects arising from the primitive branchial arches, clefts and pouches.

Etiology and pathogenesis The branchial apparatus appears around the fourth week of gestation and gives rise to multiple structures or derivatives of the ears, face, oral cavity and neck. These structures are described in more detail in other sources [14]. Anatomically, the branchial apparatus consists of a paired series of six arches, five internal pouches and five external clefts or grooves. The external grooves are of ectodermal origin and are called branchial clefts. The internal pouches are of endodermal origin and are known as pharyngeal pouches; they are separated by their branchial plates [14]. Each branchial arch is supplied by an artery and a nerve and develops into well-defined muscles, bone and cartilage. All three germ cell layers thus contribute to formation of the branchial apparatus. The arches are numbered 1–6, from cranial to caudal, and the clefts and pouches 1–5. The corresponding cleft and pouch lie immediately caudal to their numerical arch; that is, the first cleft and pouch lie between the first and second arches, the second cleft and pouch lie between the second and third arches and so on.

During the development of the head and neck, the second branchial arch gives rise to a cavity, lined by ectoderm, called the cervical sinus of His [15, 16].

A number of theories exist to explain the genesis of branchial cleft anomalies. Regauer and associates [17] proposed that the cysts arise from the endodermally derived second branchial pouch. An alternative explanation is that the cysts develop from cystic epithelial inclusions in the lymph nodes that are either of salivary gland origin or from the displaced epithelium from the palatine tonsil [18]. The various theories about the histogenesis of branchial cleft cysts have been recently reviewed [19].

Papers dealing with anomalies of the branchial apparatus do not always distinguish between the terms sinus and fistula and often use them interchangeably as synonyms. A sinus is a tract that has only one opening, either cutaneous or mucosal. A fistula is a tract that has two openings, one cutaneous and one mucosal. A cyst may occur independently or in association with a sinus or fistula [16, 20].

Most anomalies of the branchial apparatus of concern to the surgical pathologist present clinically as a cyst, fistula, sinus or skin tag. Fistulae, sinuses and skin tags occur in younger patients than cysts [21].

Epidemiology Branchial cleft cysts comprise approximately 75–80 % of all branchial anomalies, and fistulae and sinuses together account for 15–20 % of all such malformations [22]. In some series, external fistulae, sinuses and skin tags are more common than cysts [23].

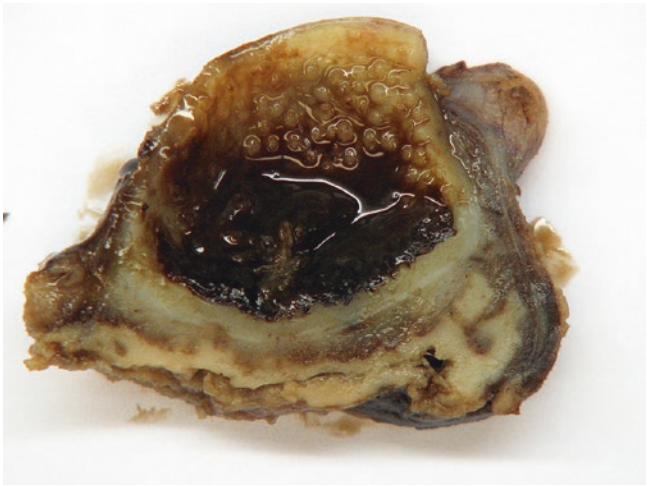

Fig. 9.2 Branchial cleft cyst presenting macroscopically as a unilocular cyst

Clinical aspects Branchial cleft anomalies have no gender preference. Most patients (75 %) are aged 20–40 years at the time of diagnosis. They are usually unilateral and unilocular (Fig. 9.2); bilateral presentation is an exceptional event [21, 24]. Since fewer than 3 % of cysts are found in patients older than 50 years, the pathologist must be careful in making this diagnosis in this age group; a metastatic cystic squamous cell carcinoma in a cervical lymph node may masquerade as a branchial cleft cyst.

Branchial cleft anomalies frequently present superimposed inflammation, although microbiological cultures obtained from aspirated inflamed fluid have only on rare occasions given rise to bacterial growths [21]. Correct preoperative diagnosis is important and should be based on clinical symptoms, radiological findings and fine needle aspiration procedure [21, 25]. Guldfred et al. [21], using this approach in a series of 126 cases of cystic lesions of the neck, found that preoperative diagnosis can have a high level of accuracy, reporting a positive predictive value of 0.856 (95 % confidence interval, 0.771–0.918) and a sensitivity of 0.944 (95 % confidence interval, 0.869–0.979). The diagnostic utility of fine needle aspiration has been confirmed in several papers [26].

Treatment and prognosis In all cases of branchial cleft anomalies, surgery is the treatment of choice [20, 21]. The presence of active inflammation at the time of surgery can increase the risk of incomplete excision and of neurological complication [21].

First Branchial Anomalies (FBA)

Etiology and pathogenesis The first branchial apparatus gives rise to the maxilla, mandible, external auditory canal and part of the middle ear structures. This process ends by the sixth to seventh week of gestation. Parotid gland and facial nerve formation starts during the sixth to eighth week of gestation. Some remnants of the first branchial apparatus can probably remain variously connected to the parotid and facial nerve in these weeks [15]. FBA are therefore usually located in close proximity to the external auditory canal and (rarely) to the middle ear and parotid gland.

Epidemiology Anomalies from the first branchial apparatus accounted for only 8 % of all branchial cleft anomalies at the Mayo Clinic [27]. Of these, 68 % were cysts, 16 % sinuses and 16 % fistulae.

Clinical aspects These anomalies occur predominantly in females and are found in all age groups. In general, sinuses and fistulae tend to develop in infants and children, whereas cysts are more common in older groups. Clinically, they may masquerade as parotid tumors or as otitis with ear drainage [27]. FBA can present as cysts, sinuses or fistulae. They can be asymptomatic or present with otorrhoea, characterized by mucous or purulent discharge in cases that become infected [28]. When the parotid gland is involved, symptoms are related to the increase in size of the lesion or the superimposed inflammatory modifications. In a case described by Sarioglu et al. [29], the intraparotideal cysts were associated with xanthogranulomatous inflammatory changes.

Pathological features According to Work [30] and Waldhausen [31], FBA can be subdivided into two types according to their relation to the facial nerve: type I when located laterally and type II when located medially to the facial nerve [32]. Type I are those that embryologically duplicate the membrane (cutaneous) external auditory canal. Accordingly, only ectodermal components are observed under the microscope. On histological examination, they are often confused with epidermoid cysts, since they are lined solely by keratinized, stratified squamous epithelium, with no adnexal structures or cartilage (Fig. 9.3); inflammation is often present in the cyst wall (Fig. 9.4). Characteristically, they are located medial, inferior or posterior to the concha and pinna. Drainage from cysts or fistulae may occur in any of these sites. The fistula tract or sinus may parallel the external auditory canal and ends in a blind cul-de-sac at the level of the mesotympanum [16, 20].

Type II deformities are composed of both ectodermal and mesodermal elements and therefore contain, in addition to skin, cutaneous appendages and cartilage (Fig. 9.5). Patients with this defect usually present with an abscess or fistula at a point just below the angle of the mandible, through the parotid gland, towards the external auditory canal. Type II defects are therefore more intimately associated with the parotid gland than are type I defects. An anomaly sometimes

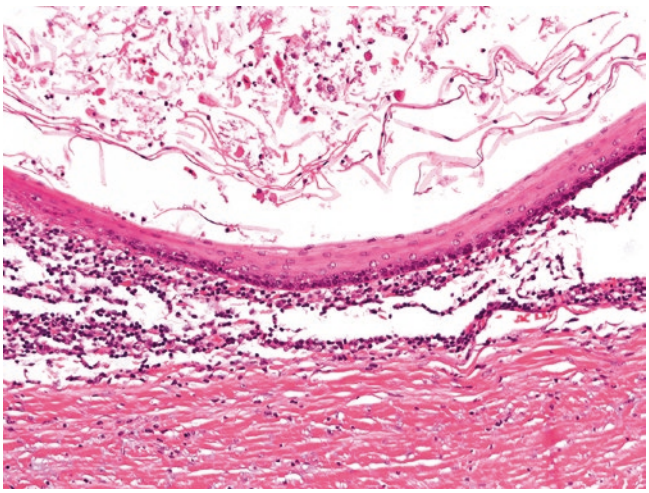

Fig. 9.3 First branchial cleft cyst, type I: wall lined by stratified squamous epithelium

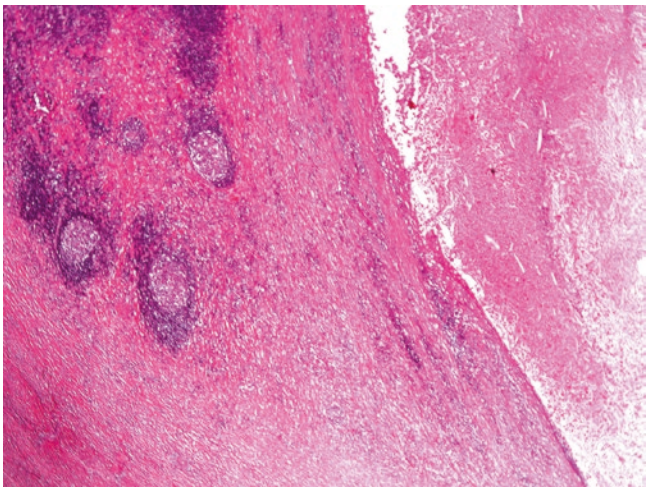

Fig. 9.4 Branchial cleft cyst, type I: inflammation is often present in the cyst wall

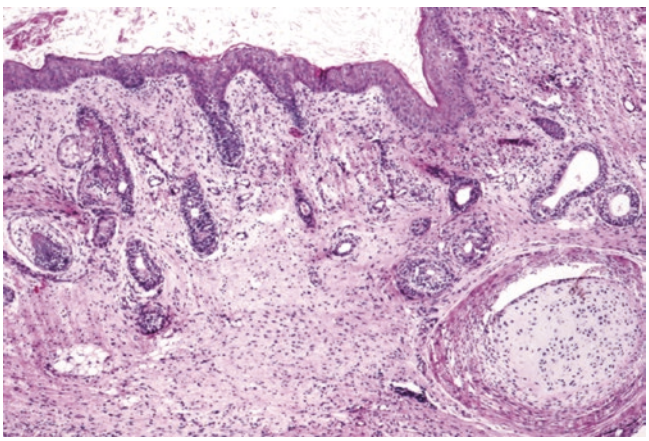

Fig. 9.5 First branchial cleft cyst, type II: squamous epithelium lining cystic cavity. Note the presence of skin appendage structures and cartilage in the stroma

cannot be distinguished as type I or type II. In those instances, Olsen et al. [27] suggested that the abnormality be classified only as to whether it is a cyst, sinus or fistula. Preoperative diagnosis can be challenging. Radiologic features are important in evaluating exactly the site of the lesion and its relation to the surrounding anatomic structures. Nevertheless, neither CT nor MR images are specific enough to differentiate FBA from other cystic lesions of the neck, especially when the parotid gland is involved [20].

Differential diagnosis Include all the cystic benign and malignant lesions of the parotid gland and cystic metastases from squamous cell carcinoma. To this purpose, fine needle aspiration cytology may be of help in differentiating in order to perform a correct preoperative diagnosis. First branchial cleft abnormalities must be differentiated pathologically from epidermal cysts (especially type I), dermoids (especially type II) and cystic sebaceous lymphadenoma.

Treatment and prognosis Complete excision is the only effective treatment. In some cases, this may necessitate a superficial parotidectomy. Nevertheless, care should be taken since surgery can be difficult due to the close relationship of FBA with the facial nerve and external auditory canal [16].

Second Branchial Anomalies (SBA)

Etiology and pathogenesis The second branchial apparatus gives rise to the facial muscles, styloid process, pinna and part of the middle ear structures. This process usually ends at the seventh gestational week [15]. The cervical sinus of His, if not completely obliterated, can probably favour the development of SBA by this time. The use of the name "branchial cyst" without further qualification generally refers to a cyst of second branchial origin.

Epidemiology Of all branchial anomalies, 92–99 % are associated with the second branchial cleft apparatus [15, 16, 20], probably because it is deeper and longer than the others [33].

Clinical aspects They can present as sinuses, cysts or fistulae. Cysts are three times more common than sinuses and fistulae in this apparatus. They typically occur along the anterior border of the sternocleidomastoid muscle from the hyoid bone to the suprasternal notch but infrequently have been described in the midline, just as a thyroglossal duct cyst may occur laterally, as a bilateral branchial cleft cyst, or even in the lateral wall of the nasopharynx [34, 35].

According to their location, SBA are subdivided into four groups [16, 20, 36]. Type I lesions are located anteriorly to the SCM and do not have contact with the carotid artery. Type II lesions, which are the most common, are found deep in the SCM. Type III lesions are located between the internal

and external carotid artery, near the pharyngeal wall. Type IV lesions are near the pharyngeal wall, in close proximity to the tonsillar fossa.

SBA are usually located beside the mandible angle but can present anywhere between the oropharyngeal fossa and the supraclavicular region. Cysts often present in adult patients, aged 30–50 [16]. Symptoms depend on the type of SBA (cyst, sinus or fistula); the cysts most frequently present as painless swellings, mobile through the surrounding tissues. Fistulae are more common in childhood. When a fistula is present, it is usually located along the anterior border of the SCM or in the supraclavicular region. SBA become symptomatic when infected [16, 20].

Radiological imaging, ultrasound, CT or MR examinations provide important clues for the preoperative diagnosis. Specifically, CT or MR images can evidence a curved rim of tissue (beak) around the cysts, which is pathognomonic of SBA [20].

Macroscopy On pathological examination of SBAs, the cysts are unilocular, usually between 2 and 6 cm in diameter.

Microscopy On histology, SBAs are lined by stratified squamous epithelium (90 %), respiratory epithelium (8 %) or both (2 %). Lymphoid aggregates with or without reactive germinal centres beneath the lining epithelium are found in the majority of cysts (75–80 %). Acute and chronic inflammation, foreign body giant-cell reaction and fibrosis are secondary microscopic changes in the wall of the cyst. In exceptional cases, the heterotopic salivary tissue may be found in the wall of the cyst [37]. Carcinoma in situ has seldom been described in the lining or the cysts [38]. Regauer et al. [39] postulated that the cysts are initially lined by an endodermally derived pouch type of respiratory epithelium, which is replaced by the squamous epithelium through an intermediate stage of pseudostratified transitional-type epithelium.

Fistulae and sinuses are found more often than cysts at birth or in early childhood. The external opening, when present, is usually located along the anterior border of the sternocleidomastoid muscle at the junction of its middle and lower thirds. The tract, if there is one, follows the carotid sheath; it crosses over the hypoglossal nerve, courses between the internal and external carotid arteries and ends at the tonsillar fossa [40].

Branchial cleft cysts have been reported infrequently in the parotid, thyroid and parathyroid glands, floor of the mouth, tonsil, pharynx and mediastinum [41]. Many of these cysts have the microscopic features of lymphoepithelial cysts (Fig. 9.6).

Differential diagnosis Thymic cyst and cystic low-grade mucoepidermoid carcinoma with prominent lymphoid stroma are considered in the differential diagnosis. The cyst's benign lining distinguishes it from metastatic cystic squamous carcinoma.

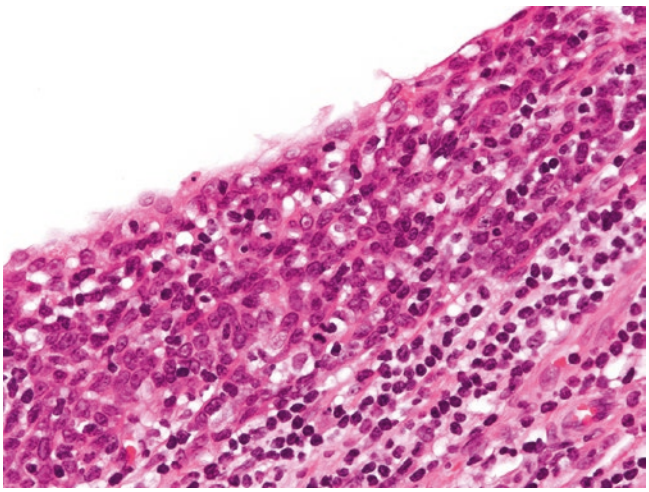

Fig. 9.6 Lymphoepithelial cyst. Note lymphocytes in the epithelium

Treatment and prognosis Complete surgical excision of the cyst, sinus or fistula is indicated. In a review of 274 patients with branchial remnants treated at the Mayo Clinic, the recurrence rate was only 2.7 % for patients with no history of surgery or infection, 14 % in those with a history of infection and 21.2 % in those who had undergone prior attempts at surgical removal [42].

Third and Fourth Branchial Anomalies (TBA and FoBA)

Etiology and pathogenesis The third branchial apparatus gives rise to the inferior parathyroid and to the thymic tissues, in addition to the internal carotid artery, ninth cranial nerve and hyoid bone. The fourth branchial apparatus gives rise to the thyroid and cuneiform cartilages, tenth cranial nerve, superior parathyroids and apex of the pyriform sinus [43].

Epidemiology Anomalies of the third and fourth branchial apparatuses are rare and together account for fewer than 5 % of all branchial cysts, sinuses and fistulae [44].

Clinical aspects A fistula in the pyriform sinus is one of the more common manifestations of a third branchial anomaly. Recurrent infections of the lower neck, including suppurative thyroiditis, and a fistulous tract into the pyriform sinus are the features of a fourth branchial cleft or pouch anomaly [44].

TBA and FoBA are rare and can present at any age from in utero to adulthood. Symptoms are often related to multiple episodes of infection [20]. Bacteria deriving from the oral cavity have been identified in cultures from TBA and FoBA [45].

At presentation, it may be difficult to differentiate TBA clearly from FoBA. Differential diagnosis is possible by analysing the relationship with the laryngeal nerve (which

derives from the fourth arch): those lesions located above the nerve are considered derived from the third branchial arch, while those located below the nerve are more probably derived from the fourth branchial arch [20]. Radiological images are important for the preoperative diagnosis [16, 20].

Third and fourth branchial sinus anomalies can be distinguished only by detailed surgical exploration. A third branchial sinus always extends from the pyriform sinus through the thyroid membrane cranial to the superior laryngeal nerve. In contrast, a fourth branchial sinus extends from the pyriform sinus caudal to the superior laryngeal nerve and exits the larynx near the cricothyroid joint [46]. Neither the fifth nor the sixth branchial arches form clefts or pouches in humans [14].

Treatment and prognosis As for other branchial anomalies, surgery is the treatment of choice.

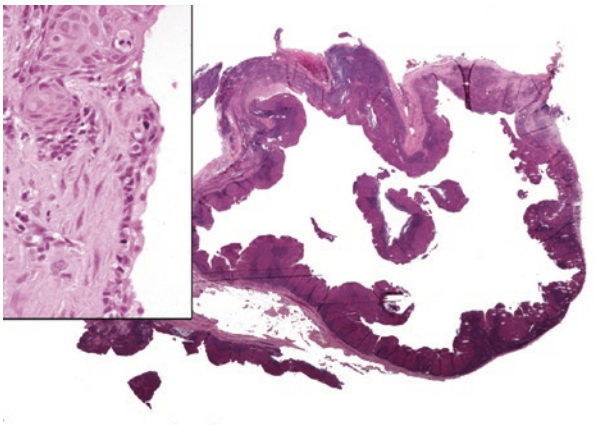

Fig. 9.7 Branchiogenic carcinoma. *Inset*: malignant squamous and respiratory epithelium lining the cyst wall

9.3.2 Branchiogenic Carcinoma

Definition Branchiogenic carcinoma or primary cervical neoplastic cysts are of interest from a historical viewpoint [39, 47]. Few of the purported examples of this entity fulfil the four criteria that Martin et al. [47] considered necessary to establish the diagnosis, which are as follows: (1) the cervical tumor occurs along a line extending from a point just anterior to the tragus, along the anterior border of the sternocleidomastoid muscle, to the clavicle; (2) the histologic appearance must be consistent with an origin from the tissue known to be present in the branchial vestigial; (3) no primary source of the carcinoma should be discovered during follow-up for at least 5 years; (4) cancer arising in the wall of an epithelium-lined cyst situated in the lateral aspect of the neck can be demonstrated histologically. These criteria were reviewed by Kafif et al. [48] who added to Martin's criteria the presence of transition aspects of the squamous epithelium, from normal to neoplastic, through dysplasia and in situ carcinoma [48]. However, this last feature has been rarely encountered [49].

The fulfilment of these criteria is practically impossible, and the actual existence of "branchiogenic carcinoma" must remain entirely hypothetical [39, 47, 50, 51]. The criteria have been criticized on the grounds that they are much too restrictive and nearly preclude a diagnosis of branchiogenic carcinoma [52, 53]. Questions about the existence of true branchiogenic carcinoma and the differential diagnosis from metastases from occult primary squamous cell carcinoma have been recently reviewed by Bradley and Bradley [54]. These authors concluded that branchiogenic carcinoma actually exists but it is exceptionally rare. Accordingly, several authors have estimated that, even accepting tentative examples of branchiogenic carcinoma, its incidence would be minuscule (0.3 % of all malignant supraclavicular tumors) [48, 52]. There is no doubt that most, if not all, of them are actually cervical node metastases with a cystic pattern. The palatine tonsil, or more generally the anatomic region of Waldeyer's ring, is notorious for producing cystic solitary metastases that resemble the usual appearance of branchial cleft cysts [39, 47, 50, 51].

Pathological features Almost all suspected branchiogenic carcinomas have been squamous cell in type, and all but one have been in the region of the second branchial apparatus (Fig. 9.7) [23, 52, 53]. The patients have been predominantly males ranging in age from 38 to 71 years [48, 52]. Nearly all of these masses have been cystic and have resided in a lymphoid matrix, hence the presumed relation to a branchial cyst. It should be obvious that neither the cystic architecture nor association with the lymphoid tissue are, in themselves, acceptable criteria for diagnosis of branchiogenic carcinoma [39, 50, 51]. The absence or presence of peripheral lymphatic sinuses and/or follicular centres in the lymphoid tissue has been used to exclude or confirm metastasis to the lymph nodes. This criterion is not valid. Branchial cleft cysts often lie within the lymph nodes and metastases can obscure the architecture of a lymph node [39, 51].

9.3.3 Thyroglossal Duct Cyst and Ectopic Thyroid

9.3.3.1 Thyroglossal Duct Cyst

Definition Thyroglossal duct cysts and sinuses are lesions located along the course of the thyroglossal duct, lined by respiratory or squamous epithelium and containing thyroid follicles in the wall.

Etiology and pathogenesis Cysts and sinuses develop during migration of the thyroid gland from the base of the tongue. The cysts are situated in the midline of the neck, usually below the hyoid bone. A fistula may develop from an infected cyst.

The thyroid begins to develop during the third to fourth week of gestation, when the embryo is about 2–2.5 mm long [14]. It is an endodermal derivative composed of two small lateral anlagen and the more substantial median anlage from the foramen caecum at the base of the tongue [16, 20]. Because of elongated cephalad embryonic growth rather than active descent, the orthotopic pretracheal location of the thyroid is caudal to the foramen caecum [14]. While the gland moves downward, epithelial remnants are left behind, attached to the foramen caecum, forming the thyroglossal duct [16, 20].

Epidemiology Thyroglossal remnants are present in 7 % of asymptomatic adults [55]. Enlargement after radiotherapy for head and neck carcinoma can occur and should not be misdiagnosed as metastasis [56].

Thyroglossal duct cysts (TDCs) are twice as common as branchial cleft cysts. In a review of 1534 cases in the literature, Allard observed that, at the time of presentation, 67 % of patients have a cyst and 33 % a fistula [34].

Clinical aspects Approximately 90 % of TDC occur in the midline of the neck, although some may occur paramedially, most often in the left. Overall, 73.8 % occur below the hyoid bone, 24.1 % are suprahyoid and <3 % are intralingual [20, 57, 58].

TDCs are the most common congenital anomaly presenting in infancy [20, 59]. Spinelli et al. [6] reviewed their experience with neck masses in children and noted that 17 (26 %) of 154 cases were TDC, and branchial cleft cysts were less common. Nevertheless, TDC can also manifest in adult patients [20]. Brousseau et al. [60] compared a series of TDC affecting adult and paediatric patients and concluded that even if presentation is more frequent during the first decade of life, a bimodal distribution is possible, with a second peak in adult patients.

Most patients with a TDC have no symptoms; they seek evaluation for a midline neck mass discovered incidentally by themselves or a family member. The most common manifestations are pain, a draining sinus or fistula, infection or dysphagia. A cyst on the floor of the mouth may cause feeding problems in newborns, whereas a cyst at the base of the tongue has, in rare instances, been responsible for sudden death in infancy [61]. TDCs are infected in 42 % of cases [60]. In children, TDC can present as otitis media [60].

A clear pattern of inheritance of TDC has not been described [60]. Nevertheless, a recent report [62] described a female baby with microdeletions of the long arm of chromosome 17, specifically deletions of 17q22q23.2, presenting several anomalies, including TDC.

Preoperative diagnosis is important to confirm the clinical diagnosis, to evaluate the presence of ectopic thyroidal tissue and to exclude the presence of a neoplastic component [63]. For this purpose, radiological imaging is important. Ultrasonography is the first imaging choice, especially in children, due to the well-known advantages (easy to perform, low cost) [63, 64]. CT scan and MRI can give useful insights for the correct diagnosis [63]. Fine needle aspiration cytology should be added to the preoperative diagnostic procedures [65].

Macroscopy TDCs range in size from 0.5 to 11 cm in diameter [66]. They can be either unilocular or multilocular and usually contain mucoid material if the cyst is not infected (Fig. 9.8a) or mucopurulent material or pus if the cyst is infected.

Microscopy The type of epithelium lining the cyst varies from case to case or even within the same surgical specimen. A columnar-to-stratified cuboidal epithelium with cilia is the most common type of epithelial lining, found in 50–60 % of cases (Fig. 9.8b). Lymphoid follicles in the wall of the cyst

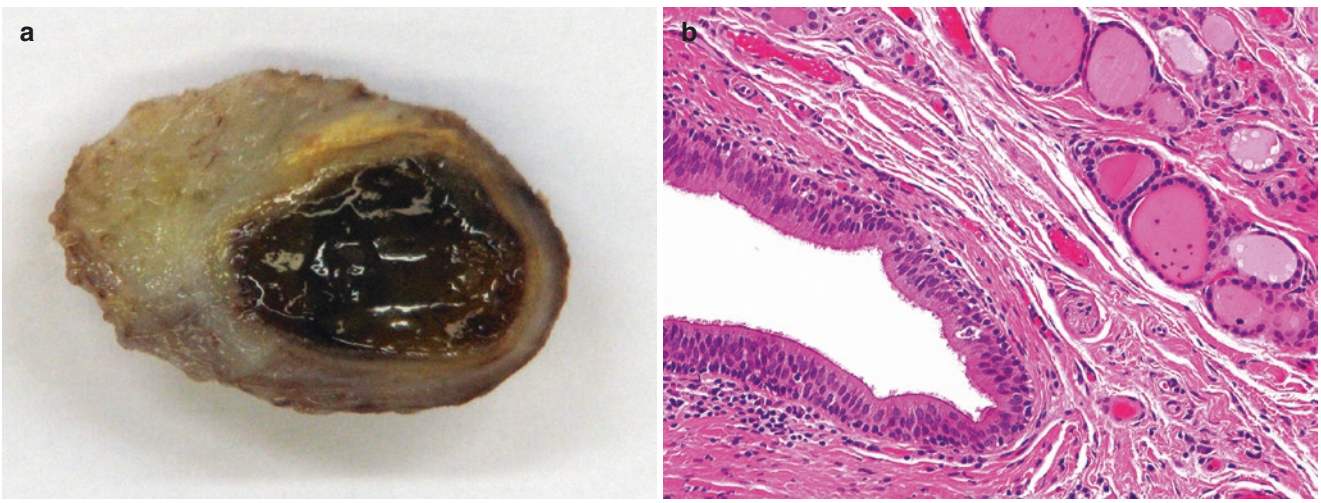

Fig. 9.8 (**a**) Thyroglossal duct cyst. (**a**) Unilocular cyst, containing mucoid material in the lumen. (**b**) Cyst is lined by respiratory type epithelium; there are thyroid follicles in the wall of the cyst

are found in 15–20 % of cases, while they occur in 75 % of branchial cleft cysts. A TDC with squamous lining and lymphoid tissue may be difficult to differentiate from a branchial cleft cyst. Immunoperoxidase staining for thyroglobulin may be of help. Ectopic thyroid tissue is identified (as collections of thyroid follicles in the soft tissues adjacent to the cyst) in 60 % of TDCs, although these figures are related to some extent to the number of tissue slides taken for histological examination and the extent of inflammatory and reactive changes present in the surrounding tissue.

Mucous glands were identified in 60 % of TDC studied by Sade and Rosen [67]. These authors believe the mucous glands to be part of the normal thyroglossal apparatus and not just glands found at the base of the tongue.

Treatment and prognosis Thyroglossal duct remnants are treated by complete surgical excision using the Sistrunk operation [68]. This consists of a block excision of the entire thyroglossal tract to the foramen caecum, as well as removal of the central 1–2 cm of the hyoid bone. If this procedure is performed, the TDC recurrence rate is less than 5 %. If the central portion of the bone is not removed, the recurrence rate is as high as 50 % [69, 70]. Most studies agree that the Sistrunk operation is the treatment of choice for TDC, both in adult and paediatric cases [16, 20, 60, 65, 71]. Nevertheless, since the Sistrunk operation is not devoid of complication [60], different treatment approaches are under study, such as marsupialization and ethanol injection [72].

9.3.3.2 Ectopic Thyroid

Definition Ectopic thyroid is defined by identification of gross or microscopic thyroid tissue outside the thyroid gland, most commonly from the base of the tongue (lingual thyroid) to the mid-lower neck superior to the orthotopic thyroid [73]. The ectopia can be complete or, more often, associated with an orthotopic thyroid. Thyroidal ectopic tissue can be identified on preoperative radiological imaging [34].

Epidemiology Hypothyroidism is a frequent finding in patients with lingual thyroid [58]. Batsakis and collaborators [74] noted a clinical prevalence of lingual thyroid of one in 10,000 individuals but an autopsy prevalence of one in ten.

Clinical aspects Patients with presumed ectopic thyroid should undergo a preoperative thyroid scan to rule out ectopic thyroid gland, because patients with an ectopic thyroid gland have no additional normally functional tissue, and thus are rendered permanently athyroid by excision of the ectopic gland [75].

Microscopy Ectopic thyroid is histologically composed of uniform, often small follicles containing minimal colloid. The microfollicles are usually intercepted by the skeletal muscle of the tongue.

9.3.3.3 Carcinoma in Thyroglossal Duct Cyst

Carcinoma in the thyroglossal duct has been reported in fewer than 1 % of TDC [76–78]. Carcinomas in TDC may be of different histological types; the most frequent is papillary carcinoma deriving from thyroideal tissue, but other types, such as thyroid follicular carcinoma and squamous cell carcinomas, have been described [77]. In addition, cases of thyroid type adenomas, such as Hurthle cell adenoma, [78] are on record.

Papillary carcinoma can be intracystic (Figs. 9.9a, b), located within the TDC [79], or it can be located in the ectopic thyroid tissue around the TDC. Yoo et al. [80] reviewed 115 cases of papillary carcinoma arising in TDC published in the literature. These tumors are typically intracystic, and

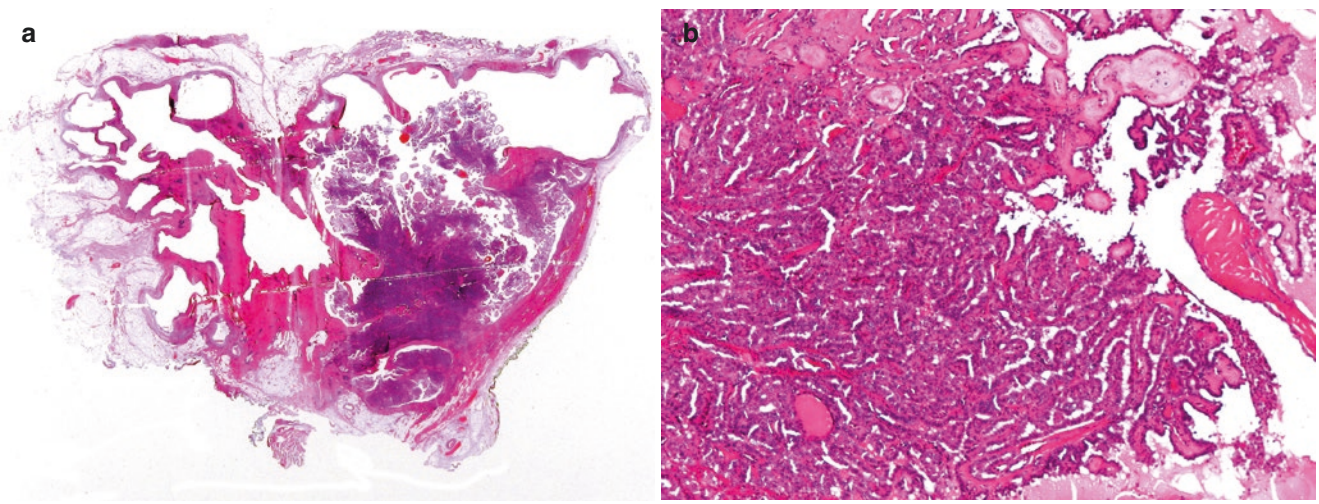

Fig. 9.9 Papillary thyroid carcinoma arising in a thyroglossal duct cyst: (**a**) The cystic cavity is enlarged and contains the papillary neoplastic growth. (**b**) Detail on papillary carcinoma

usually the thyroid gland proper is uninvolved. However, multifocal papillary carcinomas have been documented in TDCs as well as in the gland [76, 81, 82].

Fine needle aspiration cytology, associated with ultrasound examination, can be useful in performing a correct preoperative diagnosis [79, 83].

Management of thyroid carcinoma arising in TDC is fairly controversial. Numerous authors [59, 76, 80] agree that total thyroidectomy is not necessary if the gland does not show any evidence of malignancy. Nevertheless, recent papers have demonstrated synchronous thyroid involvement in 25 % [81] to 61.5 % [84] of cases, therefore suggesting that thyroidectomy should be considered [81, 84–87].

The prognosis of papillary carcinoma arising in TDC is very good, comparable to that of the thyroidal cases [82].

9.3.4 Cervical Thymic Cyst

Definition Cervical thymic cysts (CTC) are cysts containing thymic parenchyma in their wall.

Etiology and pathogenesis Faulty development of the third and fourth pharyngeal pouches results in abnormalities of the thymus and parathyroid glands.

Cervical thymic cysts (CTC) are morphologically identical to their mediastinal counterparts. They are found in the anterior triangle of the neck along the normal path of descent of the thymus, with or without parathyroid glands, and they have a fibrous band or a solid thymic cord connection to the pharynx or mediastinum.

The thymus develops as paired structures from the third branchial pouch at the sixth week of gestation. The endodermal primordium of the thymus has a ductal or luminal connection to the pouch that is known as the thymopharyngeal duct. Ventromedial and caudal growth of the respective anlage results in separation of the thymus from the pharynx. The fragmented remnants of the solid thymopharyngeal duct are thought to be the progenitors of the accessory parathyroid and thymic tissue in the neck [88]. The inferior parathyroid glands also originate from the third pouch, and their descent with the thymus explains their localization relative to the superior parathyroids, which arise from the fourth branchial pouch. By the end of the eighth week, the lower poles of the thymic anlage approach each other, but do not fuse, at the level of the aortic arch. Failures of involution or descent of any of the thymic anlage are responsible for a variety of abnormalities, such as thymic ectopia [89, 90], thymic cysts and thymoma [91]. The reader is referred to the excellent paper by Zarbo et al. [92] for the classification of these developmental abnormalities of the thymus.

Epidemiology CTC are uncommon; they account for fewer than 1 % of all cervical masses and most frequently affect children.

Clinical aspects Most cases present during the first decade of life [93]; when they appear in adulthood, the mean age at presentation is 36 years [91]. Both in children and adults, males are affected more commonly than females and most cases are on the left side [91, 94]. They can be found anywhere from the angle of the mandible to the sternum, paralleling the sternocleidomastoid muscle and normal descent of the thymus. Cervical thymic cysts usually present with neck swelling, but other symptoms as dysphagia, dyspnoea and hoarseness can also be present [91]. Correct diagnosis of a cervical thymic cyst is seldom performed preoperatively. For this purpose, correct interpretation of the radiological findings is important [90, 91].

Macroscopy The cysts range between 2 and 15 cm and may be either unilocular or multilocular.

Microscopy The epithelial lining may be cuboidal, columnar or stratified squamous. In some areas, the epithelium may be replaced by the fibrous or granulation tissue containing cholesterol clefts and multinucleated giant cells. For a cyst to qualify as CTC, the thymic tissue must be found within the cyst wall; detection of this tissue may require numerous sections.

CTC rarely have malignant potential. Moran et al. [95] recently reported for the first time carcinomas arising in CTC. This contrasts with mediastinal thymic cysts, in which malignancies are often seen. CTC have also not demonstrated pseudoepitheliomatous hyperplastic changes, as in some mediastinal cysts. On rare occasions, the parathyroid tissue is present in the cystic wall, intermingled with the thymic tissue (Fig. 9.10) [96].

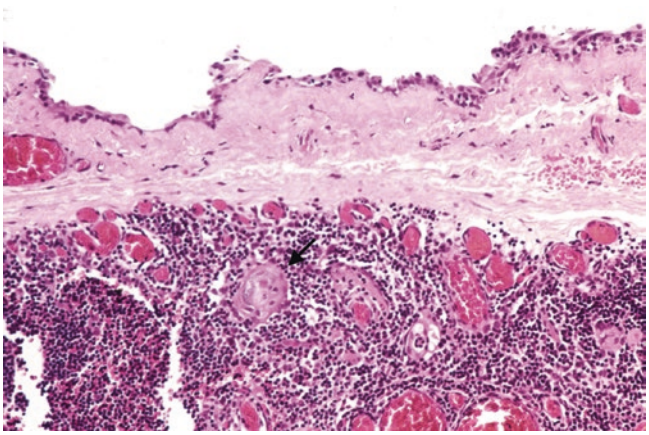

Fig. 9.10 Cervical thymic cyst. Notice Hassall's corpuscles in the wall (*arrow*)

Differential diagnosis The differential diagnosis includes other developmental cysts of the neck, as well as rare cystic presentations of Hodgkin's lymphoma, thymoma and germinoma. These three neoplasms are more likely, however, to present in the anterior mediastinum. The reader is referred to the excellent paper by May for clinical features useful in differentiating these conditions [7].

Treatment and prognosis Complete surgical excision is the treatment of choice. In children surgical treatment should avoid thymic excision.

9.3.5 Cervical Parathyroid Cyst

Definition Cysts of the parathyroid, located in the neck region. Cysts of the parathyroid glands, like thymic cysts, have several characteristic morphologic features, including a persistent hollow tract with the third or fourth branchial pouch.

Epidemiology The incidence of parathyroid cysts varies in different case series, due to different diagnostic sources. McCoy et al. [97] reported an incidence of 3 % in a series of 1769 cases undergoing surgery for hyperparathyroidism. The incidence is certainly lower, being 0.075 % when evaluated in a series of 6621 unselected patients undergoing neck ultrasound examination [98].

Clinical aspects There appear to be two distinct types of parathyroid cysts: non-functioning and functioning. The former make up the majority of these cysts and are about two to three times more common in women than in men. The mean age of patients with a non-functioning cyst is 43.3 years. Non-functioning cysts usually present as asymptomatic lesions, but, if they reach large dimensions, they can become symptomatic, with airway obstruction and recurrent laryngeal nerve palsy [99].

Functioning cysts account for 11.5–30 % of these cysts [100]. They are more common in men than women by a ratio of 1.6:1 and tend to occur in sites other than the inferior parathyroid glands, from the angle of the mandible to the mediastinum [101]. The mean age of patients with functioning cysts is 51.9 years. Functioning cysts usually develop from parathyroid adenoma or carcinoma [102] and present with symptoms of hyperparathyroidism [103]. One case of parathyroid cyst developing from parathyroid hyperplasia in a patient with multiple endocrine neoplasia type 1 has been recently reported [104].

Rare cases of parathyroid cysts have been reported in children [105, 106].

About 95 % of these cysts occur below the inferior thyroid border and 65 % are associated with the inferior parathyroid glands. Cysts have been identified from the angle of the mandible to the mediastinum, however, and they can occur in the thyroid lobe or posterior [107].

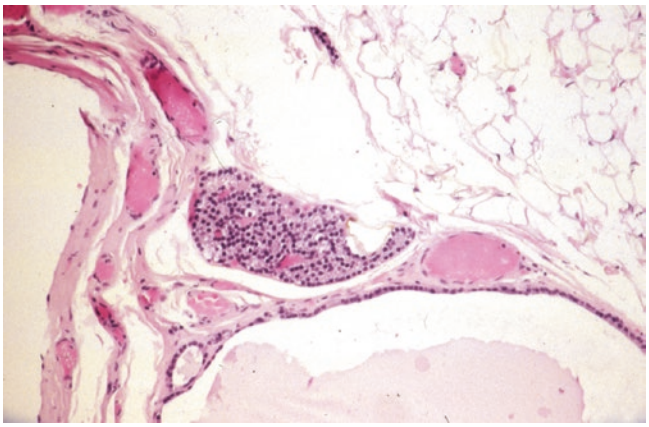

Fig. 9.11 Cervical parathyroid cyst lined by the cuboidal epithelium. The parathyroid tissue is present in the wall

Ultrasound examination, together with fine needle aspiration, is the principal diagnostic tool [106, 107]. Aspiration of clear fluid with an elevated parathyroid hormone level is a definite indication of a parathyroid cyst. The intact molecule of the parathyroid hormone should be assayed, because the N-terminal-specific assay is frequently associated with false-negative results [106, 108].

Microscopy Histologic studies show that a parathyroid cyst's wall is usually formed by a solitary layer of compressed cuboidal or low columnar epithelium, with either chief or oxyphilic cells present in the fibrous capsule (Fig. 9.11). Some cysts may not have any identifiable parathyroid tissue, but, even in these cases, a diagnosis can be established by testing the cystic fluid. Immunostaining for parathyroid hormone could be of help.

Treatment and prognosis Aspiration may be curative but persistence or recurrence of the cyst is a sign that surgical removal is in order. Functional parathyroid cysts are usually due to cystic degeneration of a parathyroidal adenoma or, more rarely, carcinoma [106]. In these latter cases, therefore, treatment is based on surgical removal of the lesion.

9.3.6 Cervical Bronchogenic Cyst

Definition A cervical cyst lined by pseudostratified columnar, ciliated epithelium of respiratory type.

Etiology and pathogenesis Bronchial cysts are derived from small buds of diverticula that separate from the foregut during formation of the tracheobronchial tree. When they occur outside the thoracic cavity, the cyst presumably arises from erratic migration of sequestered primordial cells.

Epidemiology Cervical bronchogenic cysts are uncommon congenital lesions found almost invariably in the skin or subcutaneous tissue in the vicinity of the suprasternal notch or manubrium sterni, rarely in the anterior neck or shoulder. Nevertheless, the site at presentation can be various, involving the whole head and neck area [109]. When they affect adult patients, they are more often located in the thyroidal and paratracheal region [110, 111].

Clinical aspect They are usually discovered at or soon after birth; in a recent series [109], the average age was 1.5 years (range from 5 days to 7 years), and they appear as asymptomatic nodules that slowly increase in size or as draining sinuses exuding a mucoid material. More rarely, they can produce various symptoms, such as dyspnoea, local pain and fever, as a consequence of airway compression or infection [110, 110]. They are more common in males, in some series by a ratio of 4:1 [111, 113]. Preoperative radiological imaging is useful for identifying the cyst location exactly and the relation with the surrounding tissues [37, 109, 111]. In addition, FNAC, showing a mucous background with ciliated cells, can be of help in the correct diagnosis [111].

Macroscopy The cysts range from 0.3 to 6 cm in size.

Microscopy They are lined by the ciliated, pseudostratified columnar epithelium (Fig. 9.12). If the cyst is infected, the squamous epithelium is found. The cyst wall contains smooth muscle, elastic fibres and seromucous glands. In the 30 cases studied by Fraga et al. [114], the smooth muscle was identified in 24 and seromucous glands in 16. In contrast to their intrathoracic counterparts, only two contained cartilage.

Differential diagnosis A bronchogenic cyst can be distinguished from a teratoma by a complete absence of tissues other than those that can be explained on the basis of a malformation. The lack of the ciliated epithelium distinguishes a lateral cervical cyst containing gastric mucosa from a cervical bronchogenic cyst. TDC can be differentiated from a bronchogenic cyst by finding thyroid follicles; furthermore, TDCs do not contain a smooth muscle or cartilage.

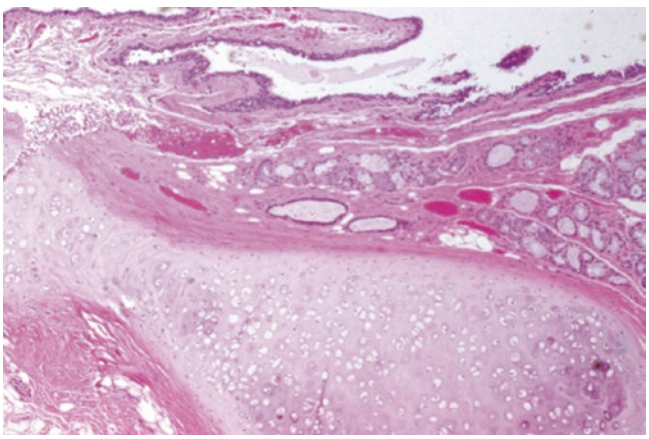

Fig. 9.12 Cervical bronchogenic cyst. The respiratory epithelium lines the cyst wall

Treatment and prognosis Complete surgical excision of a bronchogenic cyst along with its sinus tract is curative [43, 108]. One case of poorly differentiated adenocarcinoma arising in a bronchogenic cyst of the neck has been reported [115].

9.3.7 Dermoid Cyst

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Definition The term dermoid cyst should be reserved for a cystic neoplasm that originates from the ectoderm and mesoderm; the endoderm is never found in these cysts [116].

Epidemiology The head and neck area is a common site of occurrence for dermoid cysts, accounting for 34 % of cases.

Clinical aspects These cysts are located in the skin and subcutaneous tissues [117]. Most dermoid cysts affecting the head and neck region are located in the floor of the mouth; only 18 % are located in the neck area [118]. One case has been reported in the thyroidal region [119].

The position of these dermoid cysts at the midline and along the lines of embryonic fusion of the facial processes is consistent with their origin by inclusions of ectodermal tissue along lines of closure at the junctions of the bone, soft tissue and embryonic membranes [120].

Dermoid cysts in the neck account for 22 % of midline or near-midline neck lesions [116]. They have been described in the upper neck, near the thyroid cartilage, and as low as the suprasternal notch. They may occur at almost any age. More than 50 % are detected before the age of 6 years and approximately one third are present at birth [116, 117, 120]. The distribution between the sexes is approximately equal.

Macroscopy Dermoid cysts range in size from a few millimetres to 12 cm in diameter.

Microscopy On microscopic examination, they are lined by the stratified squamous epithelium supported by a fibrous connective tissue wall. Ectodermal derivatives may be seen, including dermal adnexa such as hair follicles, sebaceous glands and sweat glands.

Treatment and prognosis Surgical treatment is usually curative.

9.3.8 Unclassified Cervical Cyst

Some cysts may be difficult to classify because of apparent discrepancy between the anatomic site of presentation and the histologic features, indeterminate microscopic findings, loss of an intact epithelial lining or mixed histologic appearance. When a final determination about the type of cyst is not possible, the term "congenital or developmental cyst, indeterminate type," should be used [121].

9.3.9 Non-developmental Cysts

Most of the non-developmental cysts in the head and neck region occur in the jaw bones, oral cavity or parenchymal organs, such as thyroid gland, salivary glands and parathyroid glands. Mucoceles, ranulas and laryngoceles are considered non-developmental cysts that may occur in the neck. Ranulas are actually pseudocysts: they lack an epithelial lining. Because they mimic true cysts histopathologically as well as clinically or radiographically, however, it is reasonable and convenient to include them in a general discussion of cystic lesions. Mucoceles are discussed in the chapter on the pathology of the salivary glands.

9.3.9.1 Ranula

Definition A ranula is a special type of mucous retention cyst most commonly caused by partial obstruction of the excretory duct of the sublingual gland. Rarely, it may originate from the cervical sinus or from branchial cleft remnants.

Clinical aspects A ranula may be asymptomatic or present with symptoms related to compression or infection [122]. It produces a mass in the floor of the mouth to one side of the midline. Dissection of the mucus into the fascial planes of the neck results in a pseudocyst called plunging ranula. Simple ranulas are distinguished from mucoceles by their location and by the presence of an epithelial lining [123].

Microscopy A classic or simple ranula is a true cyst lined by a cuboidal, columnar or squamous epithelium and filled with mucoid material similar to that found in mucoceles. Inflammation is frequently detected (Fig. 9.13).

Differential diagnosis A plunging ranula may mimic other cystic or glandular swellings, such as dermoid and epidermoid cysts, TDC or cystic hygroma. Quick and Lowel [124] pointed out that no specific clinical diagnostic tests are available to distinguish these lesions. Consequently, a definitive diagnosis is dependent on postoperative histopathologic evaluation of the surgical specimen.

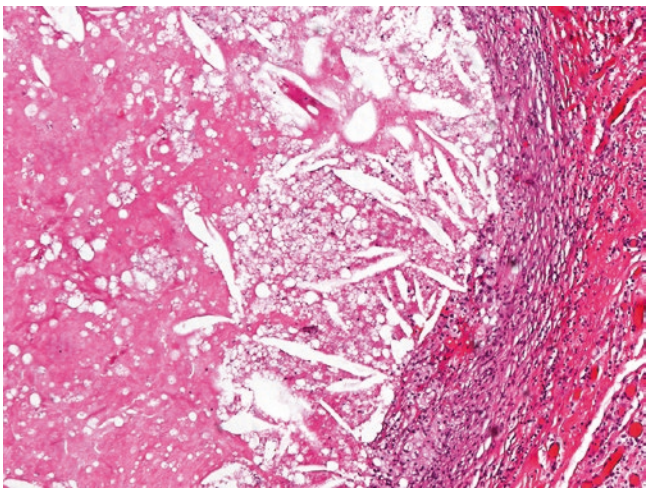

Fig. 9.13 Plunging ranula, associated with marked inflammatory changes

Treatment and prognosis The management of these lesions requires removal of the sublingual gland and excision of the ranula. The recurrence rate after this procedure is 0 %. Excision of only the ranula was followed by a 25 % recurrence rate, whereas marsupialization has a 36 % recurrence rate [125]. The recurrence rate is higher in cases of plunging ranula [122].

9.3.9.2 Laryngocele

Laryngocele is a dilatation of the ventricle of Morgagni or its appendages, filled with air or fluid [126–128]. For more details on this lesion, see Chap. 7.

9.4 Cystic Neoplasms

9.4.1 Cystic Hygroma and Lymphangioma

Definition Cystic hygroma and lymphangioma are the two ends of the spectrum of histopathologic classification of lymphatic lesions [129]. Whether these are true neoplasms or represent malformations or hamartomas is still debated but this issue is of no clinical consequence. These lymphatic lesions may be divided into three morphologic types based on the size of the vessels: capillary (lymphangioma circumscriptum), cavernous (lymphangioma cavernosum (Fig. 9.14a)) and cystic (cystic hygroma) [130]. The difference between cavernous lymphangioma and cystic lymphangioma (hygroma) is not clearly defined. Both are composed of dilated lymphatic vessels. Some consider them to be synonyms, while others define the latter as having macroscopically visible uni- or multilocular cysts. Cystic lymphangiomas seem to arise in locations with abundant loose connective tissue, allowing the lymphatic vessels to dilate and form large cystic spaces. The differentia-

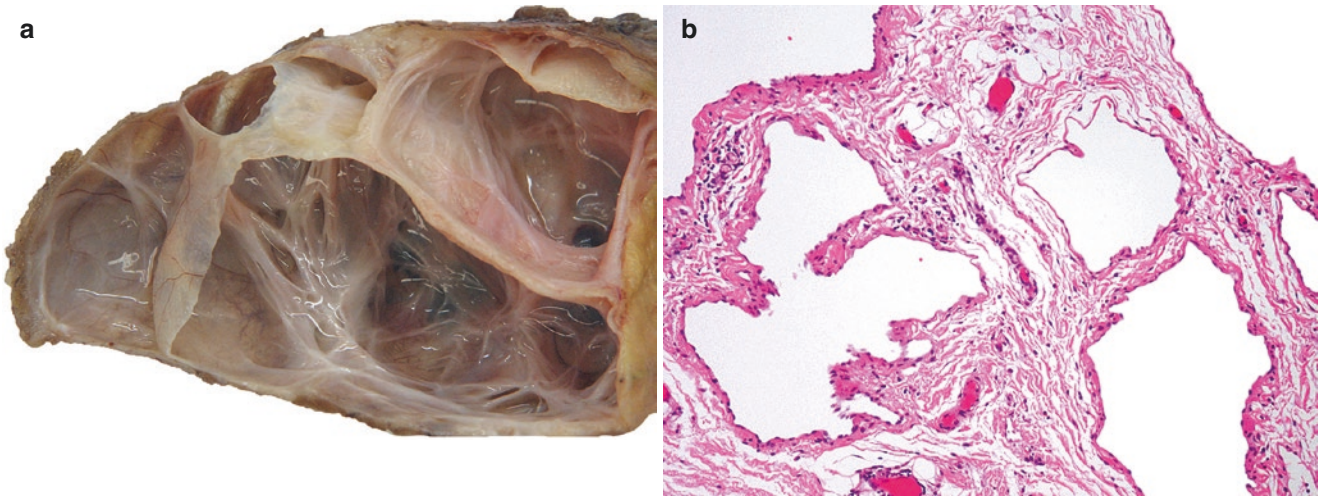

Fig. 9.14 Lymphangioma cavernosum (**a**). Macroscopic appearance: multiple cystic cavities with clear fluid. (**b**) Microscopically, cavernous lymphangioma is composed of dilated lymphatic channels, lined by flattened epithelium

tion of lymphangiomas on the basis of the size of the vessels has no clinical significance.

Epidemiology Lymphangiomas are relatively rare. Almost all lymphangiomas appear during the first 2 years of life, most commonly in the oral cavity, parotid gland, neck or axilla. Clinical aspects Lymphangiomas often lie in the lateral cervical region beneath the platysma, less frequently in the anterior cervical region, and may extend into the mediastinum. Symptoms at presentation are related to the mass effect and can comprise respiratory failure and brachial plexus compression [131, 132].

Cervical cystic hygroma (hygroma colli cysticum) is a cystic lymphangioma of the neck. It can be associated with foetal hydrops and Turner syndrome [133]. They may be detected in utero and are associated with a poorer prognosis than cystic hygromas arising as an isolated abnormality. Microscopy Lymphangiomas consist microscopically of lymphatic channels of variable size and shape lined by typical endothelial cells (Fig. 9.14b). Focal infiltrates of lymphocytes in the stroma are common.

Differential diagnosis The main differential diagnosis of lymphangiomas of the head and neck is cavernous hemangioma. Lymphangiomas contain proteinaceous fluid and thin valves, and the surrounding tissue is usually infiltrated by lymphocytes, whereas cavernous hemangiomas are filled with red blood cells and lack valve structures.

Treatment and prognosis Treatment of lymphangioma is problematic, especially in the case of large ones because of their tendency to be poorly demarcated, to be infiltrative and to involve vital structures. Presently, most cases are treated with surgery and/or sclerotherapy [134]. They may recur if not completely excised; the reported incidence rates are between 15 and 80%. A staging system for patients with lymphatic malformations of the head and neck has been proposed, based on their anatomic location and the extent of the disease, which might help to predict prognosis and the outcome of surgical intervention [133]. Nevertheless, since no agreement has been reached on the best way to treat lymphangiomas, new therapeutic approaches are under evaluation [134, 135].

9.4.2 Hemangioma

Hemangioma are a heterogeneous group of vascular lesions commonly located in the head and neck. Most hemangiomas in this region are superficial; however, they may arise within the skeletal muscle and involve the parenchymal tissue such as salivary glands and the thyroid gland. Hemangiomas are classified by morphology into capillary, cavernous, arteriovenous, venous and epithelioid types [136]. Of these types, cavernous is the one that most often simulates a cyst. Other types more often manifest themselves as solid lesions. Congenital cervical hemangiomas are usually located in the posterior side of the neck, and their diagnosis can be performed by ultrasound prenatal examination [137]. For more extensive discussion of hemangiomas see Chapter 12.

9.4.3 Teratoma

Definition Teratomas are neoplasms composed of elements from more than one of the three germ layers (ectoderm, endoderm and mesoderm).

Epidemiology Cervical teratomas represent only about 3 % of all teratomas. On rare occasions, a mediastinal teratoma can present with a cervical cystic mass [138].

Clinical aspects In the head and neck region, lesions are also found in the central nervous system, orbit, temporal fossa, oropharynx, oral cavity, nasopharynx, nasal cavity, palate and tonsil [139].

Teratomas arising in the cervical region are rare. Although they were previously divided into those arising from the thyroid gland and those arising elsewhere, this distinction has not proved to be clinically useful. The most significant clinical marker divides tumors presenting in infancy or early childhood from those presenting after the first decade of life.

Teratomas presenting in infancy or early childhood exhibit primarily benign clinical behaviour. However, such lesions are associated with a high mortality rate at the time of birth, generally because the airway and pulmonary function are compromised. Diagnosis of congenital cervical mass is possible through ultrasound examination. In addition, to reduce mortality due to airway obstruction, a new therapeutic approach has been described, called ex utero intrapartum treatment (Exit). It consists of a multidisciplinary approach to the foetus, trying to guarantee airway patency at the beginning of extrauterine life [140].

Teratomas presenting after the first decade of life are usually smaller and more likely to be malignant [141, 142].

Various systems of classification for teratomas have been proposed. The majority of these were considered by Gonzalez-Crussi [143], who presented a tentative new classification system for all teratomas that does not rely on the primary site of occurrence of the tumor.

Macroscopy On gross examination, these tumors are usually cystic, but they can be solid or multiloculated. They are commonly encapsulated, lobulated masses that measure up to 15 cm in the largest dimension.

Microscopy On microscopic examination, cervical teratomas are similar to those found in other anatomical regions. They may contain the skin, hair, fatty tissue, central nervous tissue, cartilage, bone and components of the respiratory or digestive tract (Fig. 9.15). Areas of a more immature or embryonic tissue may be present (Fig. 9.16).

It is exceedingly important to sample all potentially teratomatous tumors adequately. Specifically, solid areas with necrosis or haemorrhage should be carefully examined. It is not unusual to find, in teratomas throughout the body, small foci of malignant germ cell tumors, especially endodermal sinus tumor or choriocarcinoma. The presence of either of these two tumor types adversely affects the prognosis. It is also important for the pathologist to recognize that more immature foetal tissues have malignant potential [73, 141, 142]. Patients with these tumors require especially close clinical follow-up.

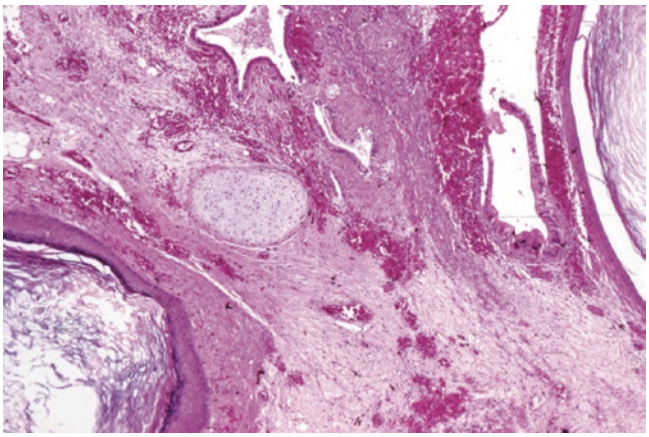

Fig. 9.15 Mature teratoma. Cysts lined by the squamous and respiratory epithelium. Cartilage is present

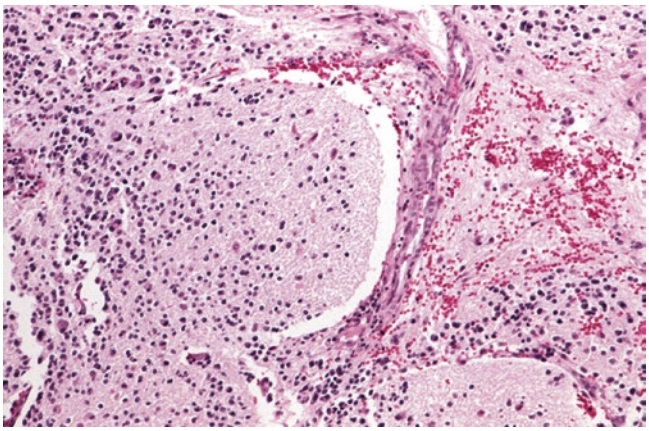

Fig. 9.16 Immature teratoma composed of primitive neuroectodermal tissue

Differential diagnosis Teratoma should be differentiated from the rare condition named foetus in foetu (FIF). Teratomas are characterized by unorganized admixture of different mature tissues. In contrast, in FIF the different tissues are organized and the vertebral axis is usually well formed. FIF is a rare condition and one case only has been described in the neck [144].

Treatment and prognosis Most authors strongly favour operative management of teratomas [141, 142]. When malignant components are found in a teratoma, the patient may need chemotherapy and/or radiotherapy in addition to surgery.

9.4.3.1 Malignancy in Teratoma

Cervical teratomas in a neonate are almost always benign, whereas the few reported cases of cervical teratoma arising in adults have been malignant [143]. To the best of our

knowledge, only rare cases of congenital cervical teratoma with metastasis have been reported [142]. Congenital cervical teratomas may present areas of immature tissue [145–147]. Malignancy in head and neck teratomas has been more frequently described in cases affecting the naso-facial region [148]. Resection seems to offer the best control in cases with aggressive biologic behaviour [145–147].

9.4.4 Heterotopic Salivary Gland Tissue and Salivary Gland Cystic Neoplasms

9.4.4.1 Heterotopic Salivary Gland Tissue

Definition The heterotopic normal salivary gland tissue (HSGT) is defined as the presence of the normal salivary gland tissue outside the usual location of the three major and minor salivary glands [149–151].

Epidemiology HSGT is a rare condition that can affect several sites of the head and neck region [150–152].

Clinical aspects In the neck, the most frequent site is the anterior border of the sternocleidomastoid muscle. It can appear as an accessory salivary gland, salivary gland tissue related to anomalies of the branchial apparatus or true HSGT [149].

HSGT most frequently presents at birth or during infancy [151], although it can sometimes be diagnosed later, during adult life. Symptoms are usually those of a draining sinus, sometimes associated with a small nodule. Colourless, saliva-like fluid is produced; secretion can become apparent in relation to a meal. On histology, HSGT is characterized by a normal serous-mucinous salivary gland with an associated duct opening onto the overlying epidermis [149, 151]. On rare occasions it can be bilateral [152]. In addition, Hsu et al. [153] reported a family with HSGT affecting three generations. However, this is the only hereditary case of HSGT presently described.

Treatment and prognosis Surgical excision is recommended to avoid infections and neoplastic transformation.

9.4.4.2 Cervical Salivary Gland Cystic Neoplasms

Definition These are cystic-like lesions consisting of the heterotopic normal salivary gland tissue or neoplasms arising in cervical lymph nodes. All the spectrum of benign and malignant tumors affecting the salivary glands can also develop from HSGT.

Epidemiology Tumors arising from HSGT are more commonly benign, with pleomorphic adenoma and Warthin tumors being the most frequent [150, 154–156]. Among malignant tumors, mucoepidermoid, adenoid cystic and acinic cell carcinomas are the most frequently reported [150]. Rare cases of carcinoma arising in pleomorphic adenoma are on record [157].

Clinical aspects This type of neoplasm presents as a painless mass, often cystic, located in the periparotid region, the upper neck or the anterior cervical triangle. Occasionally, however, these tumors have been described in the lower neck [158, 159]. In most cases, they affect adult patients. In the series reported by Zatchuz et al. [159], the age of the patients ranged from 10 to 81 years, with a mean of 45 years. Females were affected more commonly than males, with a ratio of 3:1 [159].

Microscopy Tumors that more often arise in the ectopic salivary gland tissue in the lymph nodes and simulate cysts are Warthin tumors and sebaceous lymphadenomas. Other rare types of salivary gland tumors that may resemble cervical cysts are dermal analogue tumors, mucoepidermoid carcinomas and acinic cell carcinomas [160, 161]. A diagnosis of salivary gland tumor arising from HSGT should be taken into consideration when evaluating fine needle aspiration material from neck masses. The pathology of these lesions is discussed in the chapter on salivary gland tumors.

Treatment and prognosis Surgical excision is the treatment of choice. Excision of the adjacent salivary gland may appear to be the appropriate treatment to define the site of the primary tumor, because malignant salivary tumors located within the lymph nodes suggest metastatic disease. In cases of malignant tumors, the prognosis depends on the tumor type and radical surgery. In a series reported by Daniel and McGuirt [150], one out of six patients with malignant tumors died of the disease.

9.4.5 Cervical Thymomas

Cervical thymomas are classified as being of four types: (1) ectopic hamartomatous thymoma, (2) cervical thymoma, (3) spindle epithelial tumor with thymus-like differentiation (SETTLE) and (4) carcinoma showing thymus-like differentiation (CASTLE). Of these, the first is benign, while the second can be locally aggressive. The third and fourth types are malignant [162].

Ectopic thymoma most frequently involves the thyroid [163] or the parotid gland, but examples of cervical thymoma [164–166] and thymic carcinoma [167] arising along the route of descent of the thymic tissue are on record. Ectopic cervical thymoma more often presents as a neck mass affecting adult female patients [166]. On rare occasions, it has been associated with myasthenia gravis [168] and with immunodeficiency and hypogammaglobulinemia

(Good syndrome) [169]. On histology, ectopic thymoma presents the same morphological spectrum as mediastinal thymoma [166]. This diagnosis should be kept in mind in cases of spindle cell tumors arising in the neck region, especially when fine needle aspiration cytology is performed [166, 170, 171].

9.4.6 Other Lesions

Other tumors that may appear as cervical cysts are cystic neurogenic neoplasms. In the neck, the most common location for neurinomas (schwannomas) with cystic degeneration (Fig. 9.17) is along the course of the vagus nerve or the cervical sympathetic chain.

Infectious processes often simulate cervical cysts. Such infections can be bacterial, fungal, parasitic or viral [88, 170, 173]. Amyloidosis and carotid artery aneurysms have been reported to mimic cervical cystic tumors [174, 175].

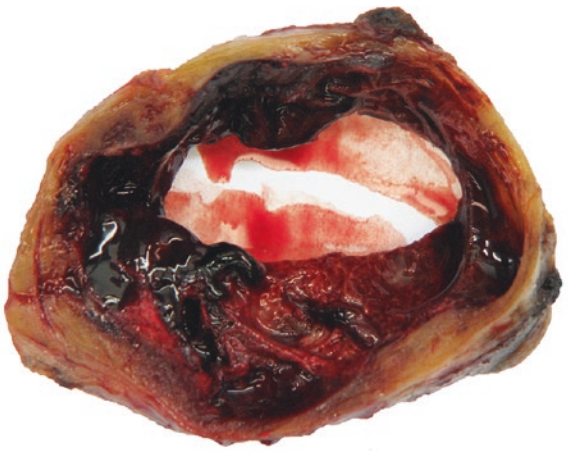

Fig. 9.17 Neurinoma (schwannoma), macroscopically presenting as a cystic, haemorrhagic neoplasm

9.5 Unknown Primary and Secondary Tumors

Definition The term "cancer of unknown primary site" (CUP) represents a heterogeneous disease entity characterized by the presence of lymphatic and/or haematogenous metastases and an inability to identify the primary tumor. In the head and neck, CUP mostly refers to metastases to the cervical lymph nodes, which may represent regional or distant metastatic disease. Metastases can also be found in various extranodal sites of the head and neck, such as the oral cavity, pharynx, salivary glands, larynx, bones, nasal cavity and paranasal sinuses, orbit and eye, skin, etc. They are described in other chapters.

Clinical features An enlarged cervical lymph node is often the first clinical manifestation of a neoplastic process in the head and neck. Cervical lymph node metastasis is the presenting symptom in 25 % of patients with cancer in the oral cavity or the pharynx, in 48 % of patients with nasopharyngeal carcinoma and in 23 % of patients with thyroid carcinoma. In approximately 10 % of patients, a primary tumor cannot be found, even after a thorough search (Table 9.2) [180–185].
A typical patient with metastatic tumor in the cervical lymph nodes is a man, older than 50 years, who smokes and drinks alcohol heavily. Patients with HPV-positive metastatic tumors present at a younger age and are frequently non-smokers [186]. They all usually present with a painless node larger than 2 cm along the jugular chain or the supraclavicular fossa. Various groups of lymph nodes can be affected by metastatic neoplasms, but the most frequently involved are the upper jugular (71 %), the mid-jugular (22 %), the supraclavicular (18 %) and the posterior cervical nodes (12 %). Approximately 14 % of patients with such disease have more than one lymph node group affected by metastases, and 10 % have bilateral lymph node metastases [180, 185].

Table 9.2 Location of lymph node metastasis and predominant sites of their primary tumors

Lymph node region affected by metastasis	Predominant site(s) of primary tumor
Sublevel IA (submental)	Anterior floor of the mouth, anterior oral tongue, anterior mandibular ridge, lower lip, skin of the face
Sublevel IB (submandibular)	Oral cavity, anterior nasal cavity, midface, submandibular gland
Sublevel IIA (upper jugular)	Waldeyer's ring, oral cavity, nasal cavity, oropharynx, supraglottis, floor of the mouth, pyriform sinus, larynx, skin
Sublevel IIB (upper jugular)	Anterior tongue, nasopharynx, tonsil
Level III (middle jugular)	Hypopharynx, base of tongue posterior pharyngeal wall, supraglottic larynx, inferior part of pyriform sinus, post-cricoid region
Level IV (lower jugular)	Hypopharynx, thyroid, subglottic larynx, oesophagus
Sublevels VA, VB (posterior cervical)	Nasopharynx, thyroid, oropharynx
Supraclavicular	Lungs (40 %), thyroid (22 %), GI tract (12 %), GU tract (8 %), all ENT regions (20 %), breast

Extracted from Refs. [176–179]
ENT ear nose throat, GI gastrointestinal, GU genitourinary

Lymphatic drainage of the head and neck region is highly predictable, and the location of the lymph node metastases may provide a clue to the location of the primary lesion. Patients with metastases in the upper and mid-level cervical lymph nodes are likely to have a primary cancer of the head and neck. In contrast, those with involvement of the lower cervical lymph nodes are more likely to have a primary tumor of the lungs, breast, gastrointestinal tract or genitourinary tract. Not all tumors metastasize according to these rules, and the possibility of an unusual metastatic pathway must be considered in the differential diagnosis. Table 9.2 shows the probable primary sites by region of metastasis [176, 185, 187].

Search for the primary tumor If the search is conducted systematically, the primary cancer can be discovered in 75–90 % of patients presenting with cervical lymph node CUP. Evaluation protocols have been outlined by several authors [182, 188], but there is poor consensus on the extent of the necessary diagnostic evaluation. Routine workup usually includes physical examination, CT or MR of the head and neck, CT of the chest and panendoscopy of the upper aerodigestive tract, with or without random biopsies. With advances in upper aerodigestive tract examination, the detection rate of the primary cancer is increasing. Introduction of PET/CT procedures has led to a better definition of the primary tumor site [189], even if the real diagnostic value is still under debate [182]. More recently, the application of transoral robotic surgery (TORS) for the resection of the base of the tongue [190, 191] has led to the identification of the primary tumor in about 90 % of the cases.

The success rates of ultimate detection of occult primaries (reported by various authors) vary between 10 and 90 % [181–183, 191]. As might be expected, occult primary tumors are significantly less likely to be detected in patients treated with radiation. Nearly 50 % of primary carcinomas originally considered occult, which are eventually found in patients after treatment of the cervical lymph node, are located in the region of Waldeyer's ring [50, 51]. The tonsillar fossa and base of the tongue are the two most common sites of origin [182, 191]. Primary cancer may be bilateral or contralateral to the neck metastasis [182]. Of originally unknown primary tumors found below the clavicle, the largest number is in the lungs, followed by the gastrointestinal tract.

In cases in which the primary tumor is not found, the most likely explanations are that either the primary lesion is so small that it cannot be detected clinically or it has regressed spontaneously. The former explanation is supported by autopsy studies that have shown that small clinically undetectable primary tumors are found in approx. 75 % of patients with CUP [192], whereas the latter consideration is speculative, since spontaneous regression cannot be proven.

Fig. 9.18 Metastatic melanoma in a cervical lymph node, macroscopically presenting as a black nodule

Macroscopic features Lymph nodes with metastases vary in size, ranging up to 10 cm in the largest diameter or more. There are a few macroscopic features that are important and may suggest the origin of metastases. For example, in cases of cystic metastases, the primary tumor is most frequently located in the palatine or lingual tonsils [43, 51, 183]. Another source of cystic metastases is papillary carcinoma of the thyroid gland [193]. The macroscopic appearance in other situations is variable; it is mostly nonspecific and is not helpful in determining the site of the primary tumor. Metastases may appear white-grey or yellow, occasionally haemorrhagic. Metastatic melanoma may exhibit a deeply pigmented appearance (Fig. 9.18).

9.5.1 Histological Type of Metastases and Immunohistochemical Analyses

One of the most important steps in diagnostic procedures in patients with CUP is histological assessment of the lesion. This can be achieved either by fine needle aspiration biopsy or excisional biopsy of the affected lymph node. The histologic typing of metastatic tumors is mainly based on light microscopic and immunohistochemical analyses, but other methods can be used, such as histochemical stainings, electron microscopy and molecular genetics.

Almost any histological type of malignancy can present as a metastasis to the cervical lymph nodes: carcinomas (squamous cell carcinoma, adenocarcinoma, neuroendocrine carcinoma), malignant melanoma, sarcoma, lymphoma, germ cell tumors and even gliomas. The first step is to classify the CUP, which in the majority of cases in the cervical lymph nodes are SCC and can be diagnosed from tissue sections stained with H&E. In less differentiated tumors, immu-

nohistochemistry is mandatory, classifying CUP into major subtypes: carcinoma, lymphoma, melanoma and sarcoma [194], using an initial screening panel of antibodies against a wide spectrum of cytokeratins (CK), common leukocyte antigen, S100, HMB45 and desmin. Only carcinoma, as the most frequent metastatic cancer of the head and neck, will be further discussed, while other tumors are dealt with in other chapters.

9.5.2 Squamous Cell Carcinoma

Squamous cell carcinoma (SCC) is by far the most common tumor type in the head and neck. In cases of metastases of an unknown primary tumor to the cervical lymph nodes, 80–85 % are of this histological type (Table 9.3). The diagnosis is based on demonstration of desmosomes and/or keratinization. In addition, immunohistochemical expression of CK 5/6 and p63 supports the diagnosis of SCC.

The origin of metastatic SCC can rarely be determined. However, there are some features that might be helpful. Cystic metastases composed of non-keratinizing SCC frequently originate from the oropharynx, particularly from the lingual or palatine tonsil [43, 50, 51]. Metastases from the tonsils are often unicystic, whereas those from the tongue are more often multicystic [43]. Since primary carcinomas tend to be located deep in the tonsils, tonsillectomy rather than biopsy is needed to demonstrate the primary neoplasm [195]. Poorly differentiated non-keratinizing carcinomas originating from the oropharynx are often HPV-related, and it is now widely accepted that the demonstration of the HPV genome in neck metastases indicates an oropharyngeal origin (Fig. 9.19a–d) [196].

In metastatic keratinizing SCC, regardless of differentiation, the site of the primary tumor cannot be suggested by its morphology alone. The location of the node can, however, be a clue to the location of the primary neoplasm (Table 9.2). In addition, the cytokeratin pattern may be of some help in determining the origin of the metastasis. SCC of the upper aerodigestive tract are positive for cytokeratins 5/6, 10, 13,14,17 and 19, whereas SCC of the lung are positive for cytokeratins 5/6, 12 and 14 in 100 % of cases and cytokeratins 17, 8/18 and 19 in 80 % of cases. Fewer than 4 % of cases are positive for cytokeratins 7 and 20. Furthermore, thyroid transcription factor 1 (TTF-1) is positive in 10–37 % of pulmonary SCC [197].

Table 9.3 Frequency of histological type of metastases from unknown primary tumors

Cervical	Supraclavicular
Squamous cell carcinoma	Adenocarcinoma
Undifferentiated carcinoma	Squamous cell carcinoma
Melanoma	Undifferentiated carcinoma
Thyroid carcinoma	Thyroid carcinoma
Adenocarcinoma	Prostate carcinoma
Salivary gland carcinoma	Sarcoma

Extracted from Refs. [177, 181, 188]

9.5.3 Adenocarcinoma

The most common location of metastatic adenocarcinomas in the neck is the lower regions, and the primary neoplasms are usually located in the thyroid, lung, gastrointestinal tract or prostate. In the upper and middle neck, on the other hand, the primary lesions are often located in the sinonasal tract and salivary glands.

Metastatic adenocarcinomas from different origins tend to have similar microscopic appearances. Immunohistochemistry is therefore needed to further delineate the adenocarcinoma's profile and to predict the possible origin of CUP. Dennis et al. [198] demonstrated that using ten different immunohistochemical markers (prostate-specific antigen (PSA), TTF-1, CDX2, CK 20, CK 7, gross cystic disease fluid protein 15 (GCDFP-15), oestrogen receptor, CA125, mesothelin and lysozyme), the site of origin can be correctly classified in 88 % of metastatic adenocarcinomas.

Staining for cytokeratins might be helpful: CK 7 is widely expressed in pancreatic, breast, lung, biliary tract and transitional cell carcinoma, whereas CK 20 is expressed in gastrointestinal carcinomas, particularly in the colon, and transitional cell carcinomas.

In some cases, immunohistochemistry may help to determine the origin of metastatic adenocarcinoma. For example, metastatic adenocarcinoma of the prostate, which may present as metastasis in the left neck, especially in the supraclavicular nodes, can be confirmed by using the PSA and prostatic acid phosphatase immunohistochemistry (Table 9.4). It should be kept in mind that salivary gland tumors may occasionally express PSA [208]. Thyroglobulin, calcitonin and TTF-1 are useful markers to probe the thyroid origin of a neoplasm of unknown origin [197].

The presence of oestrogen receptors, mammaglobin and gross cystic disease fluid protein 15 (GCDFP-15) would suggest a breast origin of adenocarcinoma. Lung adenocarcinomas are positive for TTF-1 and CK 7 and negative for CK 20 (Table 9.4) [197].

Metastatic adenocarcinomas from the colon express CDX2 and CK 20. Cancer with similar morphology can arise in the sinonasal region; they are CK 20 positive, like their counterparts of colonic origin [199].

9.5.4 Undifferentiated Carcinoma

In cases of undifferentiated carcinoma, nasopharyngeal type carcinoma and sinonasal undifferentiated carcinoma

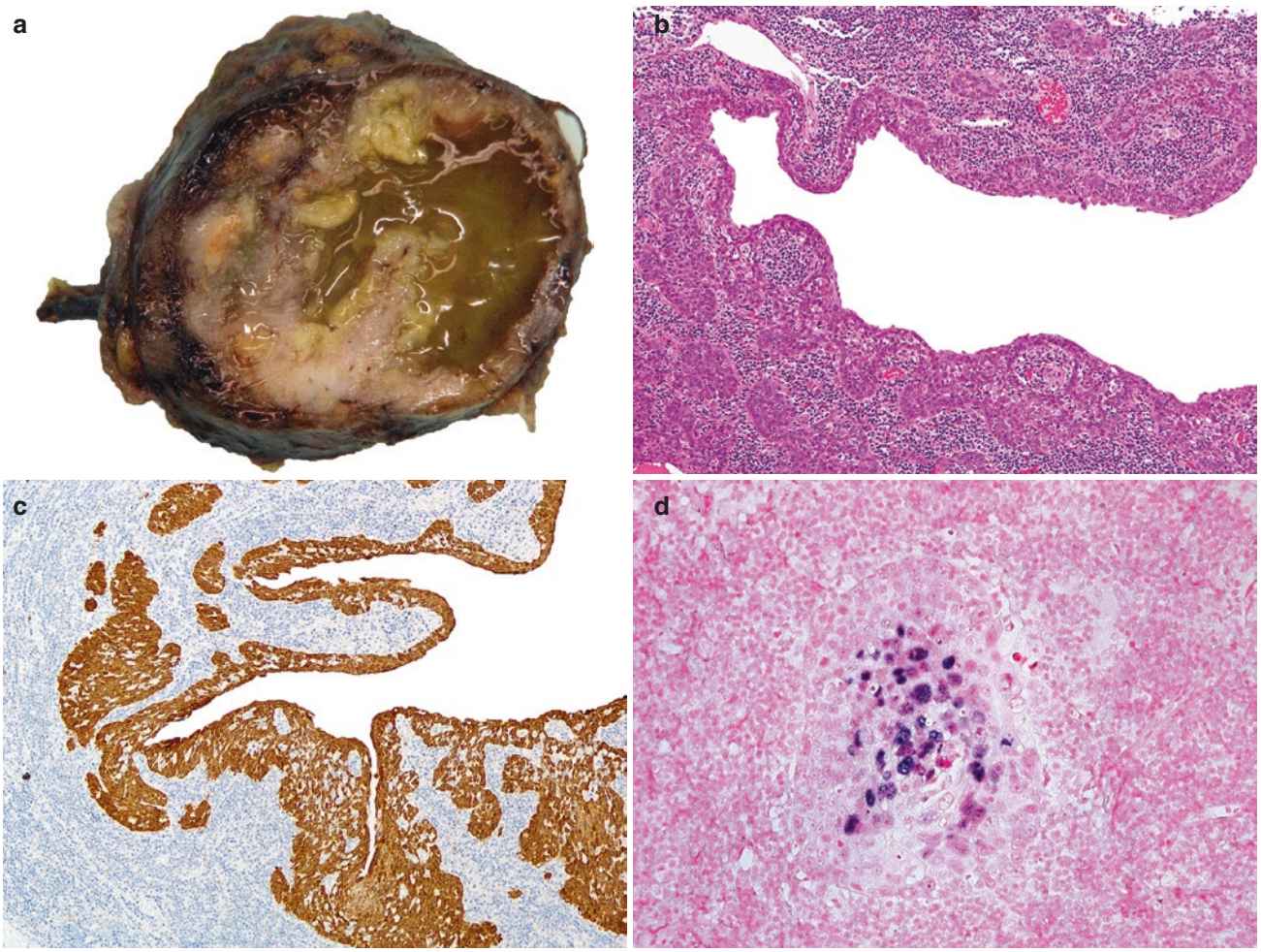

Fig. 9.19 Metastasis of squamous cell carcinoma of the palatine tonsil in a cervical lymph node. (**a**) Macroscopically, metastasis presents as a cystic lesion, with a whitish tumor tissue in the cyst wall. (**b**) Microscopic appearance: a cystic metastasis with islands of non-keratinizing squamous cell carcinoma. (**c**) Immunohistochemistry for p16: a strong positive reaction in tumor cells. (**d**) Positive in situ hybridization for HPV in tumor cells

(SNUC) must be considered. Metastases of nasopharyngeal carcinomas are usually located in the posterior neck, whereas metastases from sinonasal undifferentiated carcinoma (SNUC) are present in the upper and mid-cervical regions. If the metastases are located in the lower neck, the lung is the most likely source. In addition, testing for the presence of Epstein-Barr virus (EBV) by in situ hybridization is helpful. The presence of EBV in the tumor cells is highly suggestive of a nasopharyngeal origin of the tumor (Fig. 9.20a, b), whereas SNUC does not express EBV [200].

The pattern of cytokeratin expression is significantly different in SNUC and nasopharyngeal carcinomas, which can be a diagnostic aid. Franchi et al. [201] demonstrated that SNUC expresses CK 8 in 100 % of cases and CK 19 and CK 7 in 50 % and is negative for CK 5/6, while nasopharyngeal type carcinoma expresses CK 5/6 and CK 13 in 90 % of cases and is negative for CK 7.

9.5.5 Neuroendocrine Carcinoma

If a CUP exhibits neuroendocrine differentiation, Merkel cell carcinoma or small cell neuroendocrine carcinoma, from the lung or larynx, is the most likely diagnosis. Merkel cell carcinomas are CK 20 positive [202], and pulmonary small cell carcinomas are CK 20 negative and TTF-1 positive in 83–100 % of cases [197]. It should be kept in mind that, rarely, Merkel cell carcinomas can arise in a lymph node [209].

9.5.6 Spindle Cell Tumors

Metastatic spindle cell neoplasms most likely originate from spindle cell carcinoma, melanoma or sarcomas. Pankeratin, MART-1, HMB-45, S-100 protein, desmin, smooth muscle actin and myogenin are some immunostains

that help to distinguish these neoplasms [203]. In addition, primary spindle cell lesions arising in the lymph nodes, such as Kaposi's sarcoma, presumed tumors of the reticulum cell lineage and benign intranodal myofibroblastomas [204, 210], must be distinguished from metastatic spindle neoplasms.

Table 9.4 Immunohistochemical approach to metastasis of unknown primary tumor

Cytokeratin positive	
Keratinizing squamous cell carcinoma	CKs 5/6, 7, 20 and p63
Non-keratinizing squamous carcinoma	CKs 5/6, 7, 20 and p63
	EBV[a], HPV, p16[a]
Adenocarcinoma	CKs 7, 20
	TTF-1
	Thyroglobulin
	Females: GCDFP-15, WT-1, CA 125
	Males: PSA. Young males: AFP, HCG
Undifferentiated carcinoma	CKs 7 and 20
	Synaptophysin
	Chromogranin
	EBV[a]
Cytokeratin negative	
Various tumors	S-100 protein
	HMB 45
	Melan A
	Desmin
	Lymphoma markers
	Sarcoma markers

Extracted from Refs. [188, 197, 199–205]
[a]In situ hybridization and molecular procedures can be performed in fine needle aspirates also [206, 207]

9.5.7 Electron Microscopy

When immunohistochemical analyses are inconclusive, demonstration of specific ultrastructural features, e.g. neuroendocrine granules, premelanosomes, surface microvilli, intracellular lumina, desmosomes and tonofilaments by electron microscopy, may enable the correct diagnosis [211].

9.5.8 Molecular Genetics

Better insight into the etiopathogenesis of head and neck tumors has enabled the introduction of specific molecular diagnostic tests into routine practice. Detection of HPV 16/18 and EBV by in situ hybridization or PCR is nowadays widely used in routine work, suggesting an origin from the palatine or lingual tonsils in HPV-positive CUP and from the nasopharynx in EBV-positive CUP [212].

New genetic tests are being developed, some of which are commercially available. The aim is to use cancer mRNA or micro RNA gene profiling to compare known primary tumors with CUP, since tumors generally still express some tissue-specific genes, not only in primary but also in metastatic tumors [213]. The sensitivity of available gene expression-based assays for classifying CUP is claimed to be between 80 and 90 %. Some preliminary results seem promising, suggesting that cancers from different anatomic sites of the upper aerodigestive tract may have different genetic alterations allowing distinction between different origins within this region [214]. However, genetic tests are fairly complex and expensive for routine daily practice. It is hoped that in the future they will provide additional clinically important information for patients with CUP not adequately classified

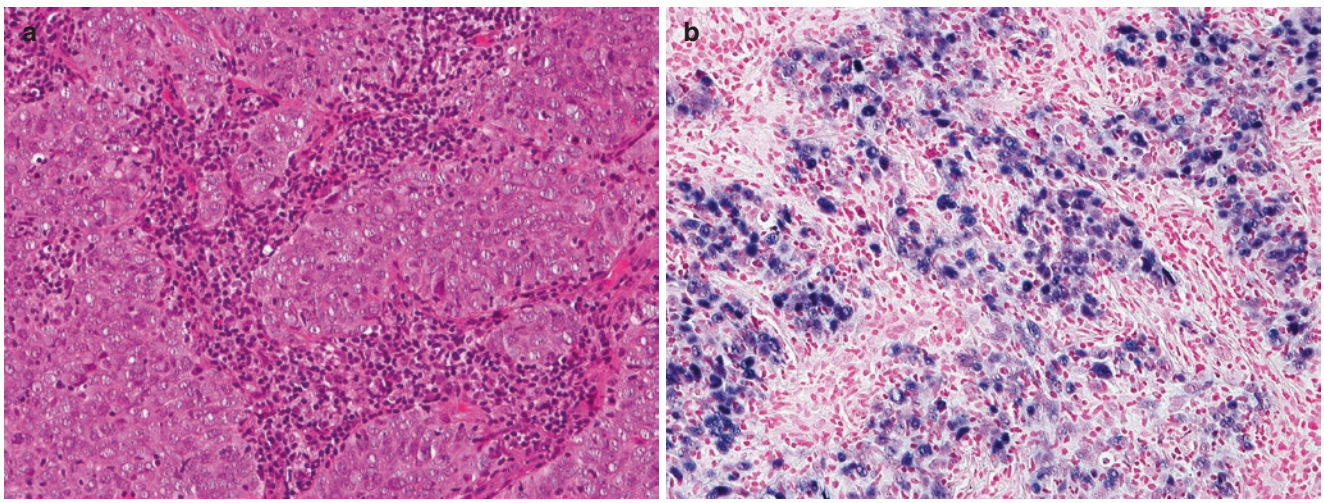

Fig. 9.20 (**a**) Metastasis of undifferentiated carcinoma of unknown primary in a cervical lymph node. (**b**) Positive in situ hybridization for EBV suggested origin in the nasopharynx, which was later confirmed by biopsy

by immunohistochemistry, thus increasing the rate of identification of primary sites [35].

9.5.9 Differential Diagnosis

Differential diagnosis in clearly malignant lesions has been described in previous sections. In addition, there are several benign non-neoplastic and neoplastic conditions in the lymph nodes that may mimic metastatic carcinoma. It is of vital importance that pathologists are well aware of them so that they are not mistaken for metastatic cancer. These benign conditions include glandular and nevus cell inclusions, primary tumors of the lymph nodes and cystic lesions of the head and neck. Microscopically, benign lesions lack atypia as well as desmoplastic stromal reaction, frequently seen in metastatic carcinoma.

Among benign glandular inclusions, heterotopic salivary gland inclusions are fairly frequently found in the cervical lymph nodes (Fig. 9.21a), particularly in the para- and intraparotid lymph nodes and less commonly in the upper cervical nodes [150]. Similarly, benign nevus cells have been found in the capsules of cervical lymph nodes [215].

In addition, benign thyroid inclusions can be present in the cervical lymph nodes. They are almost always discovered during pathological examination of lymph nodes surgically excised for another cancer. Features that may help to distinguish thyroid inclusions from metastatic thyroid carcinoma include the number and localization of thyroid follicles and cytoarchitectural characteristics. In the case of benign inclusions, the follicles are few, and they are located in the peripheral sinus and lack cytoarchitectural features of thyroid carcinoma (Fig. 9.21b).

Analysis of clonality may also be helpful in distinguishing benign inclusions from metastatic thyroid carcinoma [216]. It has been suggested that thyroid carcinoma in a lymph node (Fig. 9.22) does not necessarily represent metastasis from a thyroid gland primary but may also arise from thyroid inclusion (heterotopia) as a primary lymph node lesion [217].

Metastatic carcinoma in the lymph nodes must also be differentiated from neoplastic lesions, which rarely arise in the lymph nodes as primary tumors, for example, intranodal myofibroblastoma and salivary gland tumors. Intranodal myofibroblastoma is a benign spindle cell tumor with a favourable prognosis that must be differentiated from metastatic spindle cell tumors, such as melanoma, spindle cell carcinoma and sarcoma [183]. Among salivary gland tumors, Warthin tumor and, rarely, other tumors may arise as primary lesions in the lymph nodes, presumably from the heterotopic salivary gland tissue [150, 159, 160, 218].

Cystic metastatic SCC should be distinguished from benign cystic lesions lined by benign squamous epithelium, such as branchial cleft cysts, AIDS-related cystic lymphoid hyperplasia, benign lymphoepithelial cysts, thymic cysts and cystic cervical thymomas. In all these lesions, the bland appearance of the epithelium rules out metastatic SCC.

The differential diagnosis between cystic metastatic SCC and bronchogenic carcinoma has already been discussed in this chapter.

9.5.10 Treatment and Prognosis

Metastatic cancer in cervical lymph nodes from an unknown primary constitutes a favourable risk CUP group [219]. In terms of histological type of CUP, patients with SCC have

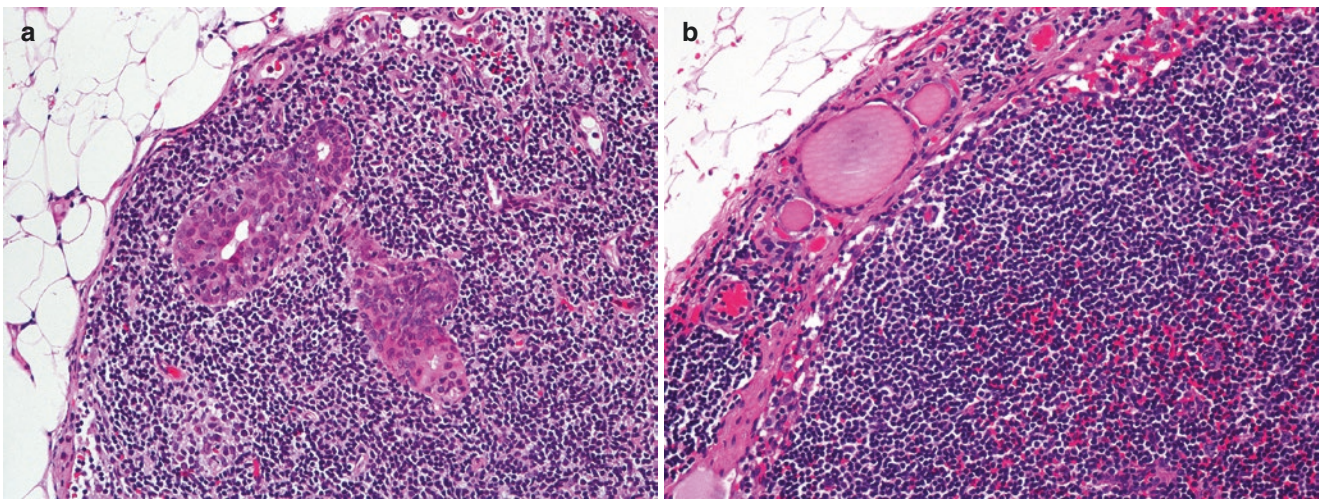

Fig. 9.21 Benign inclusions in a lymph node. (**a**) Salivary gland inclusions: ducts, lined by the hyperplastic epithelium, without atypia. (**b**) Thyroid inclusions: a few follicles in the peripheral sinus, lacking cytologic features of carcinoma

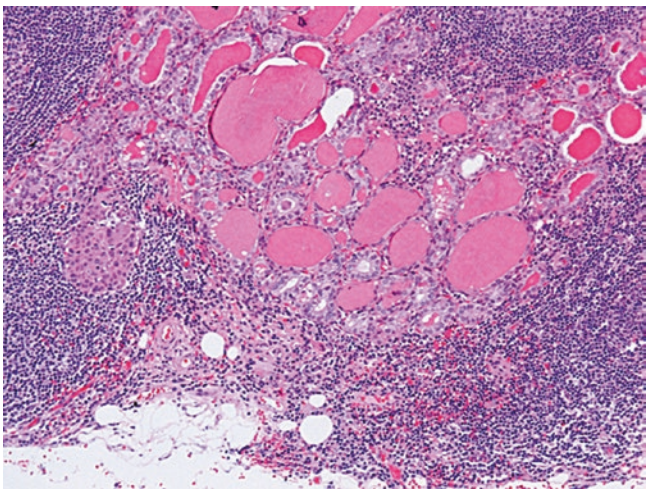

Fig. 9.22 Metastasis of papillary carcinoma of the thyroid gland (follicular variant) in a lymph node: numerous follicles within the lymph node parenchyma with cytologic features of carcinoma

the best and patients with adenocarcinoma have the worst prognosis [220]. The prognosis is better when the primary tumor is detected. In cases in which the primary tumor is not detected, the prognosis largely depends on the N stage. Factors that affect survival in these patients include the clinical stage of the neck, the extracapsular extension of tumor, the presence of recurrent or residual disease after treatment and the HPV status of CUP [221]. Balaker et al. [221] reviewed the literature on cases of neck metastases from primaries of unknown origin and reported 5-year survival rates ranging from 61.2 % in N1 cases to 26.6 % in N3 cases.

Treatment modalities are still controversial. For N1 disease, neck dissection or radiotherapy is recommended. For more advanced disease, intensive combined therapy is needed, either a combination of neck dissection and radiotherapy or initial chemo-/radiotherapy followed by neck dissection if a complete response is not achieved.

9.6 Neck Dissection

9.6.1 Classification of Neck Dissections

The American Academy of Otolaryngology – Head and Neck Surgery and the American Society of Head and Neck Surgery have classified neck dissection into four categories: radical, modified radical, extended and selective [3, 222, 223].

Neck dissection is classified primarily on the basis of the cervical lymph node groups that are removed and secondarily on the anatomic structures that may be preserved, such as the spinal accessory nerve, the sternocleidomastoid muscle and the internal jugular vein [3, 224].

The cervical lymph node groups are referred to using the level system as described by the Sloan-Kettering Memorial Hospital Group (Fig. 9.1). For more precise description of the anatomic boundaries of the different lymph node groups, see Chapter 17, Table 17.3) as well as the relevant literature [3, 225].

Radical neck dissection consists of the removal of all five lymph node regions of one side of the neck (levels I–V). This includes removal of the sternocleidomastoid muscle, the internal jugular vein and the spinal accessory nerve. Modified radical neck dissection refers to excision of all lymph nodes routinely removed by radical neck dissection, with preservation of one or more of the non-lymphatic structures (i.e. the spinal accessory nerve, internal jugular vein and/or sternocleidomastoid muscle).

The term extended radical neck dissection refers to a neck dissection that is extended to include either lymph node groups or non-lymphatic structures that are not routinely removed in a standard radical neck dissection.

Selective neck dissection is any type of cervical lymphadenectomy in which one or more of the lymph node groups that are removed in a radical neck dissection is preserved. The four subtypes of selective neck dissection are supraomohyoid, posterolateral, lateral and anterior [3, 222–224, 226].

The term "extended selective neck dissection (ESND)" has been recently introduced for patients who received chemoradiation preoperatively. It refers to procedures of selective neck dissection that include non-lymphatic structures, usually not removed [226] (Table 9.5).

9.6.2 Gross Examination of Neck Dissection Surgical Specimens

The presence of lymph node metastases is an important prognostic feature in head and neck cancer. Correct pathological management of the neck dissection specimen is therefore of outmost importance [228, 229]. The importance of the sentinel node in head and neck cancer is discussed in Chap. 17.

The following procedure pertains to standard radical neck dissections and needs to be modified for the other three types. When the main anatomic landmarks, such as the submandibular gland and internal jugular vein, are lacking in a neck dissection specimen, the surgeon must identify and label the lymph node groups. This is especially important in selective and extended neck dissections.

After the neck dissection specimen has been oriented as it appears in vivo, its overall dimensions are measured. The lengths of the sternocleidomastoid muscle and the internal jugular vein are measured separately. The jugular vein should be opened along its entire length. Tumor involvement, including thrombosis, should be noted, described and sampled adequately. The submandibular gland, the sternocleidomastoid muscle and the internal jugular vein should then be

Table 9.5 Updated classification of neck dissection

Type of dissection	Lymph node levels removed	Non-lymphatic structures resected
Radical neck dissection	I, II, III, IV, V	SCM, IJV, SAN
Modified radical neck dissection	I, II, III, IV, V	Preservation of one or more of the following: SCM, IJV, SAN
Selective neck dissection	Preservation of one or more of the following: I, II, III, IV, V. Brackets are used to denote levels or sublevels removed (e.g. SND {I, II, III})	None
	Differs according to the different sites of cancers. Includes level VI in cases of thyroid cancer	
Extended neck dissection	Resection of one or more of additional lymph node levels routinely not removed by the radical neck dissection (e.g. parapharyngeal)	Resection of one or more non-lymphatic structures routinely not removed by the radical neck dissection (e.g. carotid artery)
Extended selective neck dissection	Similar to selective neck dissection	Resection of one or more non-lymphatic structures

Extracted from Refs. [3, 222, 223, 227]. See also Chapter 17, Table 17.2

SCM sternocleidomastoid, IJV internal jugular vein, SAN spinal accessory nerve, SND selective neck dissection

divided and the node-containing fat separated into five levels: (1) sublingual and submandibular, (2) superior jugular, (3) middle jugular, (4) inferior jugular and (5) posterior. The presence of tumor in soft tissues, submandibular gland and muscle should be described. All lymph nodes visible and palpable are carefully dissected from the connective tissue with a rim of the perinodal connective tissue or fat. The number of lymph nodes (by level) should be noted; if a tumor is present, the actual size of metastases in centimetres and the presence of extracapsular extension are also noted and recorded. It is generally recognized that most masses larger than 3 cm in diameter are not single nodes but are confluent nodes or tumor in soft tissues. Midline nodes, if found, are considered ipsilateral nodes. The contralateral neck dissection is treated similarly. Nodes larger than 2–3 cm, when not macroscopically metastatic, should be completely submitted for histology. Smaller lymph nodes are submitted in toto. The tissue sections of lymph nodes submitted for processing should include a capsule of the lymph node, including a rim of the perinodal connective tissue or fat. If a group of matted lymph nodes is present, two or three sections through the nodes are often adequate to document the extent of the tumor. Tissue sections submitted for processing include all lymph nodes (by level), the submandibular gland, the sternocleidomastoid muscle and the internal jugular vein. If the neck dissection is of the extended type, sections of all extra lymph node groups and non-lymphatic structures that were removed should be submitted for tissue processing.

9.6.3 Histologic Evaluation of Neck Dissection

The major aim of the histologic evaluation of the status of lymph nodes in cases of carcinoma of the head and neck is to provide information required for staging the disease, planning further treatment and predicting patient outcome. The histologic evaluation also documents and confirms the pathologist's own gross evaluation of the dissected specimen. More importantly, increasingly important histologic parameters, such as the number, size and levels of positive nodes, the presence or absence of extracapsular spread, the presence of desmoplastic reaction, the presence of gross residual tumor and the presence of tumor emboli in intervening lymphatics, among others, can be assessed by thorough histologic evaluation. These histologic findings by themselves and in combination with other histologic parameters have been increasingly identified as important prognostic factors in disease control and survival, recurrence of neck disease and distant metastasis. The five factors that are currently indicators for adjuvant postoperative therapy can be reliably provided only by histologic evaluation; they are (1) the extranodal spread of disease, (2) the number of involved lymph nodes, (3) the number of involved lymph node regions, (4) the size of metastases and (5) the presence of desmoplastic reaction in metastatic disease [228–230].

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Eye and the Ocular Adnexa

10

Sarah E. Coupland and Marijke R. Van Dijk

Contents

S.E. Coupland, MBBS, PhD (✉)
Pathology, Molecular and Clinical Cancer Medicine,
Royal Liverpool University Hospital, The Apex Building,
Liverpool, Merseyside L78TX, UK
e-mail: s.e.coupland@liverpool.ac.uk

M.R. Van Dijk, MD
Department of Pathology, University Medical Centre Utrecht,
P.O. Box 85500, Utrecht 3508 GA, The Netherlands
e-mail: M.R.vanDijk-20@umcutrecht.nl

10.1 Introduction

10.1.1 Embryology

The embryology of the eye has been outlined in detail in classic texts, which the reader is referred to [1–3]. The embryonic development of the eye can be observed from the third week after inception when the optic sulci appear in the neural folds. The genesis of the eye is characterised by a complex expression of 'eye-field transcription factors' at the molecular level, regulating the development of the eye structures from the neuroectoderm, the surface ectoderm, and the mesenchyme.

Briefly, the primary optic vesicles develop bilaterally at the apical end of the neural folds and sulci in the fourth week

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of gestation as protuberances of the neuroectoderm. An invagination and detachment of the surface ectoderm form the lens vesicle. The secondary optic vesicle (optic cup) is formed between the fourth and seventh week of embryogenesis. The rim of the optic cup corresponds to the pupil. The non-pigmented epithelium of the ciliary body and the neurosensory retina develop from the inner leaf of the optic cup. The pigmented layers of the iris and ciliary body epithelium anteriorly and the retinal pigment epithelium (RPE) posteriorly develop from the outer leaf of the optic cup.

The formation of the primary optic vesicle is accompanied by the invagination of the hyaloid artery inferiorly into the developing optic cup. At the end of the sixth to seventh week after the hyaloid has penetrated into the optic cup, it closes. Incomplete closure of the optic cup can result in typical colobomas, which can be isolated to the iris or ciliary body but also to the retina/choroid and/or in the optic disc or in various combinations, depending on the stage of their occurrence (see below).

Congenital anomalies are developmental anomalies that are present at birth. Congenital malformations are caused by chromosomal abnormalities, mutant genes, and major environmental factors, such as infections, drugs, toxins, or radiation. In many cases, the causes are unknown. Detailed reviews of developmental anomalies associated with the eye are provided by other authors [1–3]. Notable development ocular anomalies are: synophthalmia/cyclopia, congenital cystic eye, uveal colobomas, and developmental abnormalities associated with chromosomal anomalies, e.g. Down syndrome (trisomy 21) and Patau syndrome (trisomy 13). Further, a variety of heritable disorders caused by genetic mutations have ocular manifestations, including aniridia caused by mutations in the *PAX6* gene; neurofibromatosis type 1 with a variety of ocular findings; tuberous sclerosis complex, often leading to astrocytomas and astrocytic hamartomas; von Hippel-Lindau disease, resulting in retinal hemangioblastoma and Sturge-Weber syndrome, leading to ipsilateral glaucoma and diffuse cavernous hemangioma of the ipsilateral choroid.

10.2 Anatomy and Histology

10.2.1 Conjunctiva

The conjunctiva is a thin and moist mucous membrane, covering most of the anterior surface of the eye and the inner surface of the eyelids. The conjunctiva derives from the ectoderm and can be distinguished as a surface lining distinct from the skin and cornea around the tenth week of gestation. It is composed of two to five layers of stratified columnar epithelium containing mucin-secreting goblet cells (Fig. 10.1). At the corneoscleral junction, the limbus, a gradual transition from stratified columnar to stratified squamous non-keratinized corneal epithelium is seen. At the palpebral margins, a relatively abrupt transition into the epidermis is present. The basal layer contains scattered regular melanocytes and Langerhans cells. The conjunctival stroma is composed of fibrous connective tissue containing fibroblasts, some chronic inflammatory cells, blood vessels, smooth muscle, nerves, and lymphatic channels. In the fornices, the conjunctival epithelium contains more goblet cells and the stroma can focally contain cartilage. Ectopic lacrimal gland tissue can also be present. In the medial interpalpebral area of the eye, a nodular fleshy mass is present: the caruncle. The caruncle is covered by stratified non-keratinized squamous epithelium and the subepithelial stroma contains sebaceous glands, hair follicles, and muscle fibres.

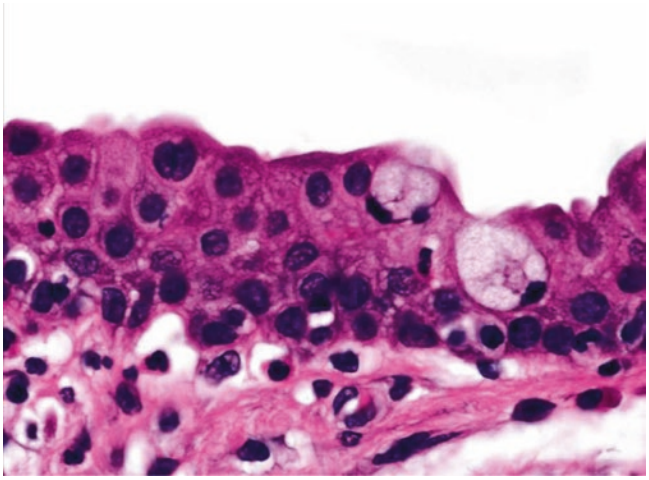

Fig. 10.1 Healthy conjunctiva with 4 layers of epithelium, scattered goblet cells, and occasional lymphocytes in the underlying lamina propria

10.2.2 Cornea

The cornea develops from the surface ectoderm, when the lens vesicle move into the optic cup (fifth gestational week), and neural crest-derived cells from the periocular mesenchyme migrate in front of it and later into the later cornea. The cornea is divisible into five distinctive structural components (anterior to posterior): the corneal epithelium, Bowman's layer, the stroma, Descemet's membrane, and the endothelium (Fig. 10.2).

The non-keratinizing stratified squamous epithelium, derived from the ectoderm, consists in the centre of the cornea of five layers, increasing to nine or ten layers in the periphery. Bowman's layer is believed to represent a modified layer of the stroma. It is composed of small collagen fibrils surrounded by mucoprotein ground substance. The corneal stroma is avascular and consists of almost parallel-

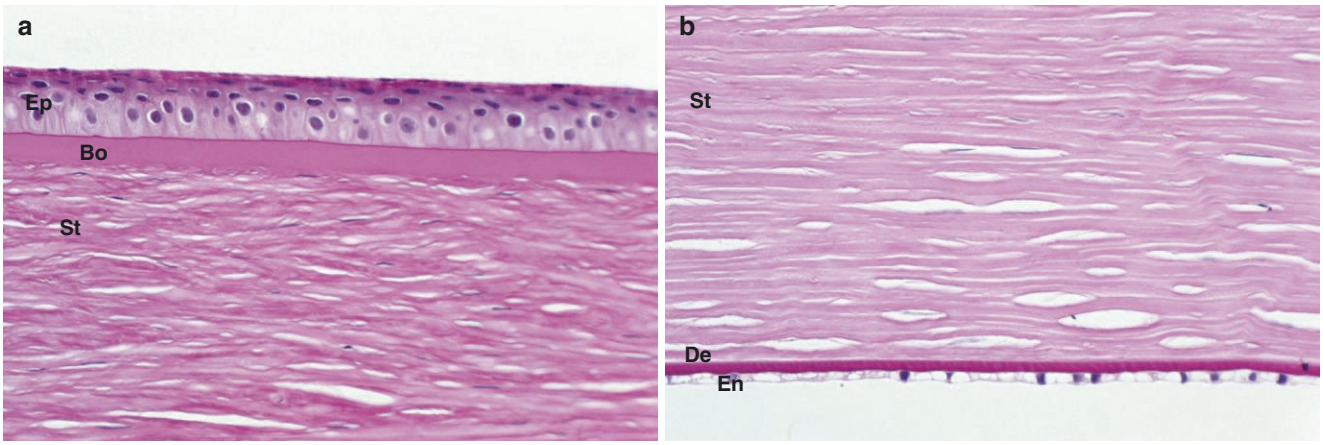

Fig. 10.2 Normal cornea with (**a**) the regular epithelium (Ep), Bowman's layer (Bo), the underlying corneal stroma (Str), and (**b**) the inner layers of the stroma, Descemet's membrane (De) highlighted in the PAS stain, and the corneal endothelium (En)

arranged collagenous lamellae interspersed with flattened keratocytes, derived from the mesectoderm. Descemet's membrane is a faintly eosinophilic staining, periodic acid-Schiff (PAS)-positive, acellular structure, formed by corneal endothelial cells, derived also from the mesectoderm. The underlying endothelium is a single layer of polygonal cells, arranged in a mosaic pattern. The corneal endothelium is essential for controlling the hydration of the cornea, acting both as a permeable barrier, limiting access of water from the aqueous to the corneal stroma, and by an active transport mechanism. Corneal endothelial cells do not proliferate, and hence their density decreases with age.

10.2.3 Intraocular Tissues

The intraocular tissues comprise the uveal tract, the retina, the lens, the aqueous, and the vitreous gel. The uveal tract (also termed tunica vasculosa) is the 'middle' layer of the eye internal to the sclera and consists of the iris, the ciliary body, and the choroid. It is a highly vascularized and pigmented tissue and provides several essential functions to the eye, including nutritive supply to almost all intraocular structures, production of aqueous, secretion of hyaluronic acid into the vitreous, and control of accommodation.

The *iris* is the anterior visible part of the uveal tract (Fig. 10.3). The iris has two components: the posterior iris pigmented epithelium derived from neuroectoderm and the iris stroma derived from the neural crest. Its major component, the iris stroma, is a loosely arranged tissue that contains pigmented and non-pigmented cells set in an abundant extracellular matrix containing bundles of type I collagen fibrils and hyaluronidase-sensitive glycosaminoglycans. The cells include melanocytes and fibroblasts. The smooth muscles of the sphincter and dilator muscles are also present within the iris stroma.

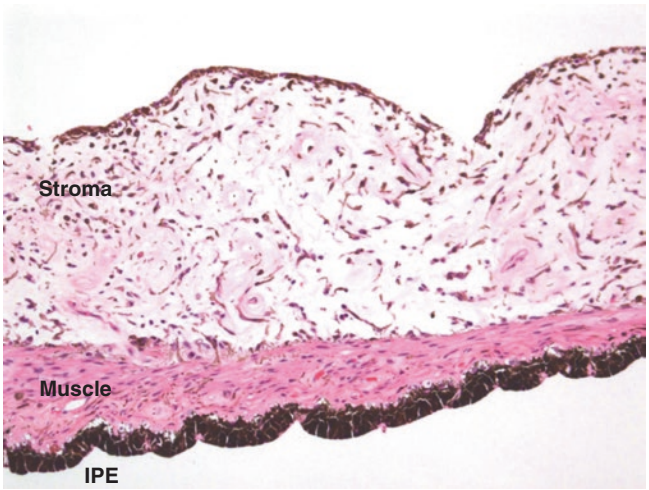

Fig. 10.3 H&E section of the normal iris with the anteriorly located iris stroma containing scattered cells including melanocytes, the smooth muscle of the dilator muscle, and the posterior iris pigmented epithelium (IPE)

The *ciliary body* is the middle part of the uveal tract, interposed between the iris and the choroid (Fig. 10.4). It has two major components: the pars plicata (also known as the 'corona ciliaris') and the pars plana. The anterior pars plicata is composed of a ring of 70–80 ciliary processes, which project into the posterior chamber. Their inner non-pigmented epithelial cells secrete the watery aqueous humour that fills the anterior chamber. The aqueous leaves the eye by passing through the trabecular meshwork and its associated drainage channels located between the iris and the peripheral inner cornea. The pars plana (or flat part) of the ciliary body is located posterior to the pars plicata and forms a circular band that extends to the ora serrata. The outer layer of the ciliary epithelium is pigmented. At the ora serrata, it continues posteriorly as the retinal pigment epithelium (RPE), whilst the inner

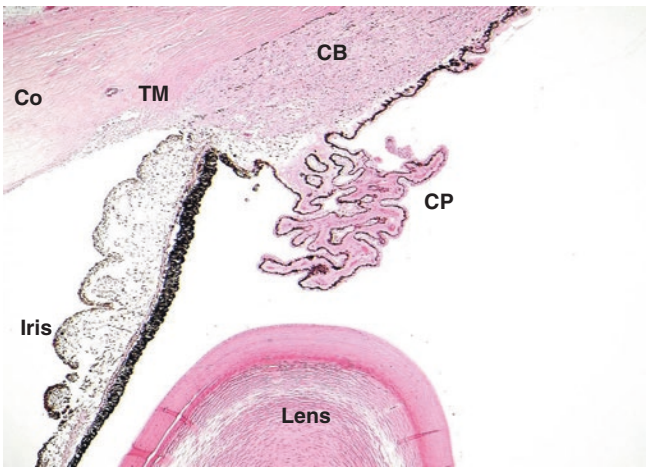

Fig. 10.4 Arrangement of the cornea (Co), trabecular meshwork (TM), iris root, ciliary body (CB), and ciliary processes (CP)

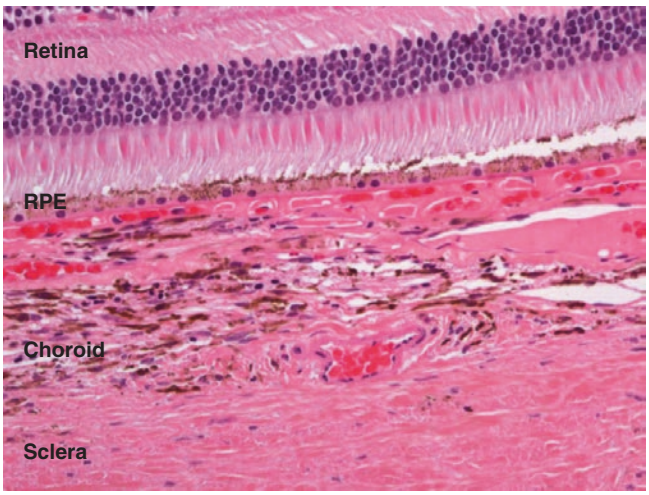

Fig. 10.5 Hematoxylin and eosin section of the normal outer retina, the retinal pigment epithelium (RPE), Bruch's membrane, the choriocapillaris, scattered normal melanocytes, and the sclera

non-pigmented ciliary epithelium abruptly thickens to form the neurosensory retina.

The stroma of the ciliary body is composed largely of smooth muscle. The ciliary muscle has circular, longitudinal, and radial parts, and its primary function is focusing (termed 'accommodation'). The longitudinal ciliary muscle of Bruecke attaches to the scleral spur, a ridge of connective tissue located directly behind the trabecular meshwork and the canal of Schlemm.

The *choroid* is the posterior part of the uvea and arises very early in the development from mesoectodermal cells around the optic cup (Fig. 10.5). It is responsible for supplying oxygen to the outer layers of the retina. It is located between the RPE and the sclera, with Bruch's membrane limiting it internally and the pigmented lamellae of the *lamina fusca*, externally. The anterior limit of the choroid is the ora serrata where the

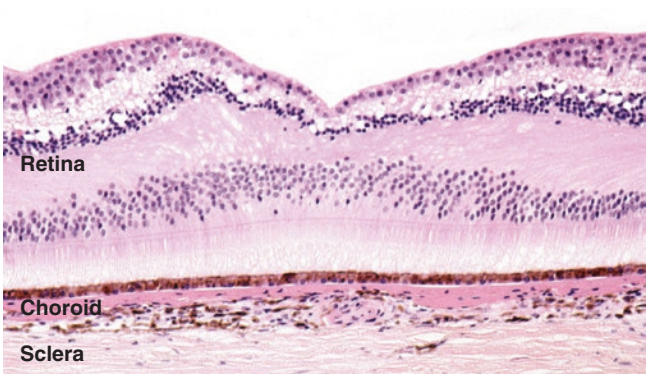

Fig. 10.6 Cross section of the normal retina in the region of the fovea with thinning of the layers

ciliary stroma merges irregularly into the choroidal stroma. The posterior part of the choroid wraps around the optic nerve and merges with its glial tissue and axons (*Elschnig-Scheide or rim*). Both Bruch's membrane and the RPE also end close to the optic nerve edge. The thickness of the choroid varies with it being the thickest at the posterior pole, measuring 0.22 mm, and decreasing considerably to 0.10 mm at the ora serrata.

The structure of the choroid is generally divided into four layers: (a) Haller's layer, (b) Sattle's layer, (c) choriocapillaris, and (d) Bruch's membrane. The choroid also consists of the choroidal stroma in which scattered lymphocytes and melanocytes are seen. It is thought that the melanin within the melanocytes aids the choroid limit uncontrolled reflection within the eye that would potentially result in the perception of confusing images.

The *retina* is a light-sensitive layer of tissue and is innermost layer of the eye (Fig. 10.6). The human retina consists of ten distinct layers, and its embryological development is quite complex (see below):

1. *Inner limiting membrane* – basement membrane produced by Müller cells.
2. *Nerve fibre layer* – axons of the ganglion cell nuclei.
3. *Ganglion cell layer* – contains nuclei of ganglion cells.
4. *Inner plexiform layer* – contains the synapse between the bipolar cell axons and the dendrites of the ganglion and amacrine cells.
5. *Inner nuclear layer* – contains the nuclei and surrounding cell bodies of the bipolar cells.
6. *Outer plexiform layer* – projections of rods and cones ending in the rod spherule and cone pedicle, respectively. These make synapses with dendrites of bipolar cells.
7. *Outer nuclear layer* – cell bodies of rods and cones.
8. *External limiting membrane* – layer that separates the inner segment portions of the photoreceptors from their cell nucleus.
9. *Photoreceptor layer* – rods/cones.
10. *Retinal pigment epithelium* (*RPE*).

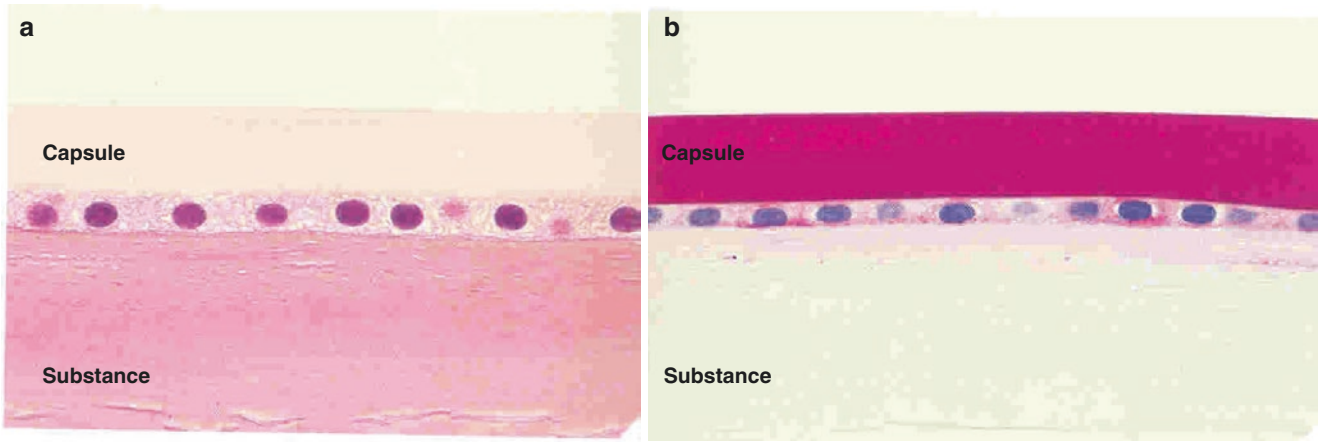

Fig. 10.7 The lens is situated in the posterior chamber behind the iris. It is composed of highly differentiated epithelial cells. (**a**) A monolayer of cuboidal lens epithelial cells is present between the anterior lens capsule, which is highlighted in the PAS stain (**b**). Comparatively the posterior lens capsule (not shown) is much thinner. The epithelial monolayer terminates at the equatorial lens bow, where the cells elongate to form the secondary lens fibres

The thickness of the retina varies in different regions with it being thinnest at the macula.

Differentiation of the neurosensory retina begins in the fourth week of gestation with three to four rows of cells with high mitotic activity. The inner third is called 'inner marginal zone' and differentiates into the nerve fibre layer. Neural and glial cells keep evolving simultaneously and develop into the inner and outer neuroblastic layers. They are separated by the transient nerve fibre layer of Chievitz, which around 10–12 weeks becomes the inner plexiform layer, except in the macule where it remains to birth. By the end of the third month, the four major horizontal layers of the retina are developed. Between the third and fifth month of gestation, the ganglion cells are the first to differentiate and axons enter the optic stalk, inducing the formation of the optic nerve. Photoreceptors arise from the outermost layer of neuroblastic cells, and the differentiation of cones starts in the future foveal area. The differentiation of cone outer segments begins at the fifth month, and the rod outer segments develop during the seventh month when also bipolar cells develop.

The development of the retina originates from the posterior area, and prearrangement of foveal organisation starts early because it is the central point from where cells extend peripherally. The foveal pit is recognisable by the seventh month, due to thinning of the inner nuclear layer. By the eighth month, the ganglion cell layers have decreased to two layers, and the inner nuclear layer is reduced to three cells or less because of lateral displacement of the remaining layers. At birth, the fovea still contains the transient layer of Chievitz, and its maturation to form the foveola begins at 4 months after birth; the remodelling continues until 4 years of age.

It is important to note that the RPE develops from the outer layer of the optic cup, and at week 6 melanogenesis begins. The RPE are the first cells in the body to produce melanin. They turn into cuboidal and cylindrical cells by the fourth month, and RPE is supposed to be functional at this time.

The *biconvex lens* is a transparent structure, the form of which is easily altered by contraction of the ciliary muscles (Figs. 10.4 and 10.7). It arises from the lens placode (surface ectoderm) under the induction from the underlying optic vesicle. The gene *PAX6* acts in the early phase as the master controller of lens development, activating other genes encoding cytoskeletal, structural, and membrane proteins. The lens consists of three parts: the capsule, epithelium, and substance (i.e. cortex and nucleus). The shape of the lens is maintained by the elastic lens capsule, which has the thickest basement membrane in the body, and it is highlighted in the PAS stain (Fig. 10.7). The inner surface of the anterior lens capsule is covered with a single layer of cuboidal epithelium. In a fixed specimen, the lens substance is opaque and rigid, caused by coagulation of the soluble crystallins.

10.2.3.1 The Aqueous Humour and the Vitreous

The aqueous humour is the fluid between the cornea and the lens, and it is important for both maintaining the pressure within the eye and providing nutrition for the central cornea and lens, which do not have their own blood supplies. The intraocular pressure is a product of the rate of aqueous production and the rate of aqueous drainage. The aqueous humour is filtrated from blood, modified in its composition, and secreted by the ciliary processes of the ciliary body in the posterior chamber. The volume of aqueous is about 0.2 microlitres, and it is entirely replaced every 1–2 h. The aqueous exits the eye via the trabecular meshwork located between the peripheral cornea and the iris root. A raised intraocular pressure typically results from poor aqueous outflow, which can lead to glaucoma (see below).

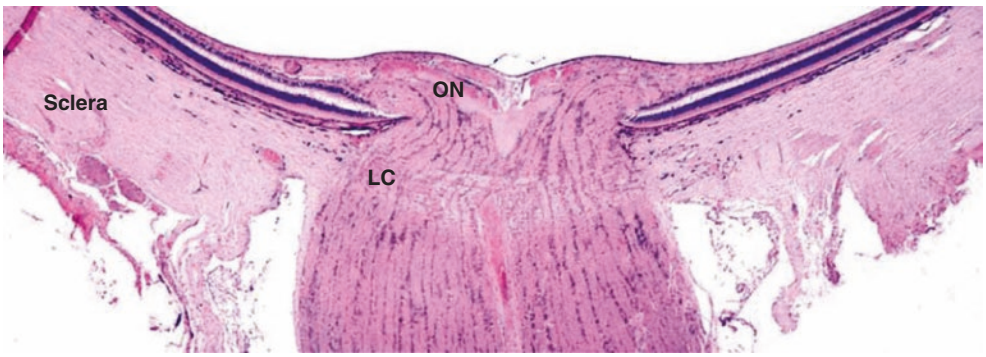

Fig. 10.8 Cross section of the optic nerve head (ON) where the axons from the retinal nerve fibre layer converge, forming a bulge. The lamina cribrosa (LC), a sieve-like plate of connective tissue, defines an ocular 'barrier', which is used for defining tumor spread (e.g. retinoblastoma. See Fig. 10.39c)

The vitreous (body) is a transparent, extracellular viscous gel, with a complicated structural framework of collagen soluble proteins and hyaluronic acid, and is composed 99 % of water. The few cells that are normally present in the vitreous gel are located predominantly in the cortex and consist of hyalocytes, astrocytes, and glial cells. Vitreous abnormalities include opacification, liquefaction, and shrinkage; it can be infiltrated by both inflammatory and neoplastic cells causing the formation of 'vitreous opacities' (see Review [4]).

10.2.4 Optic Nerve

The optic nerve develops from the optic stalk, the connection between the forebrain and the optic vesicle. The axons from the retinal nerve fibre layer converge at the optic disc. The axons in the nerve fibre layer of the optic disc form a bulge as they pass through the lamina cribrosa, a sieve-like plate of connective tissue formed by fibroblasts, in growing from the posterior part of the adjacent sclera (Fig. 10.8). This bulge is larger on the nasal side. The tissue anterior to the lamina derives blood supply from the posterior ciliary arteries; the tissue posterior to the lamina has a meningeal blood supply, derived from branches of the ophthalmic and central retinal artery.

10.2.5 Lacrimal Glands and Lacrimal Drainage System

The pale brown and ovoid lacrimal gland is located in the upper outer orbit and is responsible for secreting the aqueous layer of the tear film. The large ducts pass through the conjunctival epithelium into the superior fornix. The tear fluids drain into the canaliculi, ending in the lacrimal sac. The secretory gland is composed of lobular grouped acini, secreting solutes and glycosaminoglycans. The drainage system consists of ductules, formed by epithelial cells surrounded by myoepithelium (Fig. 10.9). The canaliculi are covered by stratified epithelium, whilst the lacrimal sac is covered by columnar epithelium. A wide variety of diseases can affect the lacrimal gland, e.g. inflammation as well as benign and malignant neoplasias (see below), requiring different treatment strategies [5, 6].

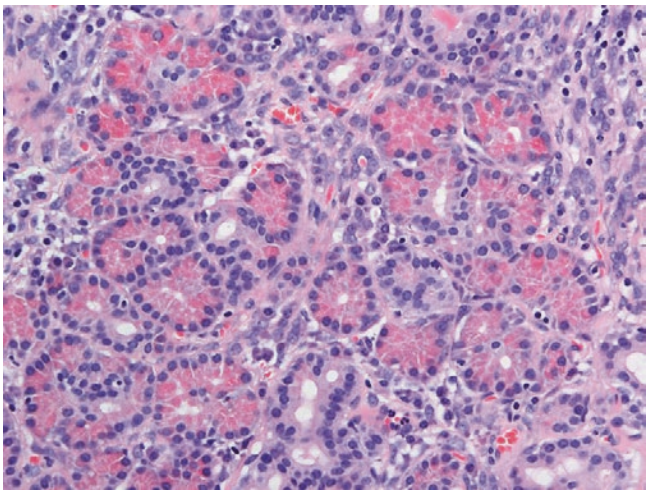

Fig. 10.9 Normal lacrimal gland with some scattered adipocytes, which increase in number within the gland with age, and occasional lymphocytes. The inner layer of tall columnar secretory cells and a relatively inconspicuous discontinuous layer of contractile myoepithelial cells comprise the acini of the lacrimal gland. Large intensely eosinophilic secretory granules called zymogen granules are found in the apical cytoplasm of the secretory cells

10.2.6 Eyelids

The eyelids are derived from the ectoderm and mesoderm, and abnormal development of the structures in the embry-

onic lid fold may result in several deformities of the eyelid and the palpebral aperture [2, 7]. They are covered by an epidermis above a thin dermis with small sweat glands and pilosebaceous units. The dermis covers the orbicularis oculi muscle, located on the anterior surface of the tarsal plate, which is composed of compact stroma. In the anterior part of the eyelids, the pilosebaceous units are much larger to form the lashes. The parts of the tarsal plates closest to the lid margins contain the large sebaceous (meibomian) glands. A transition from keratinized squamous epithelium of the outer eyelid into columnar epithelium of the tarsal conjunctiva is present in the transition zone (Fig. 10.10). Small pilosebaceous glands (Zeiss) and sweat glands (Moll) are present at the lid margins.

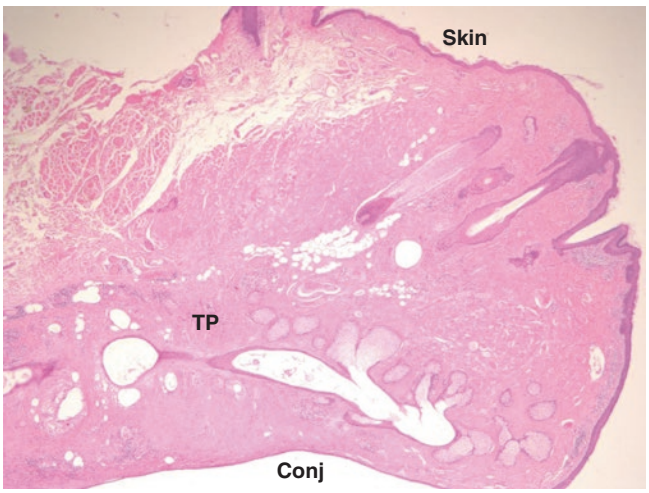

Fig. 10.10 Low power section of the normal eyelid, showing normal skin appendage structures and tarsal plate (TP) containing meibomian glands

10.2.7 Orbit

Detailed reviews of ocular and periocular embryogenesis have been provided recently and the reader is referred to these [2, 3]. Briefly, the organisation of the orbit and its bony walls is governed by the optic cup and the optic vesicle. Unlike the trunk and extremities, the orbital bone and ocular connective tissue are derived from neural crest cells, not mesoderm. The connective tissue contributions of the neural crest are collectively called mesoectoderm.

The orbital septum and globe divide the orbit into 'anterior' and 'posterior' compartments. The anterior compartment consists of the lids, lacrimal apparatus, and the surrounding anterior soft tissues. The posterior compartment is also called the 'retrobulbar space'. The cone-like structure forming this retrobulbar space consists of the six extraocular muscles and an envelope of fascia. The optic nerve is located within the intraconal space, surrounded by fibrous and fatty tissue.

10.3 Conjunctival Pathology

10.3.1 Introduction

Because conjunctival biopsies and excisions are very thin, they tend to curl inwards and fold when placed unsupported into fixation solution. Without causing compression artefacts, the surgeon should spread the conjunctival biopsy onto a piece of filter paper and let it dry for a few seconds, before placing it into a small cassette (e.g. cell safe biopsy capsule or equivalent) and then placed into buffered formalin (Fig. 10.11). Such preparatory work is of great value as it allows for perpendicular sections, which are important for the assessment of the basement membrane or basal surgical

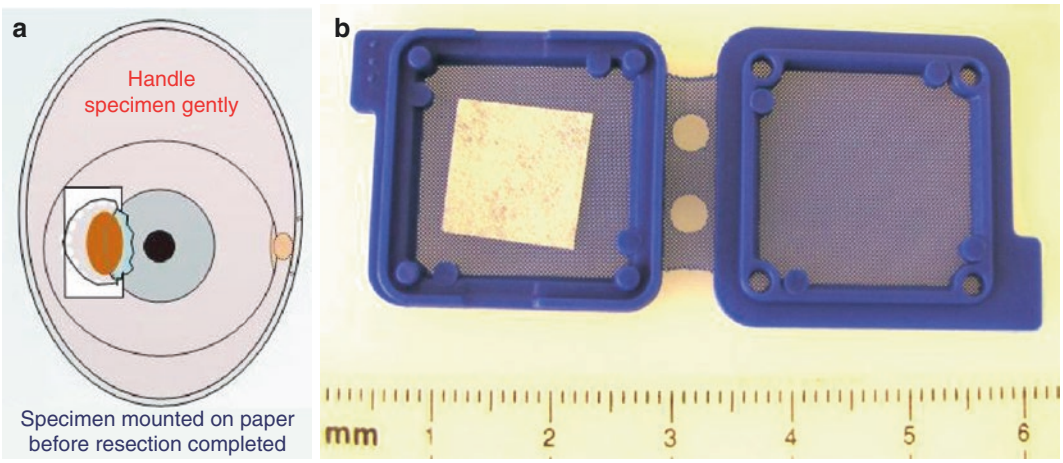

Fig. 10.11 (**a**) Diagrammatic representation of the recommended surgical procedures involved in removing conjunctival for histological assessment, with placement on a small piece of filter paper, and (**b**) this then being placed into a CellSafe capsule or equivalent, prior to placement in formalin

margin in infiltrating conditions, e.g. conjunctival squamous cell carcinoma and invasive melanoma.

10.3.2 Developmental Anomalies

Development lesions of the conjunctival include epibulbar dermoids, dermolipomas, complex choristoma, ectopic lacrimal gland and episcleral osseous choristomas. The first three entities will be discussed below.

10.3.3 Dermoid, Dermolipoma and Complex Choristoma

Definition Dermoid tumors are firm or cystic masses, typically occurring at the limbus on the temporal side. They are composed of epithelial and/or connective tissue elements, which become entrapped within embryonic clefts.

Clinical aspects Dermoids are present at birth and have little or no growth potential. The lesions can occur as isolated ocular lesions or in association with anomalies affecting other organs (Goldenhar's syndrome, mandibulofacial dysostosis, and neurocutaneous syndrome) [8–12].

Epidemiology They are the most frequent epibulbar tumors in children [13].

Microscopy On histological examination, the surface epithelium consists of stratified squamous epithelium, frequently containing skin appendages. The stromal component consists of collagen arranged in thick bundles; it contains blood vessels and nerve fibres. If adipose tissue is present, the lesion is called dermolipoma. Sometimes cartilage or lacrimal gland tissue is present, these lesions are known as 'complex choristoma' [14, 15].

Treatment and prognosis These benign lesions may be managed by observation or often surgical excision, because of cosmetic reasons.

10.3.4 Cysts

10.3.4.1 Inclusion Cysts

Definition Conjunctival inclusion cysts are acquired lesions, usually arising following surgical or accidental trauma [16–18].

Microscopy The cysts are lined by non-keratinizing cuboidal epithelium with apocrine changes and containing goblet cells. The cysts are lined by non-keratinizing cuboidal epithelium with apocrine changes and containing goblet cells. In the underlying stroma, chronic inflammatory cells may be present.

Treatment and prognosis Treatment consists of surgical removal of the lesion.

10.3.5 Degeneration

10.3.5.1 Pinguecula and Pterygium

Definition Pingueculae are raised, localised, yellowish-grey lesions that occur in the bulbar conjunctiva, close to the limbus on the nasal or temporal side of the cornea. Pterygia are similar in appearance and also develop in these areas, but involve the peripheral cornea, again mostly on the nasal side (Fig. 10.12). Pingueculae and pterygia are degenerative lesions causally related to prolonged actinic exposure.

Epidemiology The lesions are often bilateral and occur in middle-aged and elderly patients, especially in areas with high levels of sunlight.

Microscopy On histological examination both lesions are identical. The essential feature is elastotic degeneration of the collagen, resulting in a subepithelial zone of amorphous, basophilic material, which stains black with the elastica van Gieson stain (Fig. 10.12). In older lesions dystopic calcification can occur.

The overlying epithelium may show a wide variety of changes, but most frequently it is thin, atrophic conjunctival epithelium or acanthosis without cellular atypia. In the epithelium an actinic keratosis or even a squamous cell carcinoma may develop from an additional conjunctival squamous intraepithelial neoplasia (see below) [19]. Hence, these changes must always be looked for and excluded.

Treatment Pterygia are of concern because of their corneal involvement and therefore must be surgically removed.

10.3.6 Inflammatory Processes

Definition The conjunctiva is prone to inflammations with many different causes, either infectious or as a part of a non-infectious dermatological or systemic disease. Usually these lesions do not cause diagnostic problems, but a biopsy can be useful in making the correct diagnosis [20]. In this chapter, the inflammatory processes of the conjunctiva are divided into acute, chronic, and granulomatous. The only separated mentioned lesions are ligneous conjunctivitis and lesions caused by *Chlamydia* infections.

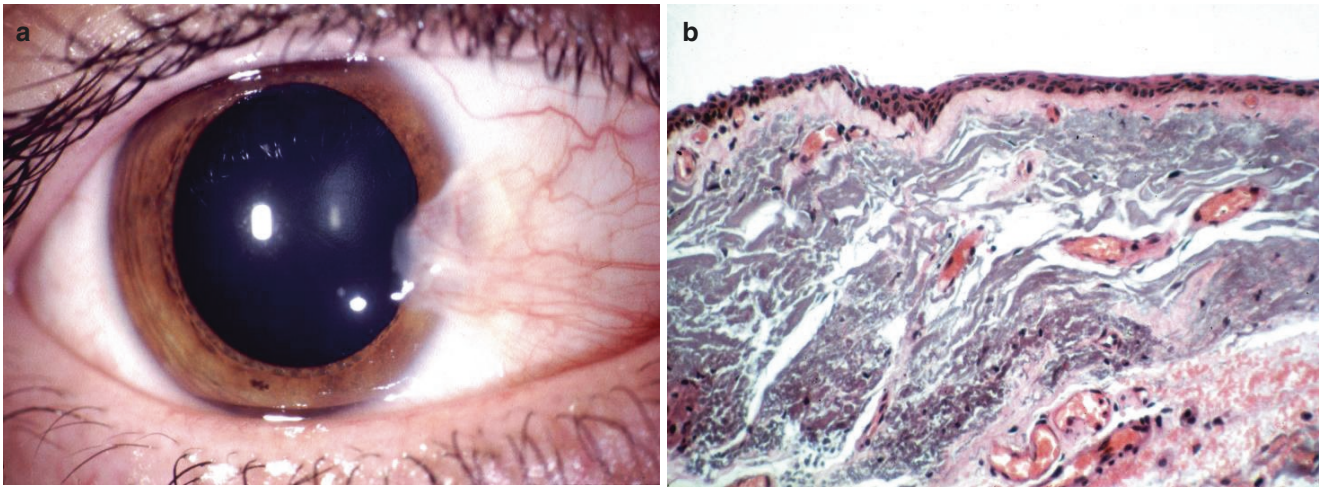

Fig. 10.12 (**a**) Clinical appearance of a pterygium with growth onto the corneal surface. (**b**) Histological sections stained with the van Gieson stain show a regular conjunctival epithelium and the elastotic degeneration of collagen, resulting in a subepithelial zone of amorphous, basophilic material, staining black grey. There can be associated variable proliferation of small capillaries depending on the age of the lesion

10.3.6.1 Acute Conjunctivitis

Clinical aspects In acute conjunctivitis there is a rapid onset of a swollen, hyperaemic conjunctiva accompanied by increased tear formation. There may be a watery (viral infections), fibrinous (bacterial infections), or mucoid (allergic reactions) discharge [21].

Microscopy The histological pattern depends of the cause of the inflammation. In viral infections the infiltrate consists mostly of mononuclear cells. In a bacterial infection many neutrophilic leukocytes can be seen [21]. When the cause is an allergic response, usually many eosinophils are found.

10.3.6.2 Chronic Non-granulomatous Conjunctivitis

Clinical aspects Chronic conjunctivitis can be caused by many infectious, immunological, and toxic agents. Anatomic aberrations (like ectropion or proptosis) can also cause inflammation.

Microscopy In chronic conjunctivitis the epithelium becomes hyperplastic and the goblet cells increase in number. Crypt-like epithelial infoldings can occur, forming subepithelial retention cysts. These cysts contain mucus in which calcification can be seen in time. The presence of perivascular infiltrate in the stroma can induce fibrous bands between the epithelium and the tarsus, which can cause surface irregularities, the so-called papillary conjunctivitis. In fact, the epithelial and stromal responses of a papillary conjunctivitis are nonspecific and can also be seen in atopic conjunctivitis and, in a more extreme form, in individuals wearing contact lenses (‘giant papillary conjunctivitis’) [22, 23]. When lymph follicles are found in the superficial stroma, it is called ‘follicular conjunctivitis’ (Fig. 10.13). The presence of these follicles can be associated with infections associated with adenoviruses [24], *Chlamydia trachomatis* [25], *Borrelia burgdorferi* [26, 27] and in patients using topical medication. In these situations, a lymphoma has to be excluded by morphological analysis and immunohistochemistry [28–31].

With long-standing inflammation, the epithelium can become atrophic, with loss of goblet cells and subsequent ‘dry eye symptoms’. The epithelium can show keratinization, resulting in a white appearance (leukoplakia). The long-standing inflammation may result in scarring of the conjunctival stroma.

10.3.6.3 Granulomatous Conjunctivitis

Granulomatous inflammation of the conjunctiva is usually associated with systemic diseases. The presence of exogenous material or parasites may cause an isolated granulomatous inflammation of the conjunctiva. In the case of a chalazion, the palpebral conjunctiva may also be affected.

Microscopy Like in other anatomic sides, a caseating necrotising granulomatous infection can be caused by mycobacteria, especially in children [32–34].

In sarcoidosis, the granulomas are usually small and sharply demarcated without any evidence of caseation [35, 36]. Serial sections and special stains can be necessary to confirm the diagnosis and rule out microorganisms.

10.3.6.4 Ligneous Conjunctivitis

Clinical aspects Ligneous conjunctivitis (‘chronic pseudomembranous conjunctivitis’) is a rare bilateral disease, mainly occurring in young girls. It presents as a subacute inflammation of the tarsal conjunctiva, often accompanied

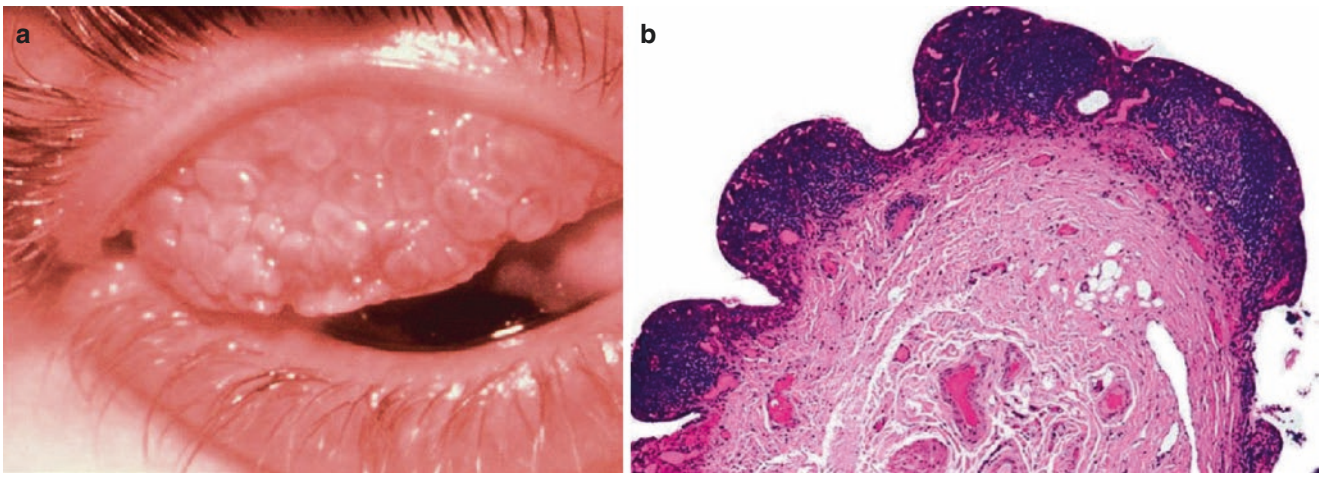

Fig. 10.13 (**a**) Clinical appearance of an advanced acute-chronic follicular conjunctivitis. (**b**) Corresponding section of such conjunctiva with prominent lymphoid infiltrates, forming reactive secondary follicles

by nasopharyngitis and vaginitis. The disease seems to be due to a defective fibrinolysin system.

Microscopy Histologically, there is granulation tissue covered with plaques of fibrinous material, later forming a hyalinised mass.

Treatment and prognosis After removal of the plaque, recurrence is common. It can be complicated by corneal involvement with the resulting keratitis possibly being complicated by perforation and ultimate loss of the eye [37–39].

10.3.6.5 *Chlamydia trachomatis* (TRIC Agent) Infection

Etiology and pathogenesis *Chlamydia trachomatis* (TRIC agent: trachoma-inclusion conjunctivitis) is an obligate intracellular pathogen of columnar epithelial cells [40]. In hot climates it can cause trachoma, primarily affecting the conjunctiva and corneal epithelium, producing a roughening of the conjunctivitis (so-called granular conjunctivitis), ultimately causing cicatrisation of this tissue. Trachoma affects commonly children in Australia, Africa, Asia, and Central and South America and is one of the world's major causes of blindness. Trachoma is spread from eye to eye by transfer of ocular discharges and, therefore, more commonly affects women than men likely due to their closer contact with children. In temperate climates chlamydial infection is venereal, with only mild conjunctival infection, called 'inclusion conjunctivitis' (see below). A third manifestation of this pathogen is ophthalmia neonatorum [41].

Clinical aspects The clinical manifestations of trachoma vary with the severity and duration of the initial infection and also depend upon environmental factors, the patient's nutritional and immune status, the number of reinfections, and the presence or absence of secondary bacterial infection. It is usually bilateral. The course of trachoma can be divided into four stages (the *MacCallan classification*) [42]. During the active stages of infection, inclusions can be found in Giemsa-stained cells scraped from the surface of infected epithelium. More easy to recognise is a positive direct immunofluorescence for *Chlamydia trachomatis* on the scraping; it can be very helpful in making the correct diagnosis [43, 44]. The diagnosis can also be made by isolation of the causative agent in cell cultures and the detection of chlamydial antibody in blood or tear fluid. In stage I there is epithelial infection by *Chlamydia trachomatis*. It is characterised clinically by the formation of conjunctival follicles and diffuse punctate inflammation of the cornea.

Microscopy The histology is indistinguishable from that of follicular conjunctivitis caused by other agents. Lymphocytes and plasma cells infiltrate the subepithelial tissue; polymorphonuclear leukocytes infiltrate the corneal and conjunctival epithelium. In stage II, the inflammatory reaction occupies the stroma, with the further formation of follicles. Large macrophages with phagocytised debris (Leber cells) are seen in the conjunctiva, accompanying the epithelial hyperplasia with round cell infiltration and subepithelial oedema [45]. In stage III, the follicles disappear and cicatrisation occurs. The fibrosis causes inversion of the upper lid (cicatricial entropion), misdirected lashes (trichiasis), and decreased tear formation. On histological examination, scattered lymphocytes and plasma cells can still be seen along with subepithelial scar tissue. In stage IV, there is spontaneous arrest of the disease, which is no longer contagious. The residual entropion and trichiasis lead to continuing corneal damage. Denuding of the epithelium leaves the cornea vulnerable to infection and further opacification as a result of scarring.

Inclusion Conjunctivitis

Definition Inclusion conjunctivitis is also called inclusion blennorrhea, chlamydial conjunctivitis, or 'swimming pool' conjunctivitis. This disease affects 4 of 1,000 (0.4%) live births. Approximately half of the infants born to untreated infected mothers will develop the disease. It is caused by *Chlamydia trachomatis*.

Clinical aspects The conjunctival involvement of the sexually transmitted chlamydial infection is mild and can even be asymptomatic. In adults, it presents as a subacute follicular conjunctivitis. It is accompanied by a chronic urethritis in the male and a symptomless cervicitis in the female. In newborns, it occurs with an acute mucopurulent discharge, 5–10 days after birth. It is accompanied by infection of the maternal vagina by the same agent. Because the extranodal lymphoid tissues are not fully developed, the conjunctivitis is more papillary than follicular.

Microscopy Like trachoma, it can be diagnosed by Giemsa staining on conjunctival scrapings. The inflammatory infiltrate is usually most dense in the lamina propria and predominantly consists of lymphocytes and plasma cells. The lymphocytes are mainly of T-cell type, with CD8+ cells being the dominant subtype. Neutrophils and macrophages are variable in number. Chlamydial inclusion bodies were uncommon in routinely stained sections. Immunohistochemistry for chlamydiae is positive in about one-third of cases.

10.3.7 Dermatological and Systemic Diseases

10.3.7.1 Keratoconjunctivitis Sicca

Definition Keratoconjunctivitis sicca was described first by the Swedish ophthalmologist Henrik Sjögren in 1933 [46, 47]. It therefore is best known as one of the symptoms of Sjögren's syndrome. However, it can also be seen in other autoimmune diseases like scleroderma or rheumatoid arthritis. Moreover, keratoconjunctivitis sicca is the most frequent cause of eye involvement in graft-versus-host disease [48–50].

Clinical aspects In this condition the cornea and conjunctiva are dry, causing a painful and gritty sensation.

Microscopy Histologically, there is atrophy of lacrimal acinar parenchyma, accompanied by fibrosis and fatty infiltration, but with preservation of the lobular architecture. There is a focal or diffuse presence of lymphocytes and plasma cells. Sometimes lymphoepithelial lesions (i.e. infiltration of lymphocytes into the overlying conjunctival epithelium) can be seen. If persistent and insufficiently treated, perforation of the cornea may occur.

10.3.7.2 Dermatologic Diseases

Many skin diseases can involve the conjunctiva. Most frequently seen are bullous diseases like pemphigus [51], bullous pemphigoid, Stevens-Johnson syndrome [52], paraneoplastic pemphigus [53, 54] and less common dermatitis herpetiformis [55], and linear IgA disease [56]. Other dermatologic diseases with conjunctival involvement are lupus erythematosus [57–59], familial chronic benign pemphigus (Hailey-Hailey disease) [60], and lichen planus [61, 62].

10.3.7.3 Metabolic Diseases

A conjunctival biopsy can be of diagnostic value in metabolic diseases with specific ultrastructural features, like galactosialidosis and different types of mucopolysaccharidoses [63, 64].

10.3.8 Tumors and Tumor-Like Conditions

10.3.8.1 Epithelial

Epithelial tumors of the conjunctiva can be divided into tumors of the surface epithelium (papilloma, squamous cell intraepithelial neoplasia, and squamous cell carcinoma) and adnexal tumors. Since the caruncle contains accessory lacrimal glands, sweat glands, hair follicles, and sebaceous glands, adnexal tumors of different kinds can be found. The histological appearances of these skin adnexal tumors are discussed in more detail in the Chap. 15.

Squamous Papilloma

Definition Squamous papilloma of the conjunctiva is a benign exophytic lesion, formed by a fibrovascular core covered by squamous epithelium.

Clinical aspects It is the most common epithelial tumor of the conjunctiva and usually presents as a red, papillomatous mass.

Etiology and pathogenesis There is a strong association between human papillomavirus (HPV) and conjunctival papillomas. HPV type 6/11 is the most common HPV type in conjunctival papilloma.

Microscopy These benign tumors histologically consist of a fibrovascular core, lined by conjunctival epithelium, possibly with squamous metaplasia (Figs. 10.14). The sessile variant shows usually only metaplastic epithelium, without goblet cells. The presence of koilocytosis is suggestive but not conclusive for the presence of HPV in conjunctival papilloma [65]. Squamous papillomas are more extensively discussed in Chap. 1.

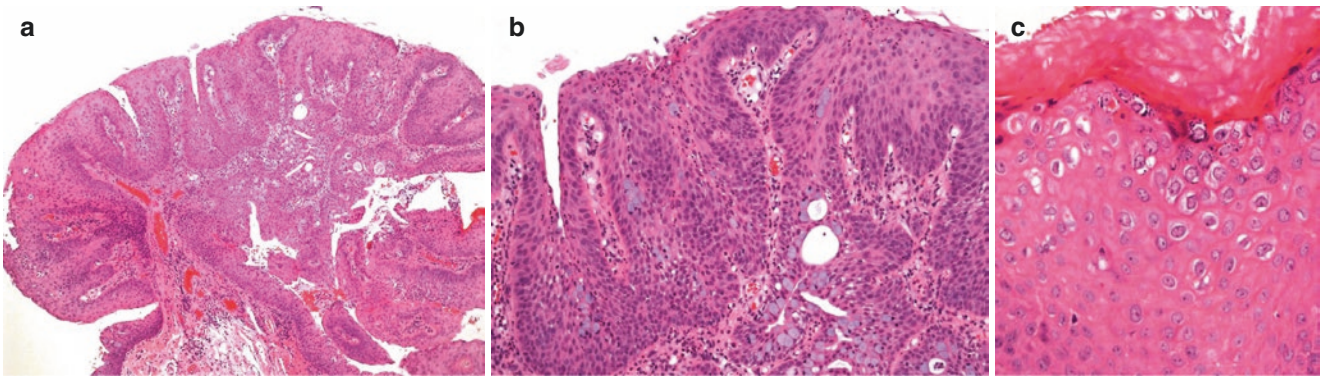

Fig. 10.14 (**a**) Conjunctival biopsy of a squamous papilloma with clear papillary-like structure and a central feeding vessel within the stalk. (**b**) Obvious acanthosis of the conjunctival epithelium, with mild dysplasia. (**c**) Focal areas of metaplasia with keratinizing squamous epithelium, and with koilocytosis

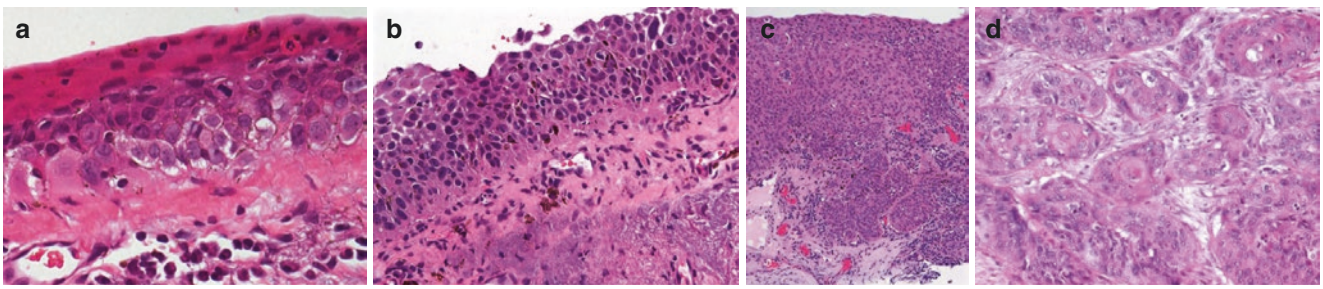

Fig. 10.15 Increasing dysplasia in metaplastic conjunctival epithelium with (**a**) mild to moderate dysplasia, (**b**) severe dysplasia, (**c**) focally invasive squamous cell carcinoma arising from carcinoma in situ, and (**d**) frank conjunctival squamous cell carcinoma with squamous 'eddie' formation

Conjunctival Squamous Intraepithelial Neoplasia

Definition Conjunctival squamous intraepithelial neoplasia encompasses a spectrum of changes of the conjunctival epithelium characterised by different degrees of squamous dysplasia on a background of keratinizing metaplasia. A variety of different terms are used, including the clinical term 'ocular squamous surface neoplasia' (OSSN), which encompasses both non-invasive and invasive squamous cell carcinoma.

Etiology and pathogenesis Conjunctival squamous intraepithelial neoplasia of the conjunctiva can be caused by sun damage (actinic keratosis) or by HPV-induced premalignant transformation (*Bowenoid* type).

Microscopy In both types of dysplasia, the epithelial cells show varying degrees of atypia, ranging from mild dysplasia to severe atypia amounting to frank carcinoma in situ (Fig. 10.15a–d). In actinic keratosis, the epithelium is thin and the stroma shows damage of elastic tissue. In the Bowenoid type, mitotic figures can be seen in the upper epithelial layers and HPV-related epithelial changes like multinucleated cells and koilocytes can be found [66–68].

Malignant Tumors of the Surface Epithelium

Epidemiology Squamous cell carcinomas (SCC) of the conjunctiva are relatively rare with an incidence of two to five per million, with the greatest frequencies being seen in countries located close to the equator, e.g. the Middle East and Australia.

Microscopy They are usually well-differentiated SCC but more aggressive variants of conjunctival SCC, like the acantholytic or adenoid type [69] and the spindle-cell type can occur infrequently (Fig. 10.16a–d). Mucoepidermoid carcinoma of the conjunctiva is a rare tumor with a poor prognosis [72, 73]. [See for more details Chaps. 1 and 5.]

Treatment and prognosis Invasive SCC is generally treated by excision of any nodules with or without adjunctive cryotherapy or topical chemotherapy. Diffuse intraepithelial disease is treated with adjuvant topical chemotherapy with mitomycin C, 5-fluorouracil (5-FU 1 %), or interferon α2b alone or in combination with all-trans retinoic acid 0.01 %. After adequate therapy, total cure is usually achieved [70, 71].

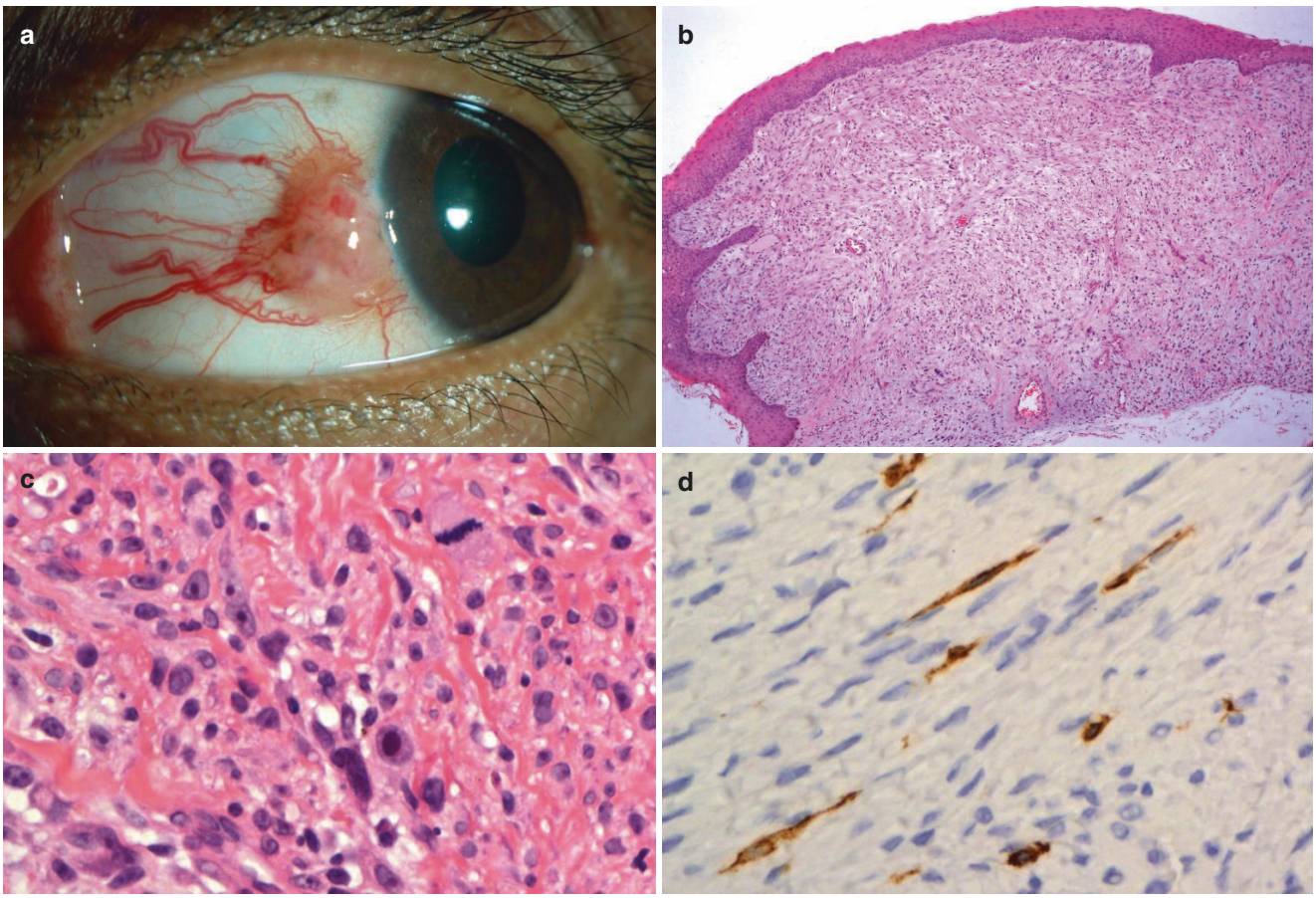

Fig. 10.16 (**a**) Clinical photograph of a patient with a spindle cell carcinoma of the conjunctiva; (**b**) the excision specimen shows a conjunctival epithelium with keratinizing squamous metaplasia; (**c**) higher power of the stroma highlights the presence of spindle cells within the stroma with obvious cellular atypia. (**d**) Pancytokeratin staining highlights the neoplastic spindle squamous cells within the stroma

Oncocytoma

Definition An oncocytoma (oxyphil cell adenoma, oxyphilic granular cell adenoma) is a benign tumor that can occur in the conjunctiva, the caruncle, the lacrimal gland, and the lacrimal sac (Fig. 10.17) [74, 75].

Clinical aspects It presents as an asymptomatic, slowly progressive swelling of the caruncle in older persons.

Microscopy The lesion consists of large epithelial cells with eosinophilic, granular cytoplasm, due to a large number of mitochondria (Fig. 10.17). The cells can be arranged in cords, sheets, or nests. Ductal and cystic glandular structures can be found. The malignant variant of this tumor, the oncocytic adenocarcinoma of the lacrimal system is very rare.

Treatment and prognosis Surgical excision is usually curative.

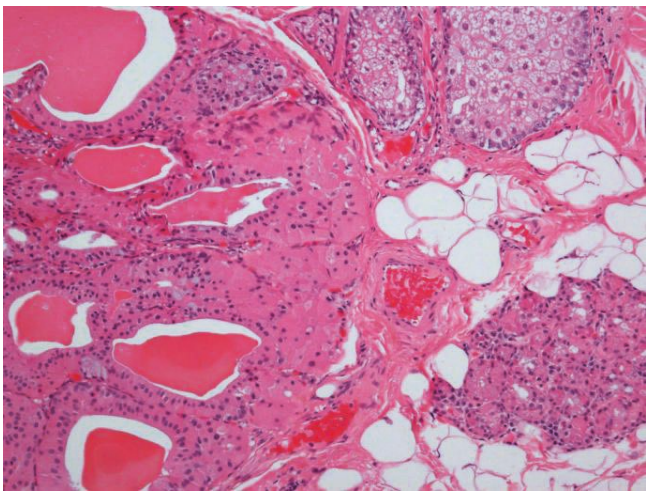

Fig. 10.17 Small oncocytoma of the caruncle with adjacent adnexal structures

Sebaceous Carcinoma

Clinical aspects This lesion is important because it can be a pitfall for both the clinician and the histopathologist. The tumor presents as a solitary nodule, that can clinically be misdiagnosed as a basal cell carcinoma, squamous cell carcinoma, Merkel cell carcinoma or even as a chalazion or a chronic blepharoconjunctivitis (Fig. 10.18) [76–78].

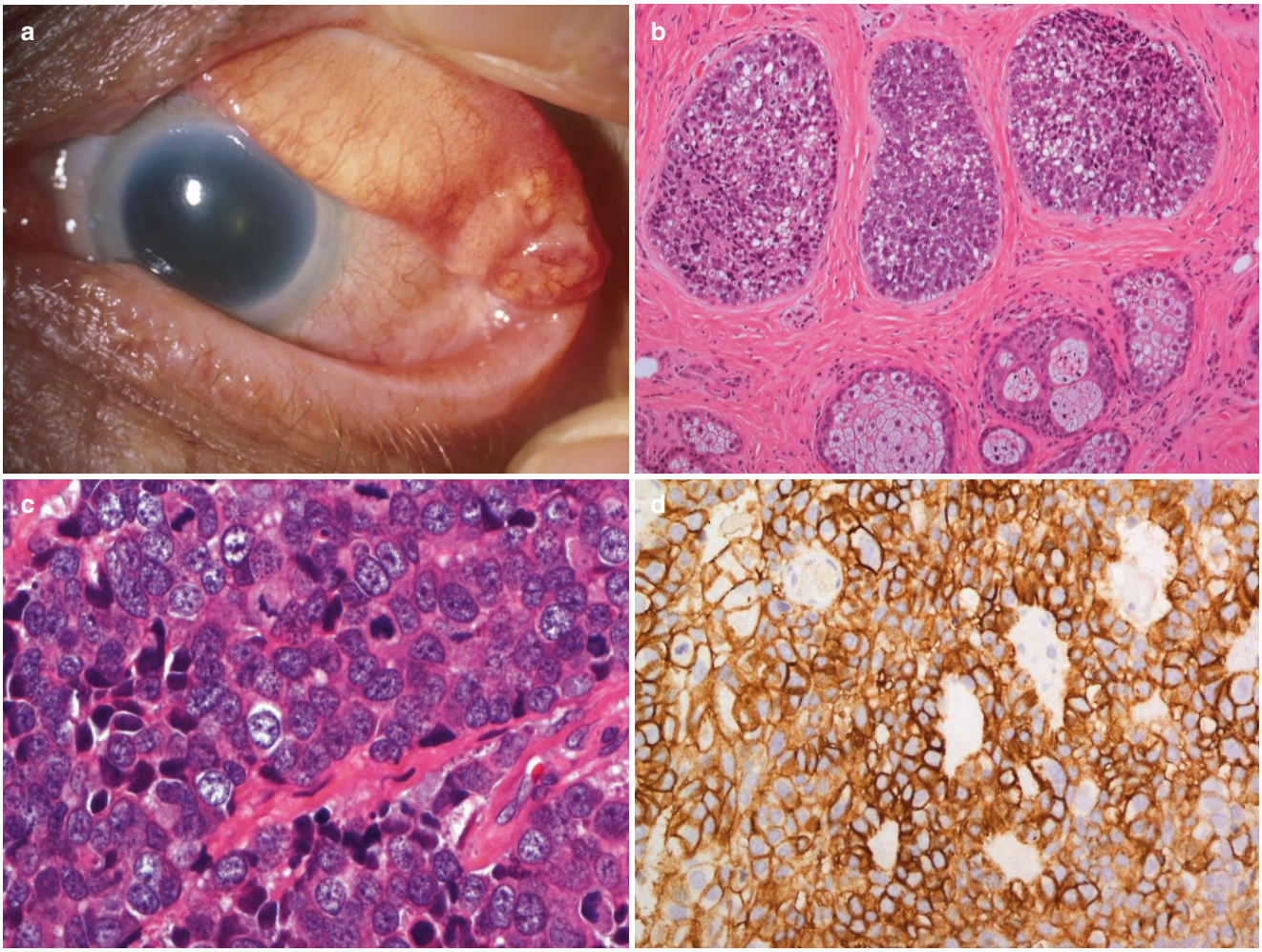

Fig. 10.18 (**a**) Clinical photograph of an elderly female patient with a nodule involving the upper palpebral eyelid. (**b**) Some sebaceous glands are filled with atypical cells. (**c**) High power of these glands demonstrates cells with small amounts of cytoplasm, nuclei with fine stippled chromatin, and moderate mitotic counts. (**d**) Immunostaining with markers such as EMA (here), Ber-EP4, and adipophilin are of value in confirming the diagnosis and in assessing the extent of pagetoid spread

Microscopy Histologically the tumor is composed of epithelial nests with varying degrees of sebaceous differentiation. The well-differentiated sebaceous carcinomas are not very hard to recognise, but the poorly differentiated ones can be overlooked and misdiagnosed easily. The intraepithelial pagetoid spread of tumor cells (which is frequently present and quite characteristic of this tumor) may be misinterpreted as dysplasia.

Immunohistochemistry Immunohistochemical stainings like EMA,BerEP4, adipophilin, and androgen receptor can help in differentiating this aggressive tumor from a squamous cell carcinoma (Fig. 10.18) [79, 80]. Recent unpublished studies of the first author demonstrate that perforin has high sensitivity in highlighting sebaceous carcinomas, particularly with pagetoid spread.

Treatment and prognosis Treatment of choice is wide excision, which can cure patients with an early stage of the lesion, with additional topical chemotherapy. However, the mortality rate from metastases is 25 %, and even higher in a poorly differentiated tumors with angioinvasive growth. Some sebaceous carcinomas occur rarely in patients in association with other malignancies, e.g. colorectal carcinoma, gastric carcinoma, and endometrial carcinoma (termed Muir-Torre syndrome).

10.3.8.2 Melanocytic Lesions

Conjunctival Nevus

Definition The most common melanocytic lesion of the conjunctiva is the compound nevus. Other types of nevi that can be found in the conjunctiva are intraepithelial, subepithelial, Spitz, and blue nevi.

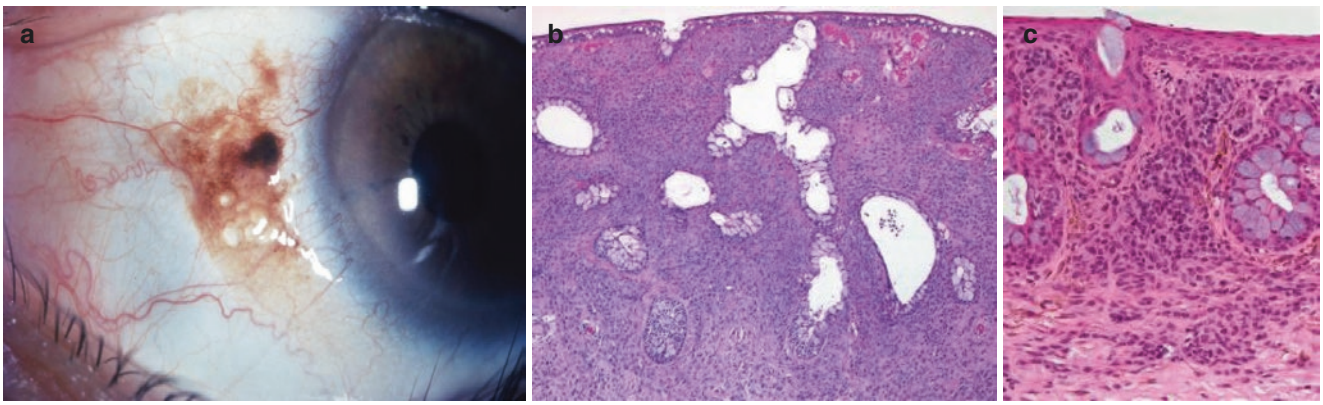

Fig. 10.19 (**a**) Clinical photograph of a conjunctival melanocytic nevus with cystic-like changes of the bulbar conjunctiva. (**b**) Low power view demonstrating the epithelial inclusion cysts within the conjunctival stroma and the surrounding nevus cells. (**c**) Differentiation of the nevus cells towards a spindle form is seen in most conjunctival nevi

Clinical aspects The nevus mostly arises in the first or second decade as a nodule in the bulbar conjunctiva.

Microscopy Their histology is similar to melanocytic skin lesions. A band of melanocytes in the basal layer of the epithelium represents the intraepithelial component. These melanocytes can be melanin-containing, but can also present as clear cells. Melanocytes can also be found in the epithelium of the inclusion cysts, which are almost invariably present. These large, mucin-containing cysts are formed by incarcerated epithelial nests and can give an erroneous clinical impression of growth. The stromal component is formed by nests of plump nevus cells with maturation to smaller cells in the deeper parts of the lesion (Fig. 10.19). Especially at young age, a considerable variation in cell size can be seen; these active lesions are easily overdiagnosed as invasive melanomas in children.

Treatment and prognosis Conjunctival nevi are usually kept under clinical observation, unless the patient would like it removed for cosmetic reasons. In the case of any change suggestive signs of malignant transformation, they can be treated like conjunctival melanoma (see below).

'Primary Acquired Melanosis' (PAM) and Conjunctival Melanocytic Intraepithelial Neoplasia (C-MIN)

Definition 'Primary acquired melanosis' (so-called PAM) arises in middle-aged or elderly patients as a stippling yellow-brown flat pigmentation of the conjunctiva. Two subgroups of PAM can be recognised: 'PAM without atypia' (also termed, benign acquired melanosis) and 'PAM with atypia'.

Microscopy In 'benign' acquired melanosis, there is hyperpigmentation of the basal layer, but there is only a mild increase in melanocytes. These melanocytes can be large, but show little or no cytologic atypia. In PAM with atypia, there is an increase in atypical melanocytes in the conjunctival epithelium. The atypia is graded mild to severe, although this grading varies considerably between pathologists, and the differentiation between severe atypia and melanoma in situ is difficult.

The term 'PAM' has been and remains controversial in the ophthalmic literature, particularly amongst eye pathologists. Recently, Damato and Coupland attempted to address this by introducing the term 'conjunctival melanocytic intraepithelial neoplasia' (C-MIN) with a scoring system to improve the reproducibility between pathologists in scoring these conjunctival melanocytic lesions [81]. The scoring system takes into account: (a) the horizontal pattern of atypical melanocytic spread, (b) the vertical extent of atypical melanocytic infiltration, and (c) the atypical cell features. The maximum score is 10. Hence, a conjunctival biopsy with purely hypermelanosis of the epithelium without any increase of melanocytes has a score of 0. A C-MIN score of 1–2 equates to 'PAM with mild atypia', C-MIN of 3–4 to 'PAM with moderate atypia', and C-MIN $\geq$5 to 'PAM with severe atypia/melanoma in situ'. The concept of 'conjunctival melanoma in situ' was included as an entity (and often as the precursor of invasive melanoma) for the first time in the seventh TNM staging system in 2010 (Fig. 10.20) [82]. This scoring system is currently undergoing validation in a multicentre collaborative European study and is being considered for use to help define 'conjunctival melanoma in situ' in the 8th and future TNM staging systems.

Immunohistochemistry Immunohistochemical stains are very useful in determining the extent of C-MIN; in particular, MelanA and MITF can highlight the atypical melanocytes (Fig. 10.20). To exclude invasive growth, use of the macrophage marker CD68 can be helpful in differentiating melanin-containing macrophages from potential infiltrating melanoma cells.

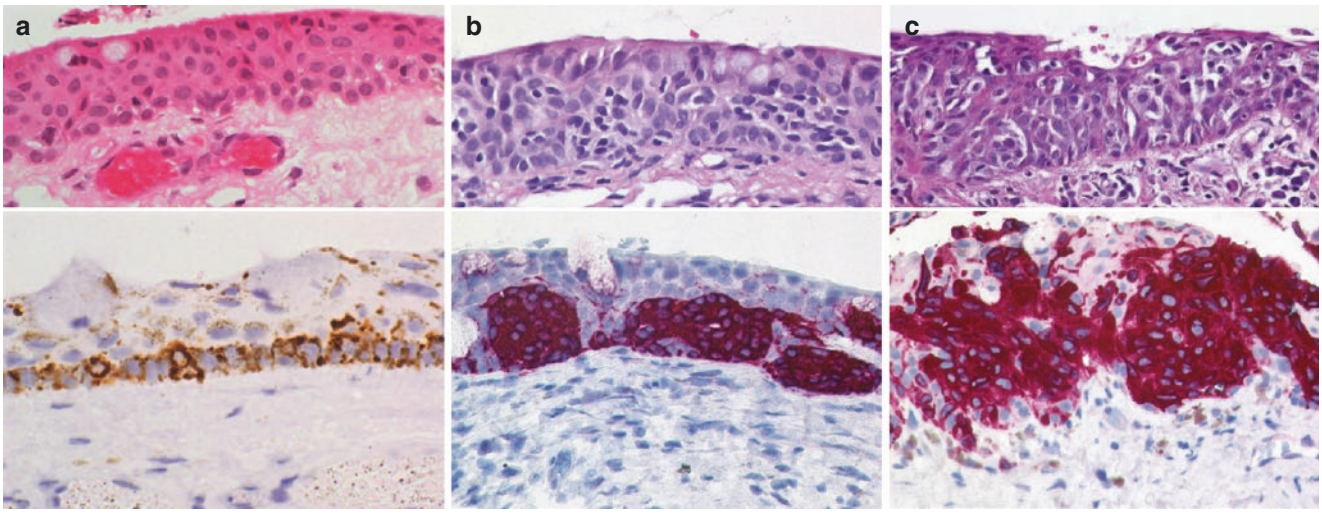

Fig. 10.20 Conjunctival melanocytic intraepithelial neoplasia (C-MIN): (**a**) A conjunctival biopsy showing melanosis only of the basal epithelial layer with some scattered melanocytes of dendritic form (PAM without atypia; C-MIN score of 2); (**b**) conjunctival biopsy with atypical melanocytes forming nests and occupying at least 50 % of the epithelium (PAM with moderate atypia: C-MIN score 5); (**c**) conjunctival biopsy with frank melanoma in situ (C-MIN score 10). The extent of epithelial involvement can be highlighted using the MelanA stain (*bottom panel*)

Treatment and prognosis Although the evolution of PAM is unpredictable, lesions without atypia tend to have a good prognosis. 'PAM with atypia' can disappear spontaneously, can remain stationary, or may progress to conjunctival melanoma in situ and ultimately invasive melanoma.

Patients with PAM without atypia are followed regularly with careful control, and no treatment is considered necessary. In the case of PAM with atypia, referral to an ocular oncology centre for further assessment and treatment is recommended. Treatment can consist of surgical excision, cryotherapy, topical chemotherapy (e.g. mitomycin C), plaque radiotherapy, or a combination of these [81].

Invasive Conjunctival Melanoma

Clinical aspects The majority of conjunctival melanomas arise within 'PAM with atypia' or in lesions given a C-MIN score of ≥5 [81]. Development of a conjunctival melanoma in a pre-existing nevus or de novo is possible, but uncommon [83].

Microscopy In conjunctival melanoma, clusters of atypical melanocytes are present in the stroma (Fig. 10.21). The melanocytes are most frequently epithelioid, but can also be spindle-shaped or have bizarre morphology. The intraepithelial component shows large, atypical melanocytes, often without ascending cells. This differs from skin melanocytic lesions, where ascending of melanocytes can be very helpful in diagnosing a malignant melanoma often without ascending. Cytological characteristics, such as high mitotic count, depth of infiltration, and lymphatic involvement, influence prognosis (Fig. 10.21).

Immunohistochemistry The melanoma cells are positive for most melanocytic markers – e.g. MelanA, MITF, SOX10, S100P, and HMB45. In contrast to conjunctival nevi where HMB45 demonstrates only superficial staining, in invasive melanomas HMB45 usually shows homogeneous positivity throughout the lesion. Cyclin D1 (BCL-1) is of use in highlighting particularly epithelioid cells within invasive melanomas. BRAF immunohistochemical staining is recommended as approximately 40–50 % of conjunctival melanomas have this mutation, and this information could be of value for further treatment of the patient, should they develop metastatic disease [84].

Treatment and Prognosis Wide surgical excision with cryotherapy to the margins is the gold standard treatment for invasive conjunctival melanoma. Patients with diffuse local disease may benefit of additional topical chemotherapy and plaque brachytherapy [81, 84]. Larger tumors are treated with radiation (e.g. plaque brachytherapy or proton beam therapy), enucleation, and sometimes orbital exenteration. The role of sentinel lymph node biopsy is uncertain; however, it may be useful when the lesion is large, and no metastasis is identified at clinical staging.

A recent analysis at a referral centre for ocular tumors demonstrated that poor prognosis was associated with incomplete excision at first treatment (particularly without the addition of adjunctive therapy in the form of brachytherapy or topical chemotherapy) and caruncular involvement [84].

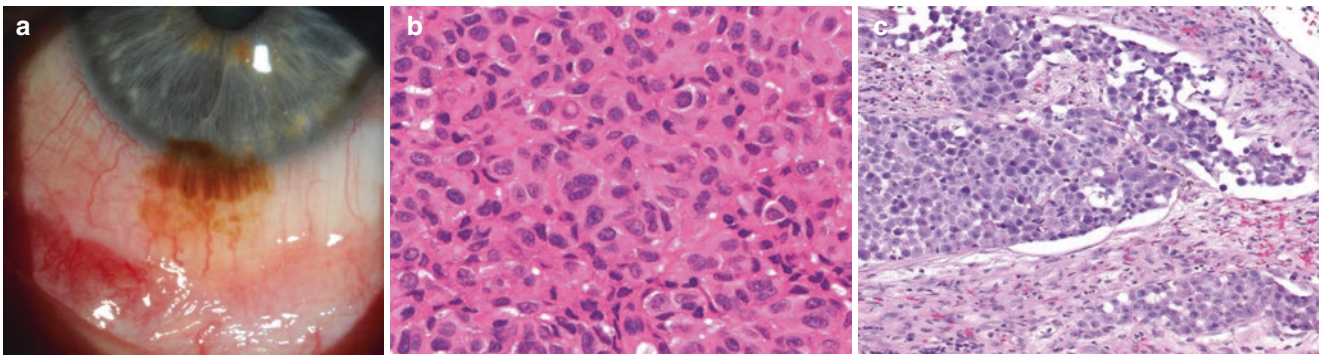

Fig. 10.21 (**a**) Clinical photograph of a partially pigmented conjunctival melanoma involving the lower lid fornices. (**b**) Histological examination shows an epithelioid cell melanoma with pleomorphic cells. (**c**) Clear involvement of conjunctival lymphatics is present

10.3.8.3 Conjunctival Lymphomas and Other Conjunctival Neoplasms

Conjunctival Lymphomas

Definition The conjunctiva contains specialised lymphoid tissue and is a part of the mucosa-associated lymphoid tissue (MALT) system acting as a barrier to both endo- and exoantigens. Reactive lymphoid hyperplasia (RLH) is a result of antigen stimulation of the conjunctival MALT system [85, 86]. RLH affects males and females equally and tends to occur in young patients. It has morphological, immunohistochemical, and molecular genetic features, which indicate that the lesion consists of polyclonal B and T lymphocytes in similar proportions.

On the other hand, non-Hodgkin lymphomas (NHLs) of B-cell type can also arise within this conjunctival MALT system [85–87] (Fig. 10.22).

Etiology and pathogenesis Extranodal marginal zone lymphomas (EMZLs) typically arise in conditions of chronic antigenic stimulation, as evidenced by the association of *Helicobacter pylori*, *Campylobacter jejuni*, *Borrelia burgdorferi*, and hepatitis C virus with EMZLs that arise in the stomach, small intestine, skin, and spleen, respectively [90]. Of note, when EMZLs are diagnosed at an early stage, removal of the antigenic stimulus may result in complete regression on the lymphoproliferation. The significance of *Chlamydia psittaci* with respect to conjunctival EMZL remains unclear: there appears to be substantial geographic variation in its association [91]. A relationship between autoantigens, present in autoimmune diseases, and ocular adnexal EMZL seems likely as evidenced by somatic mutation analyses of these tumors, which suggest an antigen selection process [92–94]. Recent evidence suggests that alterations of the *A20* gene, located on chromosome 6, are involved in the pathogenesis of conjunctival EMZL, particularly those EMZL without any evident chromosomal translocations [95]. Further, these alterations of *A20* are of clinical relevance in that complete A20 inactivation is associated with poor lymphoma-free survival, and the patients with A20 mutation/deletion required significantly higher radiation dosages than those without the A20 abnormalities to achieve complete remission [96].

Clinical aspects The clinical appearance of both conjunctival RLH and B-NHL is normally a 'salmon-patch'-like conjunctival swelling, without specific clinical signs that can aid differentiation between the two lesions (Fig. 10.22). The classical clinical signs of malignancy (e.g. growth, ulceration, invasion of surrounding tissue, feeder vessels, regional spread with lymph node enlargement) are features supporting the clinical diagnosis of lymphoma. However, whilst these features may be apparent in high-grade malignant lymphomas (e.g. diffuse large B-cell lymphoma), they are usually not present in the indolent conjunctival EMZL. Therefore, a tissue biopsy should always be considered when a conjunctival lymphoid tumor presents. The general rule should therefore be that these cases, even in children and young adults, should be suspected to be a lymphoma until proven otherwise.

Microscopy T-cell lymphomas are very rare and tend to represent secondary tumors – e.g. mycosis fungoides [88]. Between one-third and one-quarter of all ocular adnexal lymphoma are located in the conjunctiva. Conjunctival lymphoma consists of mainly four subtypes of B-NHL. The two low-grade malignant neoplasias are extranodal marginal B-cell lymphoma (EMZL) and follicular lymphoma, whilst the two high-grade B-NHLs are diffuse large B-cell lymphoma and mantle cell lymphoma. EMZL constitute about one-half of the conjunctival B-NHL [89].

Treatment and prognosis Should a conjunctival lymphoma be diagnosed, a full clinical workup should be performed. This is very important since up to one-third of the patients have either regional lymph node involvement and/or systemic involvement at the time of diagnosis without having symptoms [85]. Therefore, (even) the ophthalmologist

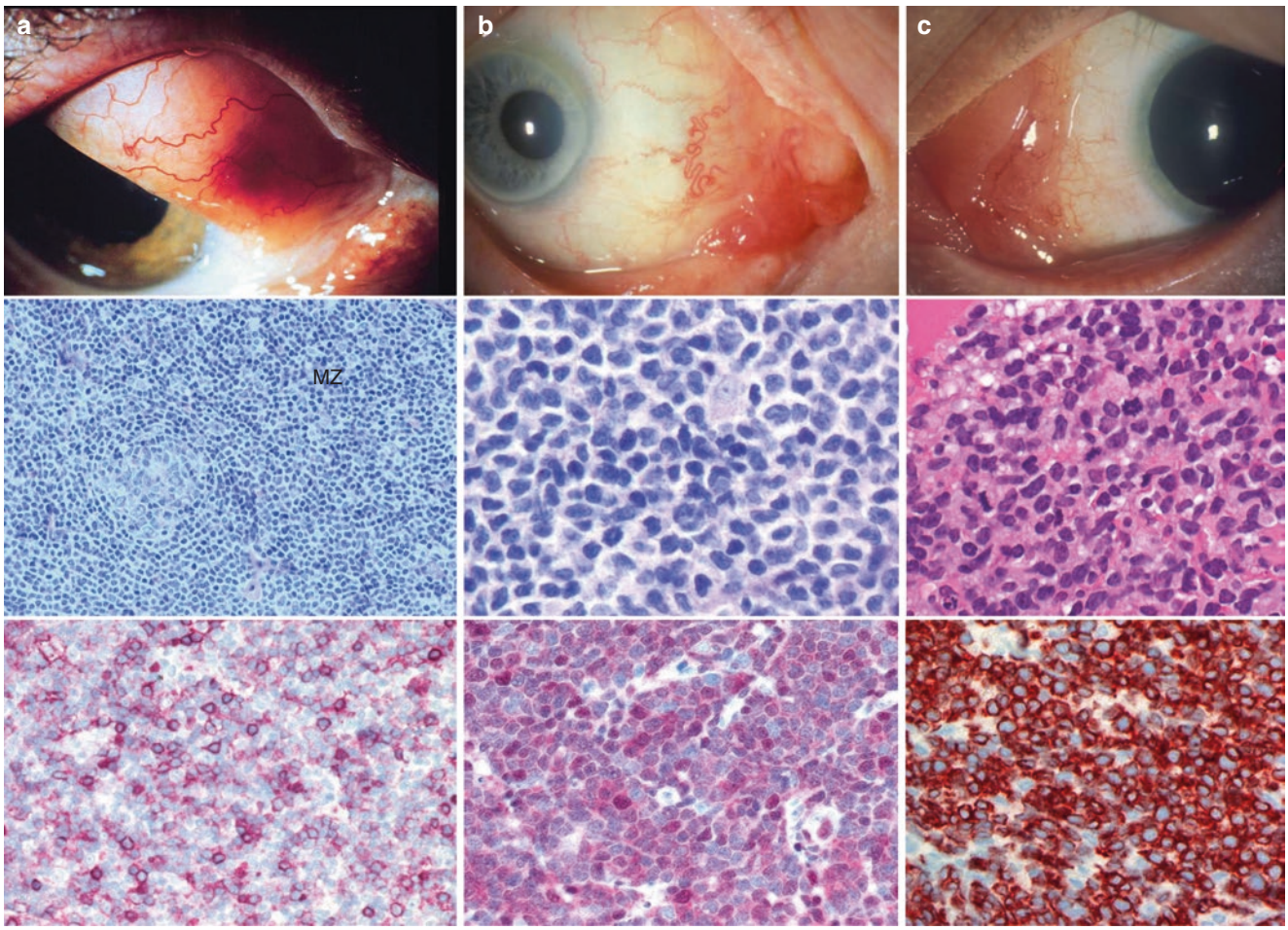

Fig. 10.22 Three examples of small cell non-Hodgkin lymphomas that require differentiation from each other using histological and immunohistological examination. Panel (**a**): Clinical photograph of a primary extranodal marginal zone B-cell lymphoma, with associated histology showing the proliferation of the neoplastic cells in the marginal zone (MZ), and aberrant positivity for the T-cell antigen. Panel (**b**): A small swelling in the region of the caruncle in an elderly male patient, composed predominantly of small cells with 'coffee-bean' like nuclei and scattered mitoses. Positivity of the cells for cyclin D1 enables the diagnosis of secondary mantle cell lymphoma involving the conjunctiva. Panel (**c**): A rapidly growing 'salmon patch' in the medial conjunctiva of a 45-year-old male. Biopsy revealed a pleomorphic lymphoid infiltrate with positivity for T-cell markers, consistent with a primary T-NHL of the conjunctiva

must probe the medical history of the patient regarding B-symptoms (i.e. night sweats, fever, and weight loss). Clinical staging of the ocular adnexal lymphomas can be performed using the conventional Ann Arbor staging system [97] and/or with the TNM-AJCC-based system, which was recently developed [98] and has been assessed by some centres [99, 100].

Treatment of localised conjunctival EMZL is usually low-dose external radiotherapy, but other options include excision only, excision and topical chemotherapy, as well as intralesional chemotherapy.

Other conjunctival neoplasms Included within this group are malignant neoplasms, which very rarely arise in the conjunctiva (e.g. angiosarcomas or leiomyosarcoma [101]), metastases to the conjunctiva (e.g. carcinomas of unknown primary tumors [102]), and those malignancies that arise in adjacent ocular adnexal locations and extend into the conjunctiva.

10.4 Cornea

10.4.1 Introduction

Inflammation and ulceration of the cornea can be caused by trauma, surgery of the eye, infectious diseases, and systemic diseases. The trauma can be mechanical or chemical or caused by heat or irradiation. The increasing incidence of cataract extraction led to an initial increase of cases with corneal damage during the surgical procedure; however, these procedures have since been improved resulting in diminishing

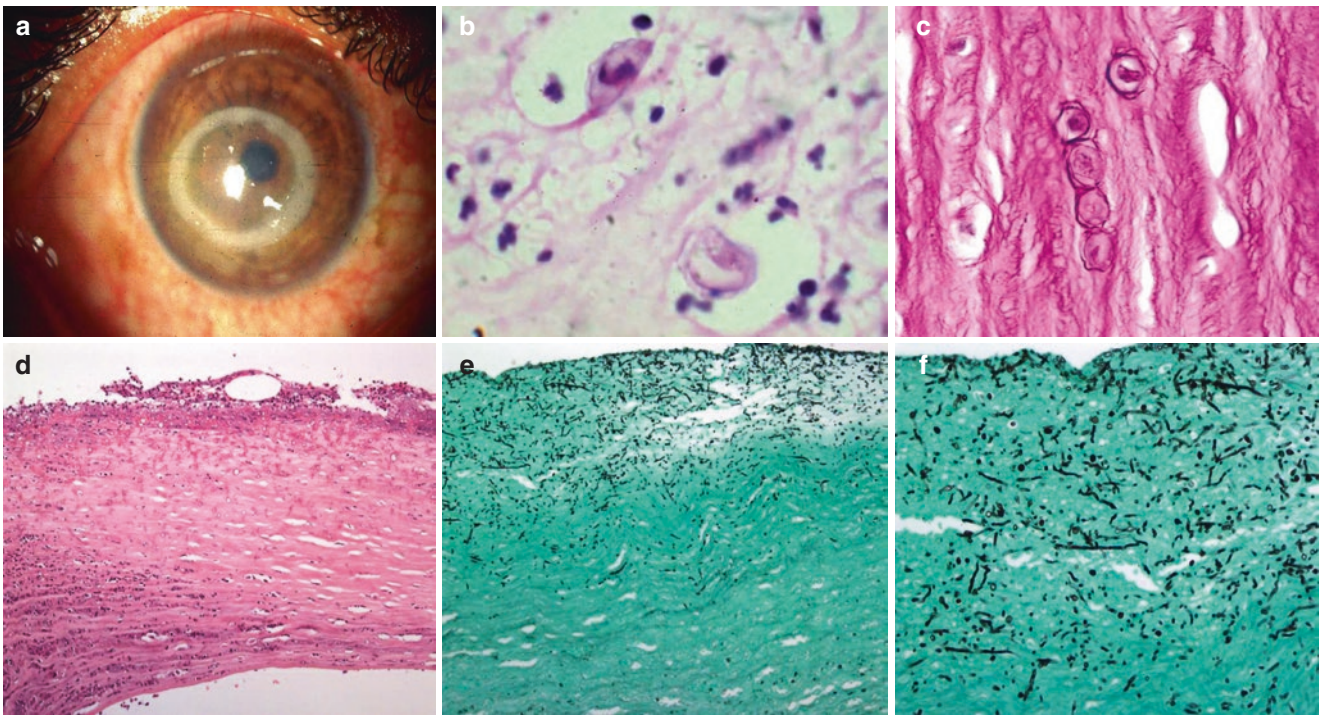

Fig. 10.23 (**a**–**c**) Clinical and histological pictures of a keratitis caused by acanthamoeba. (**d**–**f**) Severe fungal keratitis caused by *Candida*. (Photos courtesy of Dr Geeta Vemuganti)

corneal complications. Infectious keratitis can be viral, bacterial, fungal, or parasitic (Fig. 10.23). In chronic granulomatous keratitis, causes like leprosy, syphilis, and tuberculosis should be considered. Especially in immunocompromised patients, infectious causes due to unusual microorganisms should be excluded by additional stains, such as PAS, Grocott, and Gram. In corneal ulcers without a clear etiology, one has to think about keratitis induced by mechanical trauma, abuse of toxic eye drops and of cosmetic contact lenses, and indeed even of corneal tattooing [103].

Systemic diseases affecting the cornea are numerous (see below): an example of a systemic disease affecting the cornea is mucopolysaccharidosis (Fig. 10.24).

10.4.2 Keratitis and Corneal Ulcers

10.4.2.1 Herpes Simplex Keratitis

Epidemiology Type 1 herpes simplex virus (HSV) is the most common cause of viral corneal disease.

Etiology and pathogenesis After primary infection of the lip, the virus remains latent in the sensory trigeminal ganglion. Transneural migration and proliferation of the virus are triggered by stress, sunlight, or cold. The virus resides within the epithelium, leading to superficial epithelial loss in a branching pattern (dendritic ulceration) or punctate spots (punctate keratopathy). If the disease recurs, the virus may infect stromal keratocytes, causing chronic destruction with ulceration.

Microscopy Histologically, an early herpetic ulcer shows multinucleated epithelial cells with intranuclear viral inclusions. DNA in situ hybridisation, immunohistochemistry, and PCR are modern techniques replacing transmission electron microscopy, which was used to demonstrate the herpes virus particles previously [104]. In end-stage HSV keratitis, epithelial changes are no longer present, and only extensive fibrosis with scarring is seen. Often the Bowman's layer is focally replaced by fibrosis, rendering the cornea at high risk for perforation.

10.4.2.2 Corneal Ulceration Due to Systemic Disease

Systemic vasculitides like systemic lupus erythematosus, polyarteritis nodosa, and Wegener's granulomatosis can cause peripheral corneal ulceration due to vascular occlusion by immune complex deposition in the limbal vessels [105, 106]. In rheumatoid arthritis, corneal ulceration can occur due to the release of collagenases [107].

10.4.3 Keratoconus

Definition Keratoconus is a degenerative disorder of the eye in which structural changes within the central cornea

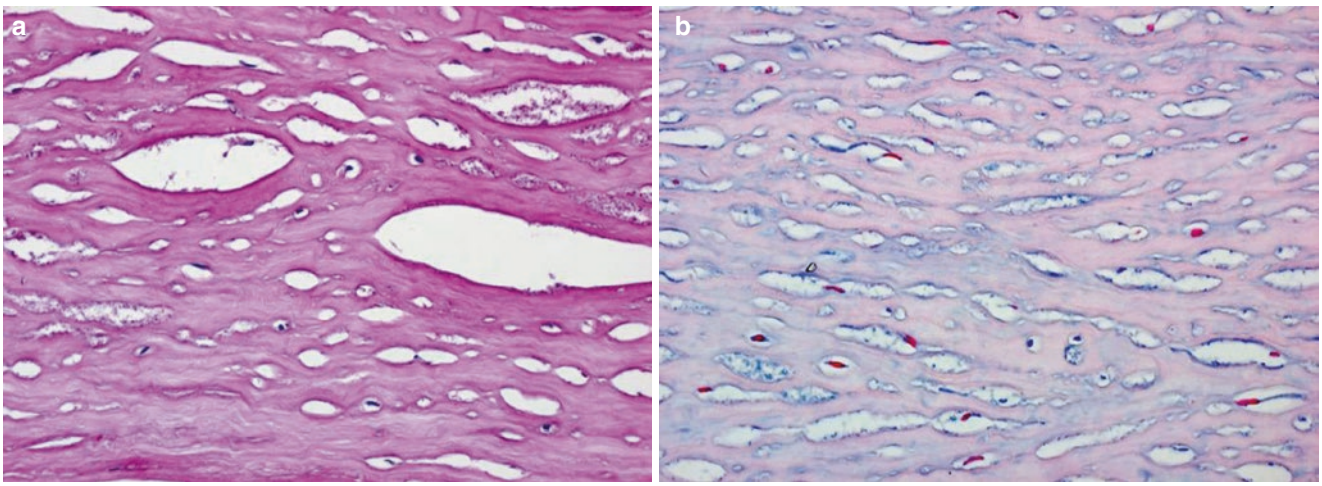

Fig. 10.24 (**a**) Corneal specimen of a patient with mucopolysaccharidosis. Deposits are seen between the collagen fibres causing disruption to the transparency of the cornea. (**b**) Alcian blue staining confirmed the suspected deposits. The family history revealed the diagnosis of mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome) (Photos courtesy of Prof. Stefan Seregard)

cause it to thin and change to a more conical shape than the more normal gradual curve (Fig. 10.25). It ultimately results in severe astigmatism and visual disability.

Epidemiology Estimates of the prevalence for keratoconus range from 1 in 500 to 1 in 2,000 people. This condition presents typically at puberty and has been found in association with systemic disorders, e.g. Marfan's syndrome, Down's syndrome [108, 109], neurofibromatosis, Ehlers-Danlos syndrome [110–112], and atopic dermatitis [113–115]. It can also be seen in combination with ocular disorders such as aniridia, cataract, and retinitis pigmentosa [116–118].

Etiology and pathogenesis The etiology and pathogenesis are unclear; however, a genetic predisposition to keratoconus has been observed, with the disease running in certain families, and incidences reported of concordance in identical twins. The frequency of occurrence in close family members is not clearly defined, though it is known to be considerably higher than that in the general population, and studies have obtained estimates ranging between 6 and 19 %. Two studies involving isolated, largely homogenetic communities have contrarily mapped putative gene locations to chromosomes 16q and 20q. Most genetic studies agree on an autosomal dominant model of inheritance.

Microscopy At histological examination the corneal epithelium can be either atrophic or hyperplastic (Fig. 10.25). The most striking finding is interruption of Bowman's membrane, with focal downgrowths of epithelium or upgrowths of corneal stroma in the breaking spot. The breaks may be narrow and the pathology is often restricted to a narrow 1–2 mm zone; sometimes serial sections are required to find the lesion. At the edge of the conus, iron can be found deposited in the epithelium (Fleischer's ring). The axial stroma shows mucoid degeneration; the peripheral stroma is of normal appearance. In severe cases, rupture of Descemet's membrane and the endothelium can occur, resulting in inflow of water and appearance of cystic spaces. Treatment and prognosis: Keratoconus can be treated by a number of means, including contact lenses as well as using a variety of surgical techniques.

10.4.4 Hereditary Corneal Dystrophies

10.4.4.1 Introduction

Corneal dystrophies are inherited, bilateral disorders that can be divided into epithelial, stromal, and endothelial abnormalities. Routine stains for a suspected corneal dystrophy must include PAS, Masson, Alcian blue, Congo red, and trichrome stains. In end-stage dystrophies, a keratopathy can develop, in which all layers of the cornea are involved. Many patients with corneal dystrophies have a point mutation in a gene on chromosome 5q31 [119–121]. The corneal dystrophies were classified in 2008 by the International Committee for the Classification of Corneal Dystrophies [122]. Since then, there have been further developments, reviewed by Vincent [123].

10.4.4.2 Epithelial Dystrophies

Definition Epithelial corneal dystrophies are Cogan's microcystic dystrophy, Meesmann's dystrophy, and Reis-Buckler ring dystrophy [124].

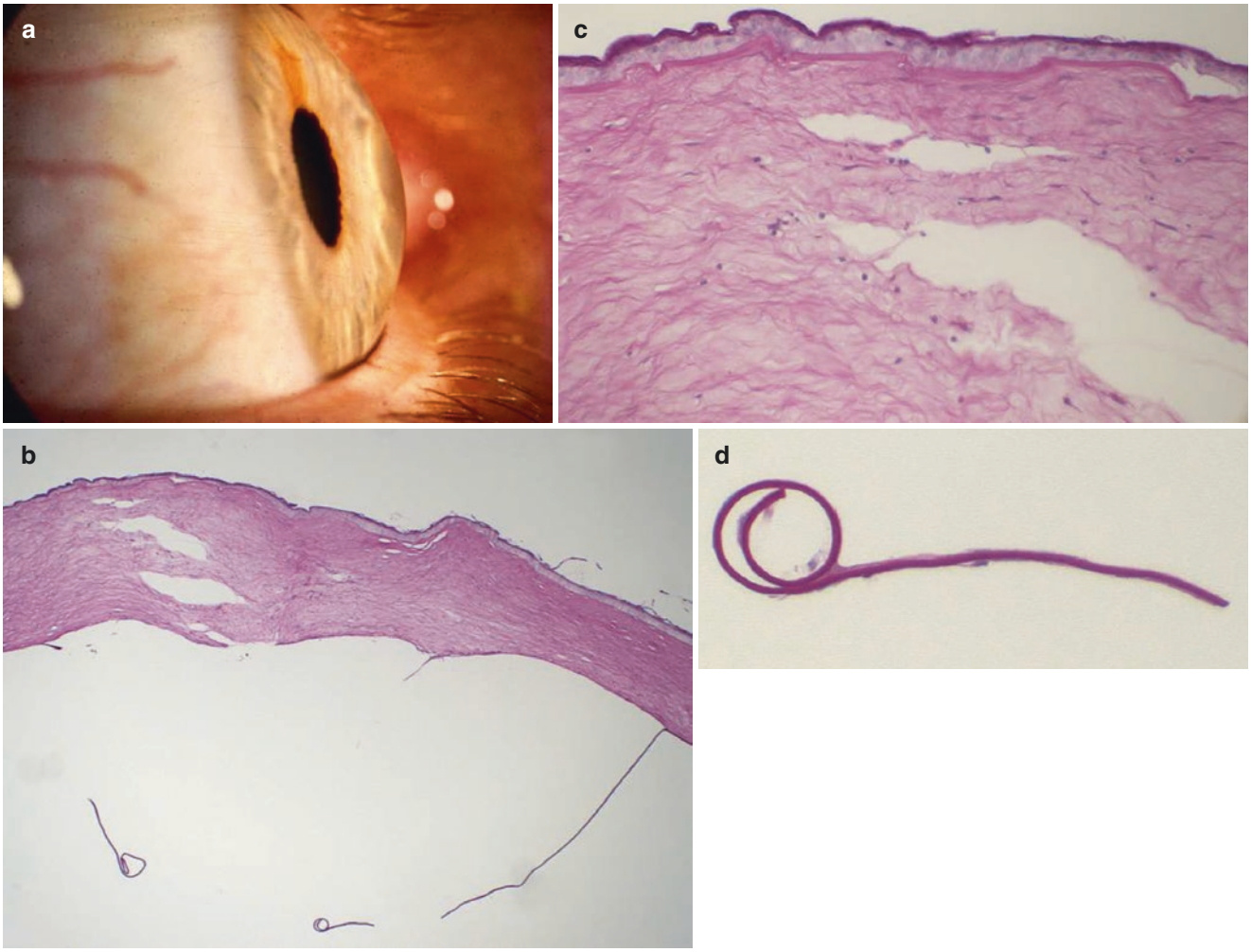

Fig. 10.25 (**a**) Clinical photograph of keratoconus. (**b**) Low power view demonstrates thinning of the cornea and oedematous changes. (**c**) Higher power view shows an irregular epithelium and Bowman's layer, with focal thickening and thinning. (**d**) The oedema is caused by the detached and rolled Descemet's membrane, which is covered by a very thin layer of endothelium (Photos courtesy of Prof. Claudia Auw Haedrich)

Clinical aspects The epithelial dystrophies present with photophobia and/or foreign-body sensation. The most common form of epithelial dystrophy is Cogan's microcystic dystrophy, affecting females of middle age.

Microscopy A thickened and folded basement membrane with epithelial cysts containing necrotic debris is characteristic. Bowman's membrane is not involved. In Meesmann's dystrophy small layers of the basement membrane can be found between the epithelial cells. Reis-Buckler ring dystrophy is a bilateral, autosomal dominant dystrophy, not only affecting the epithelium but also the anterior corneal stroma. The epithelium is oedematous and atrophic, Bowman's membrane is interrupted, and the anterior stroma contains abnormal fibrous tissue. Histology is not specific and transmission electron microscopy, which will show electron-dense rods in the superficial stroma, is necessary to confirm the diagnosis [125].

10.4.4.3 Stromal Dystrophies

Stromal dystrophies are granular dystrophy, lattice dystrophy, Avellino dystrophy, and macular corneal dystrophy. Their relevant features can be summarised as follows:

Granular dystrophy is an autosomal dominant disorder, presenting in early childhood with discrete, opaque granules in the otherwise transparent anterior corneal stroma. Histologically non-birefringent hyaline bodies are present in the stroma. The deposits are strongly positive with Masson stain (Fig. 10.26) [126].

Lattice dystrophy is an autosomal dominant disorder, clinically presenting in early childhood (type I) [127] or in the second decade (type II) with linear opacities [126, 128]. Histologically, eosinophilic deposits are found in the corneal stroma. They consist of amyloid and are strongly Congo Red positive [126, 128]. The disease is treated by keratoplasty.

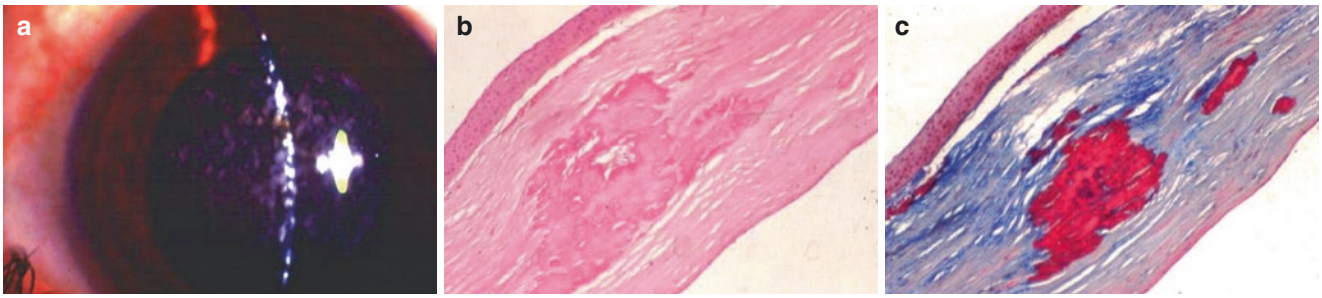

Fig. 10.26 (**a**) Clinical photograph of the slit lamp view of a patient's cornea with granular dystrophy. (**b**) The corneal button shows amorphous substances within the stroma. (**c**) Using the Congo red stain, this material is birefringent. These findings are typical of 'granular corneal dystrophy' caused by the mutation in the *TGFB1* gene located on chromosome 5q31

It has to be noted that the presence of amyloid in the corneal stroma can also be seen in chronic inflammatory conditions; these secondary amyloid deposits should not be confused with lattice dystrophy. *Avellino dystrophy* is a combined granular-lattice dystrophy with both hyaline and amyloid deposits in the corneal stroma, that was first described in the Italian village of Avellino [129].

Macular corneal dystrophy presents in childhood as a bilateral process with irregular opacities between cloudy corneal stroma. It is a disease leading to loss of vision, inherited as an autosomal recessive trait. The disease is considered to be a localised metabolic disorder with production of excessive amounts of acid mucopolysaccharide by fibroblasts [126, 130]. The disease is not associated with systemic mucopolysaccharidoses.

10.4.4.4 Endothelial Dystrophies

Fuchs dystrophy affects elderly patients and is quite common in routine histopathology. It presents clinically with bilateral diffuse cloudy and oedematous stroma. Histologically the corneal epithelium is oedematous, Descemet's membrane is thickened, and there is reduction of the endothelial cell population. A PAS stain sometimes shows lamination of Descemet's membrane. On the posterior surface of Descemet's membrane, nodular excrescences can be seen.

10.4.5 Failed Previous Grafts

Etiology and pathogenesis Many corneal diseases can be treated by keratoplasty. Complications are immunological graft rejection, formation of a retrocorneal fibrous membrane, epithelial downgrowth, and recurrence of the original disease.

Rejection of the graft can occur immediately after the operation or many years later.

Microscopy Histologically there is vascularisation of the corneal stroma, accompanied by a lymphocytic infiltrate, composed predominantly of CD4+ T cells. Formation of retrocorneal fibrous membranes occurs following fibroblastic metaplasia of keratocytes at the posterior edges of the host-graft junction. This complication diminishes with the advances in microsurgery and the introduction of new surgical procedures such as deep anterior lamellar keratoplasty (DALK) and Descemet's stripping automated endothelial keratoplasty (DSAEK). It is important for the pathologist to know about these new procedures and what tissue types will be sent to the laboratory for assessment.

Epithelial downgrowth refers to 'invasion' of either conjunctival or corneal epithelium along the surgical route, as a result of implantation. This epithelium extends through the corneal stroma into the anterior chamber, ultimately growing over the corneal endothelium and into the chamber angle, causing secondary angle closure. Due to the immunosuppressive environment within the eye, there is no rejection of this 'foreign' tissue, and the patients present with glaucoma. Immunohistochemical stains help to differentiate between corneal and conjunctival epithelium: cytokeratin (CK) 12 is restricted to corneal epithelium, whilst CK19 and MUC2 are considered to be conjunctival specific.

Prognosis Over a longer period of time, recurrence of the original disease is common in patients with corneal dystrophies.

10.5 Pathology of Intraocular Tissues

Pathology of the intraocular tissues can be divided into developmental anomalies, inflammatory processes, trauma, degeneration, and tumors.

10.5.1 Developmental Anomalies

10.5.1.1 Congenital Glaucoma

Epidemiology Glaucoma (i.e. increased pressure within the eye with the associated risk of optic atrophy and blind-

ness) due to congenital malformation is rare. Associations with systemic disorders like neurofibromatosis [131–133] and Sturge-Weber syndrome [134, 135] have been described.

Pathogenesis A malformation of the trabecular meshwork (the filter-like structure through which the aqueous humour exits before passing into Schlemm's canal and ultimately the systemic circulation) is termed goniodysgenesis. This or persistence of embryonic tissue in the chamber angle can cause an outflow obstruction. The corneoscleral envelope of the infant is distensible, so that a raised intraocular pressure can produce an enlargement of the globe (buphthalmos).

Microscopy A hypercellular trabecular meshwork with hyaloid degeneration is the best visible histopathological finding in these specimens with congenital glaucoma [136].

10.5.1.2 Retinopathy of Prematurity

Pathogenesis In premature children requiring artificial breathing with high oxygen pressures to survive, disordered neovascularisation at the periphery of the retina can occur [137].

Clinical aspects A white retrolental fibrous membrane can form in most extreme cases, causing bilateral blindness (retrolental fibroplasia). At the end stage of the disease, neovascular glaucoma caused by fibrovascular membranes developing between the iris and the posterior cornea resulting in angle closure can lead to enucleation. The distorted retina is macroscopically visible, most frequently forming a straight band behind the lens.

Microscopy Histological findings are dependent on the stage of disease but vary between a fragile neovascular membranes on the surface of the retina to frank retinal gliosis and optic atrophy [138].

10.5.1.3 Persistent Primary Hyperplastic Vitreous

Pathogenesis In the embryo, the lens is supported posteriorly by a mass of vascular tissue, the primary vitreous. If this primary vitreous fails to involute before birth, embryonic fibrovascular tissue persists in the anterior or posterior part of the vitreous and becomes hyperplastic.

Microscopy In persistent anterior primary hyperplastic vitreous, a retrolental fibrovascular mass can adhere to and possibly penetrate the posterior lens capsule and lens cortex, causing an autoimmune inflammatory response (Fig. 10.27). In persistent posterior hyperplastic primary vitreous, the fibrovascular mass damages the optic disc.

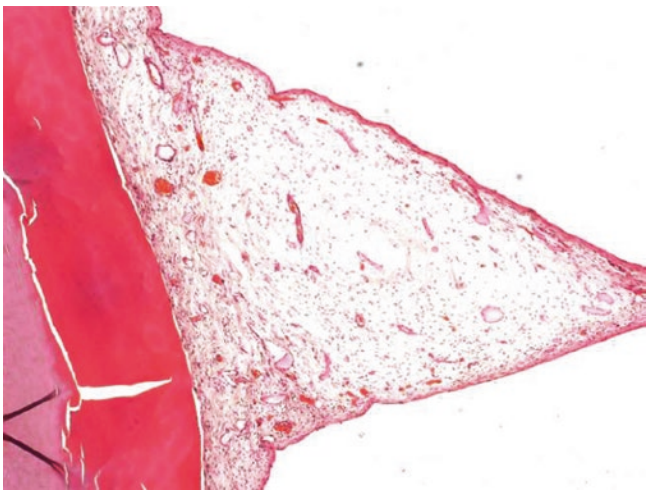

Fig. 10.27 Anterior primary hyperplastic vitreous, causing a retrolental fibrovascular mass

10.5.1.4 Retinal Dysplasia

Pathogenesis Failure of organisation of the layers of the retina leads to retinal dysplasia, in which nests of neuroblastic cells form rosettes within the retina. Clustering of rosettes leads to thickening of the retina, which may become detached.

Genetics Retinal dysplasia is a common feature of trisomy 13, trisomy 18, and other chromosomal disorders. As an isolated entity, retinal dysplasia is very rare [139].

10.5.1.5 Aniridia

Epidemiology Aniridia is a rare bilateral disease that is inherited as an autosomal dominant trait (85 % of cases) or occurs sporadically. An association of the sporadic form of aniridia has been demonstrated to be associated with Wilm's tumor of the kidney (Miller syndrome) and occurs in 13 % of cases.

Etiology and pathogenesis Most cases of aniridia are caused by mutations in the PAX6 gene on the short arm of chromosome 11. Miller syndrome is caused by deletions in 11p that include both the PAX6 gene and the tumor-suppressor gene WT. Because this locus lies close to the gene for nephroblastoma (Wilms' tumor), the recognition of a child with sporadic aniridia should alert physicians to the increased risk of development of Wilms' tumor [140, 141].

Macroscopy The designation 'aniridia' is actually a misnomer: these cases actually represent extreme hypoplasia of the iris, which are hidden clinically by opaque limbal tissues. Associated lens opacities and lens colobomas may occur, and foveal aplasia similar to that seen in albinism has been described.

10.5.1.6 Congenital Rubella Syndrome

Pathogenesis Maternal rubella infection during the first trimester of pregnancy can affect the development and function of the entire eye. It can manifest as congenital cataract, disciform keratitis, retinopathy, microphthalmus, or open-angle glaucoma [28, 142]. In societies with immunisation, the condition is rare.

Microscopy The histologic findings in the lens are characteristic, but it should be mentioned that the features may not yet be apparent in foetal eyes after early elective termination of pregnancy [143]. The central nucleus of the lens, which is normally free of cells, shows pyknotic nuclei. There is an abrupt transition from the central nucleus into the normal peripheral cortex of the lens [144].

10.5.2 Intraocular Inflammation

10.5.2.1 Acute Inflammation

Etiology Acute endophthalmitis or panophthalmitis can occur as a post-operative complication or following a trauma [145, 146]. Endocarditis or injection of contaminated material in drug addicts can be the cause of metastatic bacterial or fungal infection, especially in immunocompromised patients [147].

Microscopy Histology shows an extensive infiltrate of leucocytes with destruction of intraocular tissues. The pathogen can sometimes be found in Gram, PAS, or silver stains. Acute necrotising retinitis and a low-grade uveitis can be seen in cytomegaloviral and herpes infection. The characteristic eosinophilic nuclear inclusion bodies can be found in the cytoplasm and nuclei of infected cells. Immunohistochemical staining for anti-cytomegalovirus can be helpful in identifying the virus. The inclusions of herpes simplex retinitis can also be visualised with DNA in situ hybridisation.

10.5.2.2 Chronic Non-granulomatous Inflammation

Clinical aspects Chronic non-granulomatous inflammation of the uveal tract (i.e. uveitis) is a poorly understood condition. It can be divided into anterior uveitis, with risk of secondary angle closure glaucoma, and posterior uveitis with risk of degeneration of retinal pigment epithelium.

Microscopy The histology is non-specific, with only a moderate infiltration of lymphocytes in the uveal tissues.

10.5.2.3 Granulomatous Inflammation

Definition The specific granulomatous inflammations of the intraocular tissues can be divided into infectious and autoimmune causes. The autoimmune diseases are sarcoidosis, sympathetic ophthalmitis, and lens-induced uveitis.

Treatment and prognosis Whilst many of these diseases may be appropriately treated with immunosuppressive medication, the management of infectious uveitis is antimicrobial therapy. Inappropriate immunosuppressive therapy may be disastrous for patients with an infection. Chorioretinal biopsy may provide useful information for determining the diagnosis and guiding the subsequent management of patients with progressive chorioretinal lesions of unknown etiology [148].

Infectious

Epidemiology The most important causes of infectious granulomatous inflammatory diseases of the intraocular tissues are tuberculosis and toxoplasmosis.

Ocular Tuberculosis is relatively rare, occurring mainly on the Asian subcontinent. Toxoplasmic retinochoroiditis is caused by *Toxoplasma gondii* and occurs in neonates infected in utero or in adults who are typically immunosuppressed (Fig. 10.28a).

Microscopy Tuberculosis shows caseating granulomas, in which tubercle bacilli can be found in a Ziehl-Neelsen staining. Toxoplasmic retinochoroiditis can show a wide variation in the pattern of tissue destruction. The disease can be limited to a low-grade uveitis and retinal lymphocytic perivasculitis. In more severely affected eyes focal, sectorial or total retinal destruction can be seen (Fig. 10.28b). *Toxoplasma* cysts can be found in the retina and optic nerve. In the most severely affected eyes, the retina is necrotic and calcified [149].

Sarcoidosis

Clinical aspects In patients with systemic sarcoidosis, ocular involvement can occur. Most frequently ocular structures affected are the retina, the uveal tract, and the optic nerve.

Microscopy Histology shows sharply demarcated, non-caseating granulomas, surrounded by a T-cell-rich lymphocytic infiltrate [150, 151].

Sympathetic Ophthalmitis

Clinical aspects Sympathetic ophthalmitis is an uncommon, but feared complication because of its potential to blind both eyes. It can result not only from penetrating trauma or ocular surgery but also from non-penetrating ocular procedures.

Pathogenesis The condition seems to be caused by a T-cell-mediated autoimmune response.

Microscopy Histology of the enucleated traumatised eye shows a granulomatous uveitis with a thickened choroid with non-caseating granulomas with a few plasma cells and eosinophils, very similar to sarcoidosis [152]. Fine melanin granules can be seen in the cytoplasm of the histiocytes. The granulomatous inflammatory reaction can spread around small nerves.

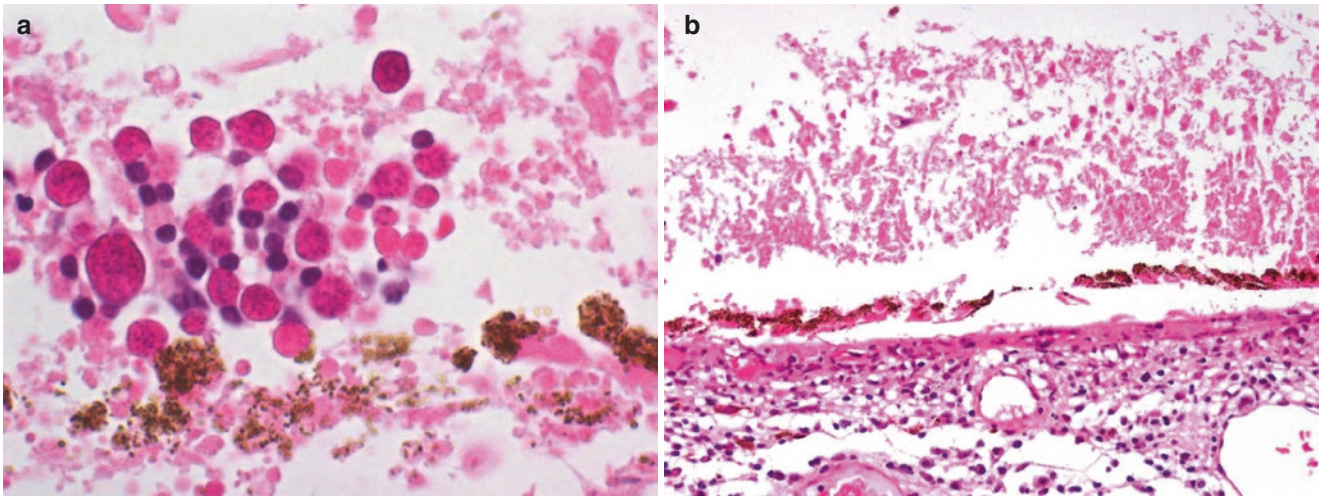

Fig. 10.28 (**a**) High power magnification of an enucleation specimen of an eye of a 50-year-old male who was immunosuppressed and severely affected by extensive combined ocular and cerebral toxoplasmosis. (**b**) The same case demonstrating a focal area of complete retinal necrosis, with the adjacent underlying RPE being detached and the choroid chronically inflamed

Treatment and prognosis Sympathetic ophthalmitis can be treated with immunosuppressive therapy. Infectious causes must be ruled out before starting this treatment. Early enucleation of the traumatised eye reduces the risk of occurrence of sympathetic ophthalmitis in the non-traumatised ('sympathetic') eye.

Lens-Induced Uveitis

Definition Lens-induced uveitis or 'phacoanaphylactic' endophthalmitis is a chronic endophthalmitis with a zonal granulomatous inflammation surrounding a ruptured lens.

Etiology and pathogenesis Most cases occur after trauma, surgical or non-surgical. Normally, small amounts of circulating lens proteins maintain a normal T-cell tolerance for lens proteins. Lens-induced uveitis develops when a breakdown occurs of this normal T-cell tolerance. Immune complexes play an important role in the tissue damage associated with the ensuing inflammation.

Clinical aspects The condition may result in vision-threatening intraocular inflammation that is poorly responsive to medical management.

Microscopy Leaking of lens proteins through an intact lens capsule may result in a lymphoplasmacytic anterior uveitis [153, 154]. The inflammation can be confined to the anterior aspect of the eye, but the choroid can also be involved.

Treatment and prognosis Surgical removal of the lens material is generally indicated shortly after the injury in an effort to save vision.

10.5.3 Trauma

Etiology and pathogenesis Mechanical injury is the most frequent cause of trauma of the eye. It can be caused by many different forces, such as broken glass, airguns, knives, firework fragments, and squash- and golfballs. Chemical, toxic, and radiation damage is less often seen.

Microscopy A wide range of foreign bodies can be found in those eyes (Fig. 10.29). The inflammation is usually mild or absent. The most important mission for the pathologist is to confirm the extent of irreparable damage. The lens is often absent or prolapsed through the corneal wound, a retinal tear shows the side of penetration, the vitreous is usually hemorrhagic, papilloedema is often present, and the retina can show exudative detachment.

Treatment and prognosis Traumatised eyes can be enucleated at three different moments in the time following the injury. The first moment to make the decision for enucleation is immediately or within a day or two following the injury. The globe is ruptured, a massive introcular bleeding is present, and there is evidence that repair will not restore visual function. To decrease the risk of sympathetic ophthalmitis, the eye will be enucleated in an early phase. When attempts to repair the eye are made, a mild uveitis or endophthalmitis may develop. Within a period of 2–3 weeks, the inflammation should diminish. If not, many ophthalmologists will make the decision to enucleate the eye to avoid the risk of 'symphathetic ophthalmitis' of the fellow eye. In those eyes, removed within a few weeks after the trauma, reparative changes like fibrous ingrowth of the corneal wound can be found. The blood in the vitreous will show organisation.

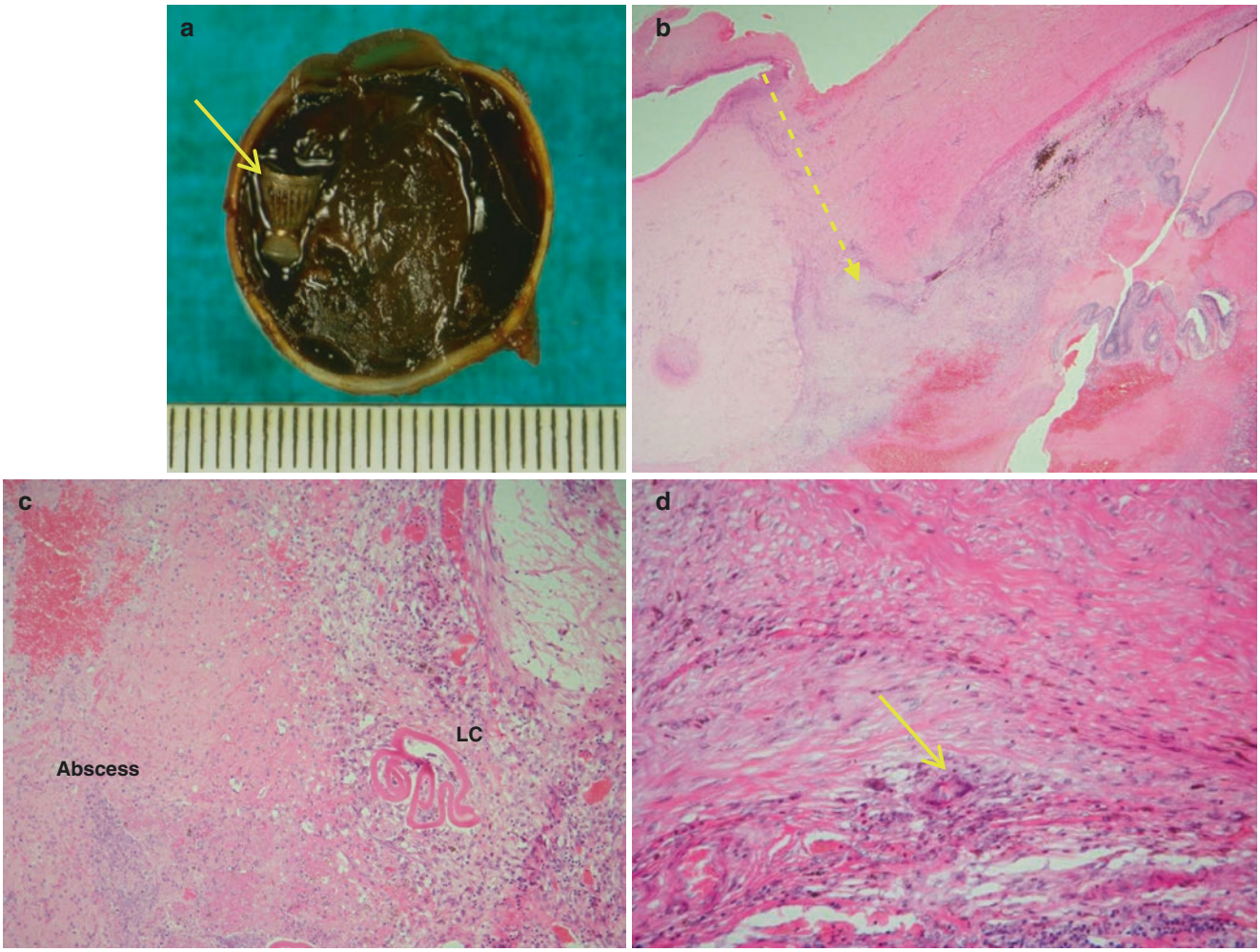

Fig. 10.29 (**a**) Enucleated traumatised eye showing with extensive haemorrhage in the posterior part of the eye and with a foreign body in situ (Courtesy of Dr Richard Bonshek). (**b**) Section of an enucleated eye showing the angle of perforation of the eye ball, with scarring at the limbus and destruction of the ciliary body and ciliary processes, detachment of the retina, and extensive haemorrhage. (**c**) High power of the same eye, showing the posteriorly displaced lens capsule remnant (LC) as well as an acute necrotising infection. (**d**) Higher power reveals some multinucleate macrophages, which contain birefringent material

Traumatised eyes with a residual vision and without inflammatory complications can become hypotonic and atrophic over years. Secondary glaucoma can develop and the eyes are removed because of pain or for cosmetic reasons. Frequently the secondary changes are very complicated and the primary pathology is not visible anymore. At macroscopic examination, the site of the trauma can be identified by presence of scars, suture tracks, or just episcleral thickening. Post-traumatic glaucoma is most often caused by secondary angle closure. Furthermore dislocation of the lens and lens-induced uveitis can be seen. The retina is usually partially or totally detached and is thickened by reactive gliosis.

10.5.4 Degeneration

10.5.4.1 Glaucoma

Etiology pathogenesis, and definition As mentioned above, the normal intraocular pressure (ca. 13–21 mmHg) in the corneoscleral envelope is maintained by a balance between the aqueous inflow and the resistance in the outflow system. The fluid is produced by the ciliary epithelium, passes through the pupil to the chamber angle, and leaves the eye via the trabecular meshwork and the canal of Schlemm to the collector canals in the sclera, draining into the episcleral venous system. Impaired outflow can cause high intraocular pressures, and when the intraocular pressure is

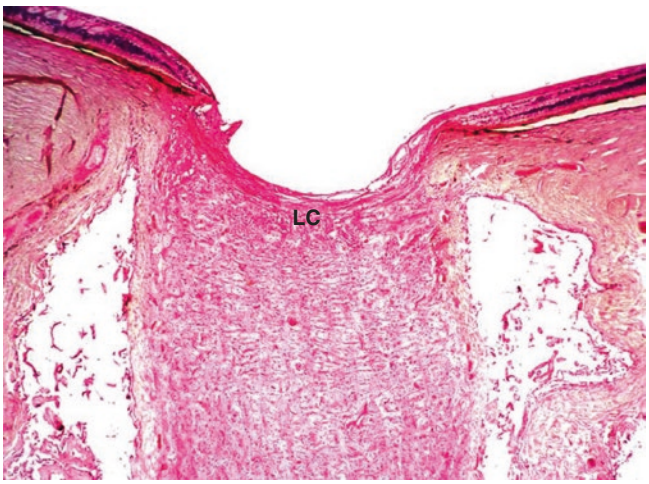

Fig. 10.30 Cross section of an optic nerve affected by advanced glaucoma, showing the classic 'cupping' of the optic disc to the level of the compressed lamina cribrosa (LC), with the degenerative changes of the axons in this area

high enough to cause damage to the intraocular tissues, the term 'glaucoma' is used. The primary cause for glaucoma can in most cases not be detected in the tissue without the complete clinical history.

Clinical aspects Depending upon the rapidity of the rise in pressure, glaucoma causes tissue damage. An intraocular pressure of 80 mmHg over a short time period (acute glaucoma) can cause severe corneal oedema, infarction of the iris, necrosis of the lens, and retinal oedema. When the pressure rises over a longer period of time, more chronic changes can be found. The trabecular tissue shows degenerative changes, the iris stroma and ciliary body become atrophic and fibrotic, and the nucleus of the lens becomes sclerotic. Atrophy of the optic disc is visible by 'cupping' and atrophy of fibres down to the level of the lamina cribrosa, which becomes bowed posteriorly (Fig. 10.30). Both the choroid and retinal pigment epithelia are able to withstand high pressures and will only show atrophy and fibrosis in end-stage disease.

Morphology Pathological examination of enucleated glaucomatous eyes is often complicated by previous surgical procedures. Glaucoma can be divided into four subgroups.

Primary Open-Angle Glaucoma

'Primary open-angle glaucoma' occurs predominantly in the elderly and is caused by an acquired unilateral or bilateral disease of the trabecular meshwork, visible at histopathological examination by hyalinisation of the trabecular meshwork.

Primary Angle Closure Glaucoma

In primary angle closure glaucoma, the aqueous outflow is obstructed by apposition of the iris to the inner surface of the cornea and the trabecular meshwork. The acute form of the disease occurs unilateral in middle-aged and elderly patients and presents with a rapid and painful rise in intraocular pressure. Both in acute and chronic angle closure glaucoma, three ageing processes seem to cause the closure of the angle: shrinkage of the eye, reduction in depth of the anterior chamber, and increased size of the lens.

Secondary Open Angle Glaucoma

Particulate or cellular elements present in the trabecular meshwork can cause outflow obstruction. Examples are iatrogenic glaucoma (caused by silicone oil, topical steroids, or viscoelastic substances used to coat lens implants), haemolytic glaucoma, lens protein glaucoma, post-traumatic glaucoma, and glaucoma in association with tumors (caused by necrotic cells of malignant melanomas and retinoblastomas). The outflow system can also be blocked by melanin pigment granules released from iris stroma or the pigment epithelium when the iris is traumatised or becomes atrophic. Infarction of a ciliochoroidal melanoma may also cause pigment dispersion and clumping of melanomacrophages may block the aqueous outflow pathway.

Secondary Angle Closure Glaucoma

In secondary angle closure glaucoma, an iridotrabecular or iridocorneal contact is present. This is most frequently caused by neovascular glaucoma, in which neovascularisation with fibrosis of the iris occurs, for example, in retinopathy of prematurity. Other causes are epithelial downgrowth (described above), end-stage inflammatory disease, retinal detachment, tumors (e.g. ciliary body melanomas), or trauma.

10.5.4.2 Cataracts

Prolonged exposure to ultraviolet light seems to be an important cause of cataracts, a frequent disorder in middle-aged to elderly patients. An extracted lens should be fixed, embedded in paraffin wax, and cut in two halves in anteroposterior direction. Slides can be made by sectioning the cut surface. Cutting the lens before processing can cause artefacts. In cataracts of the elderly degenerated lens, fibres form discrete globules and the epithelium covering the inner surface of the anterior lens capsule may extend to the posterior part of the lens. Similar observations are made in cataracts in children [155]. Other degenerations of the lens include pseudoexfoliation syndrome and siderosis (Fig. 10.31).

10.5.4.3 Phthisis Bulbi

A long period of time after a trauma or an inflammatory disease, the total eye may lose the pressure maintaining it and become atrophic. When the choroidal and retinal anatomy remains preserved, this is called 'atrophia bulbi'.

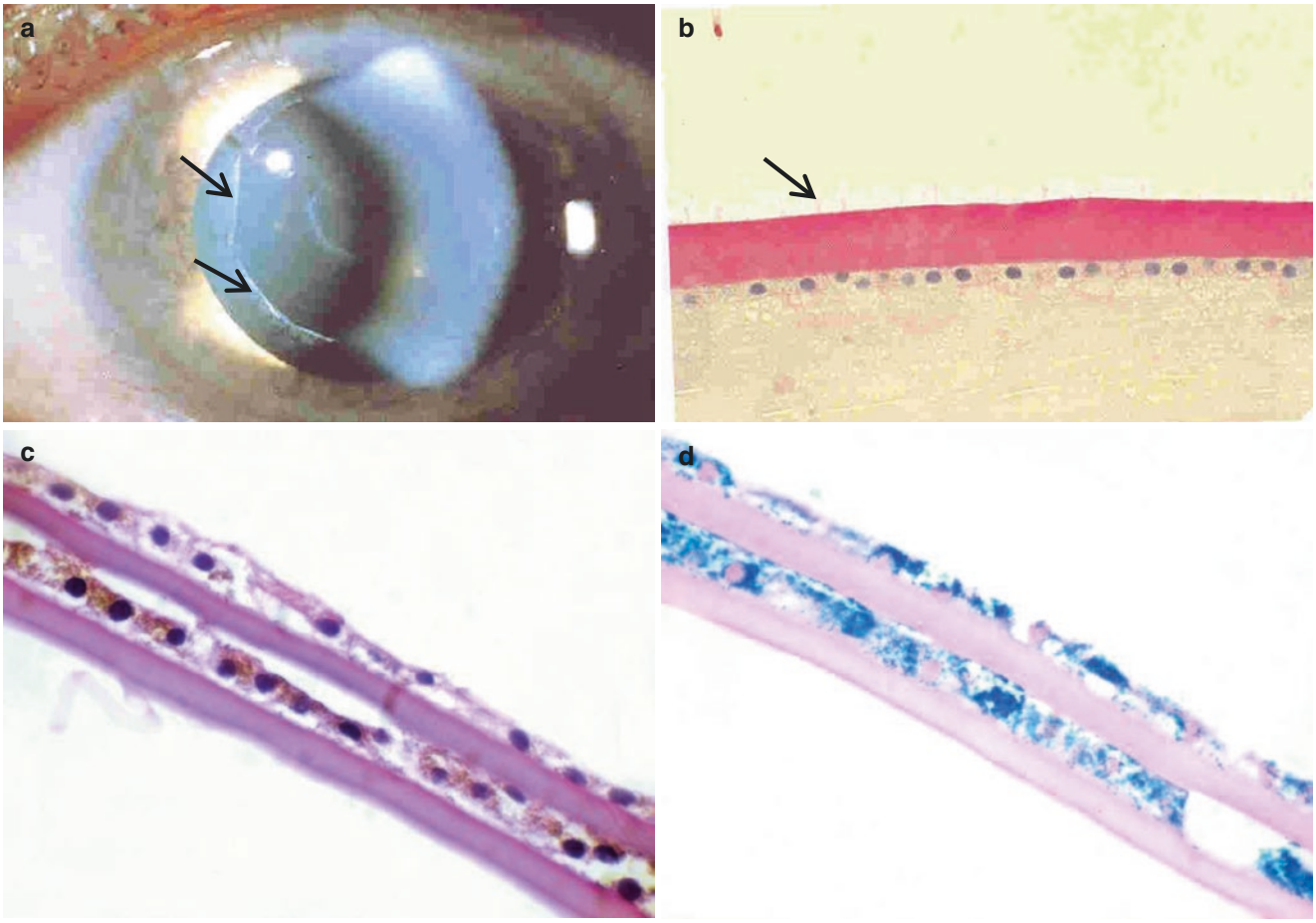

Fig. 10.31 (**a, b**) Degenerative changes of the lens associated with pseudoexfoliation syndrome, an age-related condition, manifesting itself in the eyes by the accumulation of microscopic granular amyloid-like protein fibres (*arrows*) (**b**). (**c**, **d**) Siderosis of the lens capsule can caused by scattered foci of rust-coloured material on the anterior surface of the lens and can be caused by intraocular haemorrhage or intraocular foreign bodies containing iron. The iron can be highlighted using the Prussian blue reaction

As soon as disorganisation of choroid and retina occurs, this condition is called *phthisis bulbi*. A reactive cell proliferation dominates the histology. This proliferation can be fibroblastic (trauma of cornea, sclera, choroidea, or iris) or gliotic (retinal damage). Further, proliferations of RPE or ciliary body epithelium can be seen and is often associated with dystopic calcification and, sometimes, even ossification. The optic nerve is usually completely atrophic.

10.5.4.4 Retinal Vascular Disease

Etiology and pathogenesis Loss of vision caused by ischaemic disease of the retina is common. It can be due to several different vascular disorders. Most frequently it is caused by central retinal vein occlusion, diabetes, or occlusion of a branch vein. More rare are vasculitis, retinopathy of prematurity, radiation retinopathy, central retinal artery occlusion, hypertension, and disseminated intravascular coagulopathy.

Clinical aspects Occlusion of an artery causes white infarction, whilst occlusion of veins leads to haemorrhagic infarction. The ischaemic area can vary between focal (occlusion of branch vessels), segmental, or total (occlusion of the central retinal vein or artery) (Fig. 10.32). Ischaemia of the retina with damage of retinal vasculature shows leakage of red cells, followed by neovascularisation and formation of microaneurysms and epiretinal membranes (Fig. 10.33). In the final stage, secondary angle closure glaucoma caused by neovascularisation on the surface of the iris (Fig. 10.34) and cataract formation, resulting in a not only blind but also painful eye. Those globes are often enucleated to relieve pain.

Microscopy At microscopic examination, the proliferation of endothelial cells in the retina is the most striking finding. Sometimes a CD34 and GFAP staining are necessary to differentiate the vascular proliferation from reactive gliosis (Fig. 10.35).

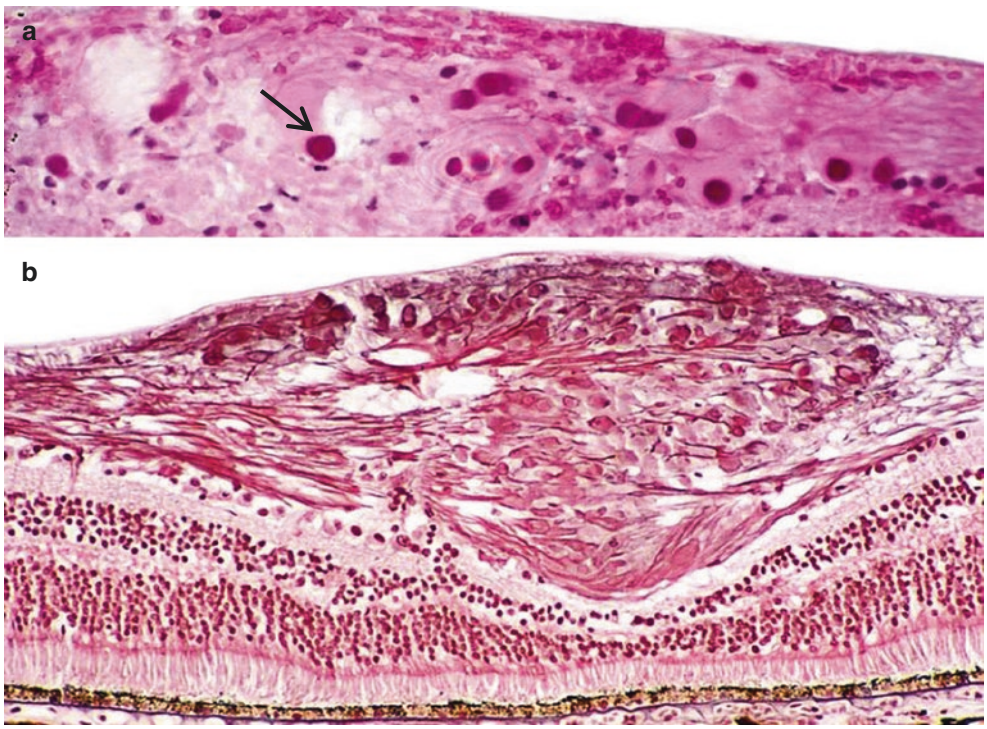

Fig. 10.32 (**a**) Cross section demonstrating a retinal microinfarct with associated degenerative changes in nerve fibre layer, with formation of so-called cytoid bodies (*arrow*). (**b**) With time, the ischaemic changes in the retina progress to fibrosis and formation of epiretinal membranes. (Images courtesy of Karin Loeffler)

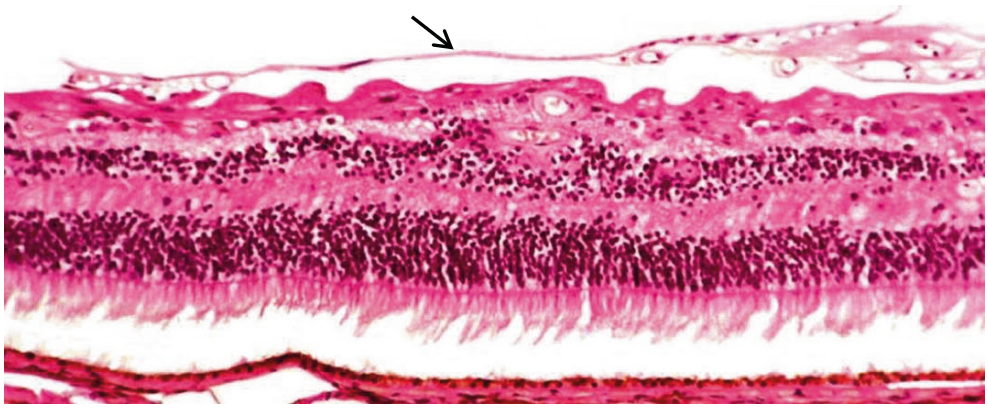

Fig. 10.33 Cross section of an epiretinal membrane (i.e. on the inner surface of the retina) in an eye with diabetic retinopathy. These are composed of fibroblasts and scattered macrophages, ultimately causing traction, wrinkling, and a detachment of the retina

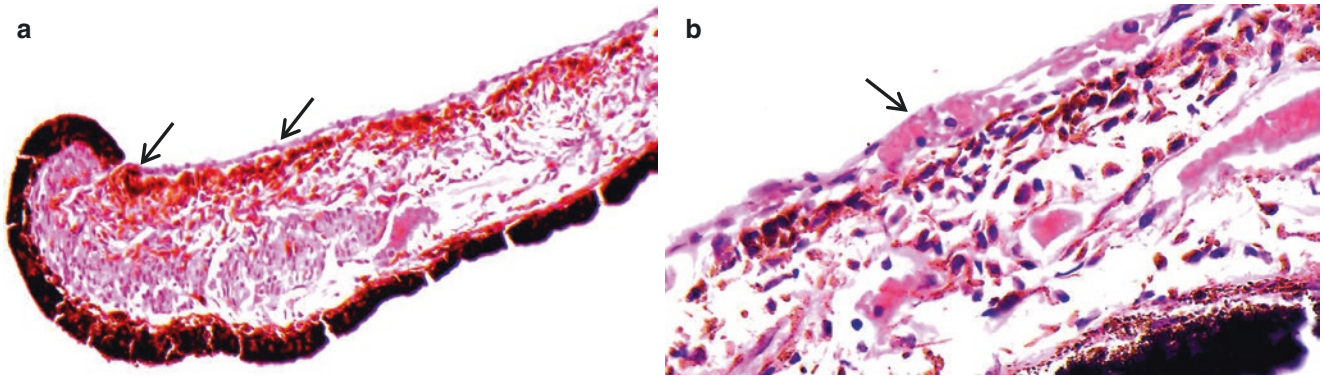

Fig. 10.34 (**a**) Ectropion of the iris with the formation of very fragile neovascular membranes on the anterior surface (*arrows*), causing its leaf to turn outwards (i.e. towards the cornea). Such membranes, seen in higher power in (**b**), form as a result of various stimuli, including diabetes mellitus and radiation treatment of large choroidal melanomas with release of substances from the ischaemic or 'toxic' tumor

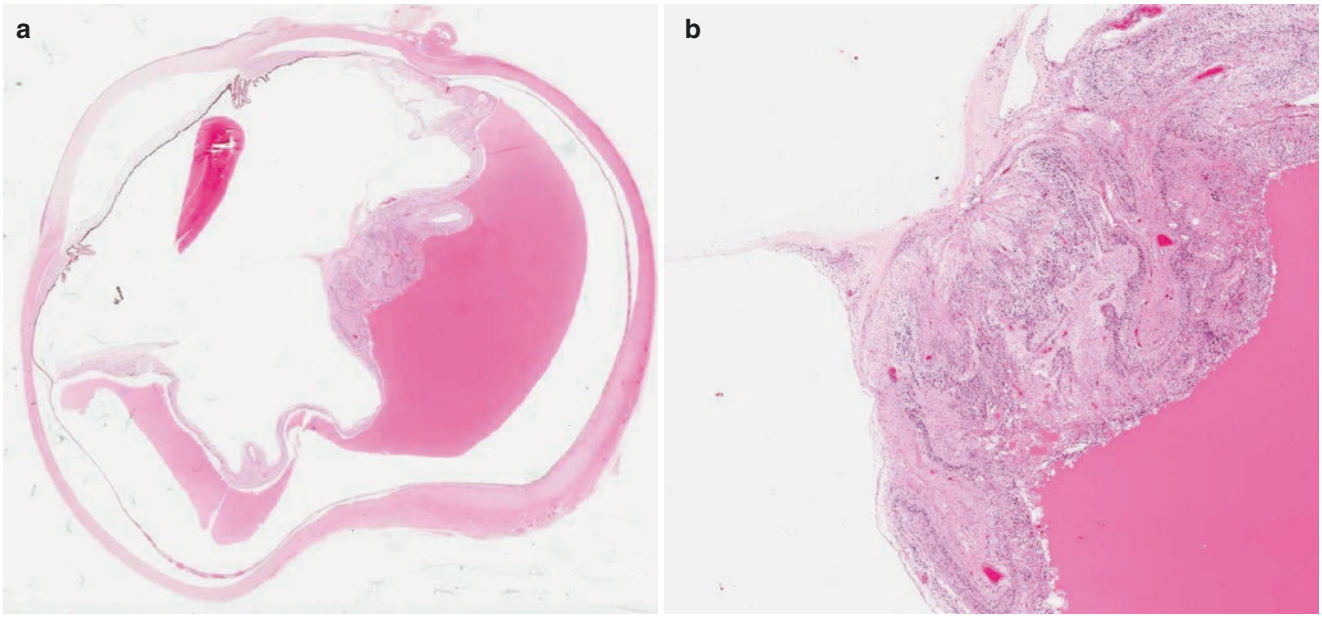

Fig. 10.35 (**a**) Enucleation of an eye with advanced degenerative changes as a result of diabetes with total retinal detachment and (**b**) with formation of a retinal 'pseudotumor'. Underneath the retina is an extensive exudate, containing only scattered macrophages and RPE debris

10.5.4.5 Retinal Detachment

Several degenerative conditions predispose to retinal detachment. The separation between the neural retina and the retinal pigment epithelium can be caused by traction, exudate, or the so-called 'rhegmatogeneous' detachment. Traction detachment occurs when the vitreous shows fibrosis or gliosis, following trauma or by neovascularisation (Fig. 10.35). Accumulation of fluid between the layers of the retina is called exudative detachment. It can be caused by processes with excessive permeability of retinal or choroidal vessels, like inflammatory or neoplastic disorders. In rhegmatogeneous detachment, passage of fluid from the vitreous cavity to the subretinal space is present. It occurs through a hole in the retina, caused by degeneration or a minor trauma. Enucleated eyes with retinal detachment usually show many signs of previous surgical intervention. The most important information for the surgeons is whether the retina survived the separation and reattachment or not and if a reason for surgical failure can be found.

10.5.4.6 Retinitis Pigmentosa

Epidemiology and pathogenesis Retinitis pigmentosa (RP) inherited degenerative eye disease that causes severe vision impairment, due to the progressive degeneration of the rod photoreceptor cells in the retina. This form of retinal dystrophy manifests initial symptoms independent of age; thus, RP diagnosis occurs anywhere from early infancy to late adulthood. There are multiple genes that, when mutated, can cause the RP phenotype. Inheritance patterns of RP have been identified as autosomal dominant, autosomal recessive, X-linked, and maternally (mitochondrially) acquired and are dependent on the specific RP gene mutations present in the parental generation. In 1989, a mutation of the gene for rhodopsin, a pigment that plays an essential part in the visual transduction cascade enabling vision in low-light conditions, was identified [156]. Since then, more than 100 mutations have been found in this gene, accounting for 15 % of all types of retinal degeneration [157]. Most of those mutations are missense mutations and inherited mostly in a dominant manner.

Clinical aspects Retinal architecture remains best preserved at the macula, so the patient often is left with tunnel vision.

Microscopy Histological examination shows retinal atrophy with proliferation of Müller cells (retinal supporting cells at the outer side of the retina), replacing the outer nuclear layer. The RPE proliferates and can surround small hyalinised vessels in the retina. A marked variation in the extent of retinal degeneration can be seen in two relatives with retinitis pigmentosa [158].

10.5.5 Tumors and Tumor-Like Conditions

10.5.5.1 Melanocytic

Melanocytes in the uveal tract – comprising the iris, ciliary body, and the choroid – can give rise to both benign and

malignant tumors. Racial differences may reflect themselves by variance in prominence and enlargement of melanocytes in choroid, ciliary body, and iris. It is very important for pathologists to be aware of those differences.

Nevus

Iris Nevi

Epidemiology Iris nevi present as pigmented macular lesions, very slowly progressive, and often completely static over years.

Clinical aspects When the clinical presentation is not suspicious, in most cases the lesion will not be excised. For that reason, nevi of the uveal tract are most commonly incidental findings.

Microscopy Histology shows a symmetrical lesion, located in the anterior part of the iris stroma and usually composed of small spindle cells with small, uniform nuclei. Large nucleoli and especially mitotic figures are suspicious for a melanoma.

Ciliary Body Nevi

Epidemiology In the ciliary body, nevi are very rare and typically are seen as incidental findings in eyes enucleated for other reasons [159].

Microscopy The histology is comparable with iris nevi: spindle-shaped cells without atypia and without mitotic figures. A rare variant of a ciliary body nevus is called a 'melanocytoma' (also termed magnocellular nevus), which are heavily pigmented benign tumors (Fig. 10.36).

Choroidal Nevi

Choroidal nevi are observed more often clinically than histologically, but are composed of spindle-shaped cells that are often heavily pigmented, with uniform nuclei in the absence of mitotic figures (Fig. 10.36). Depigmentation of the slides may be helpful in evaluating cytological details. They can also occur at the optic nerve head as nodular lesions. Very occasionally, they undergo malignant transformation to melanomas.

Uveal Melanoma

Epidemiology and pathogenesis Uveal melanomas can arise in the iris, ciliary body, and the choroid, with the majority occurring in the latter (Fig. 10.37). They occur predominantly in fair-skinned individuals in the sixth and seventh decades of life, with a frequency of six cases per million individuals per year in Europe [160]. Most choroidal melanomas are single and confined to one eye, although exceptions with bilateral choroidal melanomas, and indeed multiple tumors within one eye, do occur. They are thought to arise from pre-existing nevi in the choroid, although residual nevi are rarely seen on histological examination of a melanoma.

Clinical aspects Choroidal melanoma patients present with a variety of symptoms, including decreasing vision in one eye, photopsia (i.e. perceived flashes of light), and a mass seen through the lens or with secondary closed angle glaucoma in ciliary body tumors. In rare and advanced cases, extraocular spread may occur into the orbit causing pain and proptosis. The main clinical differential diagnosis for choroidal melanoma is metastasis of another malignancy to the eye.

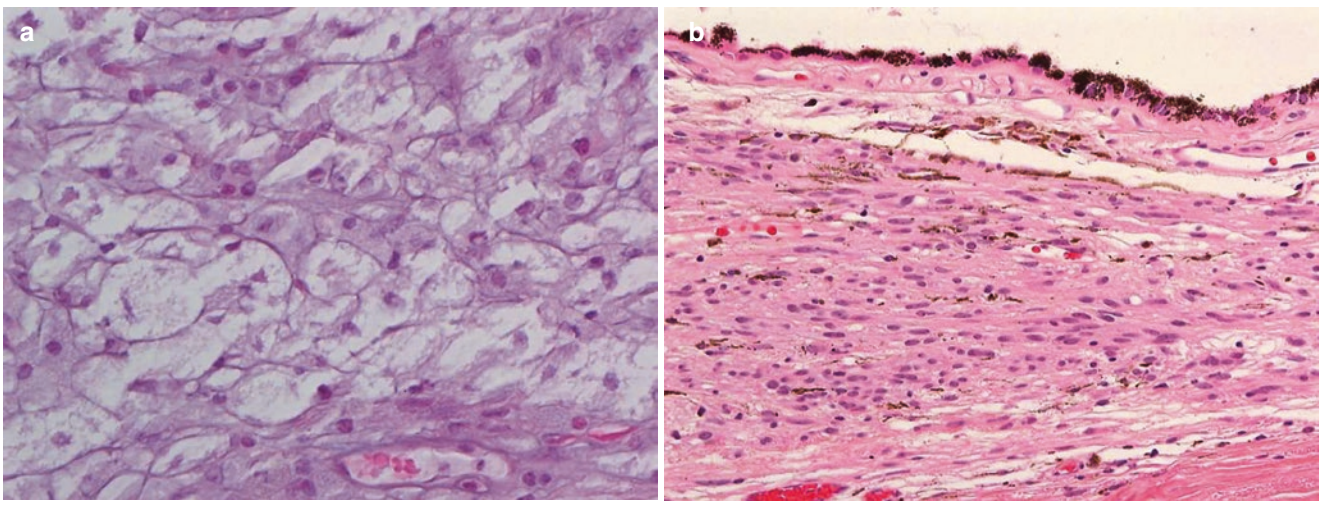

Fig. 10.36 (**a**) Bleached stain of a magnocellular nevus of the optic nerve head. (**b**) Typical benign choroidal nevus composed of bland spindle cells with large amounts of cytoplasm and scattered pigmented melanocytes

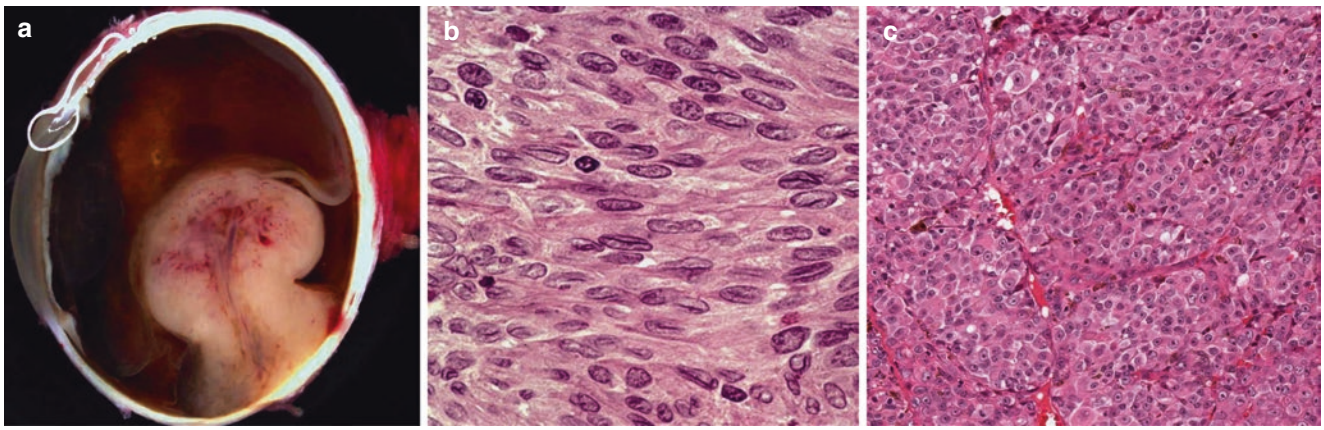

Fig. 10.37 (**a**) Macroscopic photograph of an enucleated eye with a non-pigmented choroidal melanoma with the classic 'mushroom' shape, caused by perforation of Bruch's membrane and strangulation of the tumor around its centres. The overlying retina is detached. (**b**) Spindle cell choroidal melanoma. (**c**) Epithelioid cell choroidal melanoma with prominent 'connective tissue loops' which can be highlighted in the PAS stain

Macroscopy At macroscopic examination of an enucleated eye with a choroidal melanoma, it is important to locate the tumor before cutting the eye. The tumor can be located by palpation and transillumination. If possible, the main histological section should contain the centre of the pupil, the optic nerve, and the centre of the tumor. Small pigmented nodules can be seen on the external surface of the sclera in case of trans-scleral extension of the tumor. The sample taken at this point sometimes needs section at multiple levels to demonstrate the tumor passing through the scleral canals.

Microscopy Microscopically, the melanomas consist of spindle-shaped cells, epithelioid cells, or a combination of both (mixed cell type) and are classified according to the modified Callender system (Fig. 10.37) [161]. The spindle-shaped cells are closely packed elongated cells, often with pronounced nucleoli and a few mitotic figures. In epithelioid lesions, the cytoplasm is more eosinophilic and mitotic figures are easily found. Melanin pigment usually is present, but amelanotic lesions can be seen. The final report should include the origin of the tumor (choroid, ciliary body, iris), the thickness of the tumor (in mm), the cell type (spindle cell type, epithelioid cell type, or mixed cell type), and presence or absence of extraocular growth. Should the latter be present, it should be measured in accordance with the 7th edition of the TNM staging system [162].

Immunohistochemistry Uveal melanoma cells are positive for typical melanocytic markers, such as MelanA, MITF, HMB45, and SOX10. Lack of nuclear BAP1 protein expression correlates well with the underlying inactivating deletions in the gene [163].

Genetics For extensive details, the reader is referred to Coupland et al. [164]. Signalling pathways known to be disrupted in uveal melanoma include: (1) the retinoblastoma pathway, probably as a result of cyclin D1 overexpression; p53 signalling, possibly as a consequence of MDM2 overexpression; and the P13K/AKT and mitogen-activated protein kinase/extracellular signal-related kinase pathway pathways that are disturbed as a result of *PTEN* and *GNAQ/11* mutations, respectively. Characteristic chromosomal abnormalities are common and include 6p gain, associated with a good prognosis, as well as 1p loss, partial, or complete loss of chromosome 3 and 8q gain, which correlate with high mortality. These are identified by techniques such as fluorescence in situ hybridisation (FISH), comparative genomic hybridisation (CGH), microsatellite analysis (MSA), multiplex ligation-dependent probe amplification (MLPA), and single nucleotide polymorphism (SNP) arrays. UM can also be categorised by their gene expression profiles as 'class 1' or 'class 2', the latter correlating with poor survival, as do BRCA1-associated protein 1 (*BAP1*)-inactivating mutations.

Treatment and prognosis Current treatments for choroidal melanoma include radiation (e.g. plaque brachytherapy, proton beam radiation, transpupillary thermotherapy) and/or surgery (transscleral tumor resection, endoresection, and enucleation) and are usually effective in the local control of the disease [165]. However, up to 50 % of choroidal melanoma patients develop metastases within a median time of 5 years following diagnosis of the ocular tumor. The liver is the site of first metastasis in 90 % of patients, with a median survival in patients with metastatic disease ranging from 3 to 12 months. The risk of metastasis can be determined by the combination of clinical, histomorphological, and genetic features of the tumors [164]. In particular, the choroidal melanomas with high risk of metastasis are those with the following features: a large basal diameter, involvement of the

ciliary body, epithelioid cell morphology, high mitotic count, closed connective tissue loops, and the complete or partial loss of chromosome 3 with polysomy 8q [165].

10.5.5.2 Lymphoid

Our understanding of intraocular lymphomas has advanced with progress in lymphoma classification systems, which are based on morphological, immunophenotypical, and genotypical features of each entity. This knowledge is being 'fine-tuned' with technological advances in molecular pathology, and improved surgical techniques now provide more tumor material for investigation. Essentially, the intraocular lymphomas can be divided into four major types: (a) vitreoretinal lymphoma, (b) primary choroidal lymphoma, (c) primary iridal lymphomas, and (d) secondary intraocular (usually choroidal) lymphomas. For a review on the varying intraocular lymphomas, see Coupland and Damato [166].

Vitreoretinal Lymphoma

Vitreoretinal lymphoma (VRL) is a high-grade lymphoma, usually of B-cell type, and is associated with a poor prognosis because of its tropism for the central nervous system (CNS) (Fig. 10.38). Diagnosis is on the basis of morphology and immunocytology, with adjunctive investigations including, clonality analysis, cytokine ratio, as well *MYD88* mutation detection [167, 168]. Immunophenotyping and somatic mutation analysis suggest derivation of most VRL from an

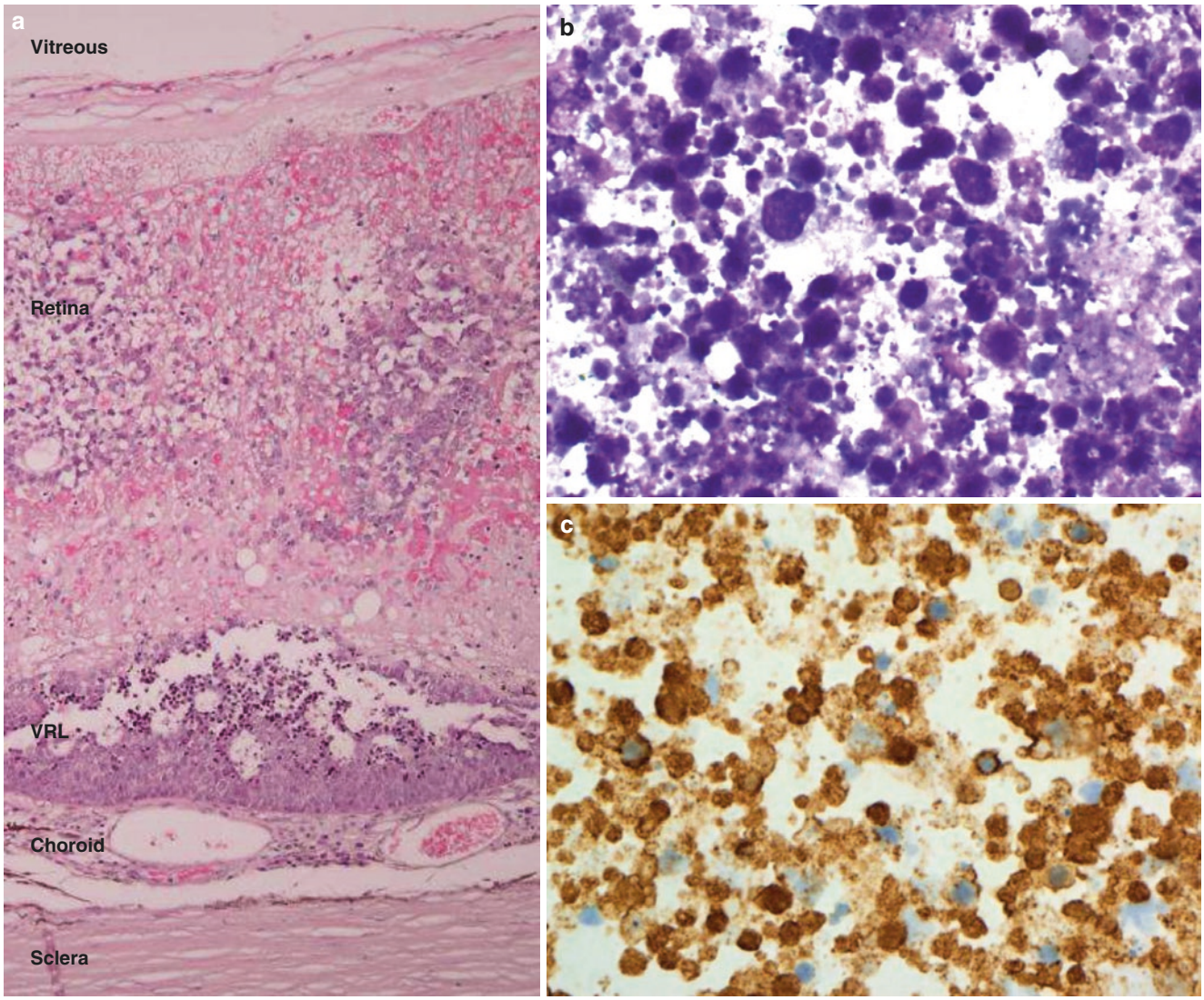

Fig. 10.38 (**a**) Cross section of the retina in an enucleated eye from a patient with vitreoretinal lymphoma (VRL). The atypical lymphocytes are seen within the necrotic retina and in clumps in the region of the 'subretinal' space between the outer part of the retina and Bruch's membrane. (**b**) High power of a cell dense cytospin of a vitrectomy performed for VRL, demonstrating large atypical blasts with minimal cytoplasm and dense chromatin, on a 'dirty' necrotic background. (**c**) CD20 positivity of the tumor cells, confirming them to be of B-cell origin

early post-germinal centre B cell. Chromosomal translocation data would suggest, however, that a subgroup of these neoplasms arises from germinal centre B cells, and these are associated with a better prognosis.

Primary Choroidal Lymphoma

Primary choroidal lymphoma is a low-grade B-cell lymphoma with morphological, immunophenotypical, and genotypic features similar to MALT lymphomas elsewhere in the body. The putative cell of origin is a post-germinal centre (memory) B cell [166]. These lymphomas are not associated with CNS disease, and the patients tend to have a good prognosis.

Primary Iridal Lymphomas

Primary iridal lymphomas are very rare, with an equal distribution of B- and T-cell types and with a variable clinical course, most patients succumbing to their disease as a result of systemic dissemination. Primary lymphomas limited to the ciliary body are exceptionally rare [166].

Secondary Choroidal Lymphomas

Secondary choroidal lymphomas represent ocular manifestations of systemic lymphoma or leukaemia and tend to represent advanced disease [166].

10.5.5.3 Retinoblastoma and Pseudoretinoblastoma

Definition Retinoblastomas are tumors originating from pluripotent germinal retinoblasts.

Epidemiology Retinoblastoma is rare but nevertheless the most common intraocular malignant tumor of childhood, clinically presenting as a white mass behind the lens, resulting in the so-called 'cat's eye' reflex. Vision in the eye is impaired, leading to strabismus. The tumor affects children younger than 5 years and can be unilateral or bilateral (30 %). Retinoblastoma in adults is extremely rare.

Clinical aspects Lesions with the typical clinical presentation but of other kind are called *pseudoretinoblastoma*. These can be other tumors (astrocytic hamartomas, hemangioblastoma), congenital malformations, or inflammatory conditions (especially solitary toxocara granuloma). The accuracy of clinical diagnosis has been improved by radiology, ultrasonography, CT scanning, optical computer tomography (OCT), and nuclear magnetic resonance, and this has resulted in more accurate and earlier diagnosis.

Molecular genetics The retinoblastoma gene, located on chromosome 13q14, is a tumor-suppressor gene. Retinoblastoma can be inherited (bilateral tumors in the first 2 years of life) or sporadic (unilateral tumors in children aged 2–5 years). One-third of the patients with a sporadic retinoblastoma show a *RB1* germline mutation and can transmit the disease to their offspring. Compared with the general population, carriers of germline mutations in the retinoblastoma gene, who survive retinoblastoma, are at increased risk of early-onset second cancers, particularly sarcomas and brain tumors.

Somatic amplification of the *MYCN* oncogene is responsible for some cases of the non-hereditary, early-onset, aggressive, unilateral retinoblastoma [169]. Although *MYCN* amplification accounted for only 1.4 % of retinoblastoma cases, researchers identified it in 18 % of infants diagnosed at less than 6 months of age. Median age at diagnosis for *MYCN* retinoblastoma was 4.5 months, compared with 24 months for those who had nonfamilial unilateral disease with two RB1 gene mutations.

Macroscopy Retinoblastomas can grow in an endophytic (growing into the vitreous), exophytic (growing into the subretinal space, leading to retinal detachment), or diffuse (a rare pattern with widespread nodular thickening of the retina) manner. Infiltration of the choroid can occur and should be graded as recently proposed by the 'International Retinoblastoma Staging Working Group' [170]. Transscleral spread of retinoblastoma is uncommon. The tumor usually spreads into the optic nerve, potentially posterior to the *lamina cribrosa*. For this reason, it is important to take transverse blocks of the cut surface of the optic nerve, before cutting the enucleated eye.

Microscopy Histological examination will show a small blue round cell tumor with a high mitotic count, areas of necrosis, as well as foci of calcification. The tumor cells have ill-defined cytoplasm and inconspicuous nucleoli. Homer Wright and Flexner-Wintersteiner 'rosettes' are frequently seen and apoptosis is common (Fig. 10.39). Glial differentiation is rare. Immunohistochemistry of retinoblastomas will show positivity for S100P, synaptophysin, and GFAP. Use of these markers can be helpful not only in identifying the tumor but also in identifying the spread of the tumor, for example, posterior to the lamina cribrosa and along the meninges (Fig. 10.39).

Treatment and prognosis The priority of retinoblastoma treatment is to preserve the life of the child, then to preserve vision, and then to minimise complications or side effects of treatment. The exact course of treatment will depend on the individual case and will be decided by the ophthalmologist in discussion with the paediatric oncologist and parents of the child. Treatments include enucleation, external beam radiotherapy, brachytherapy, laser photocoagulation, systemic chemotherapy, as well as intra-arterial chemotherapy. The cure rate of retinoblastoma is more than 90 % in

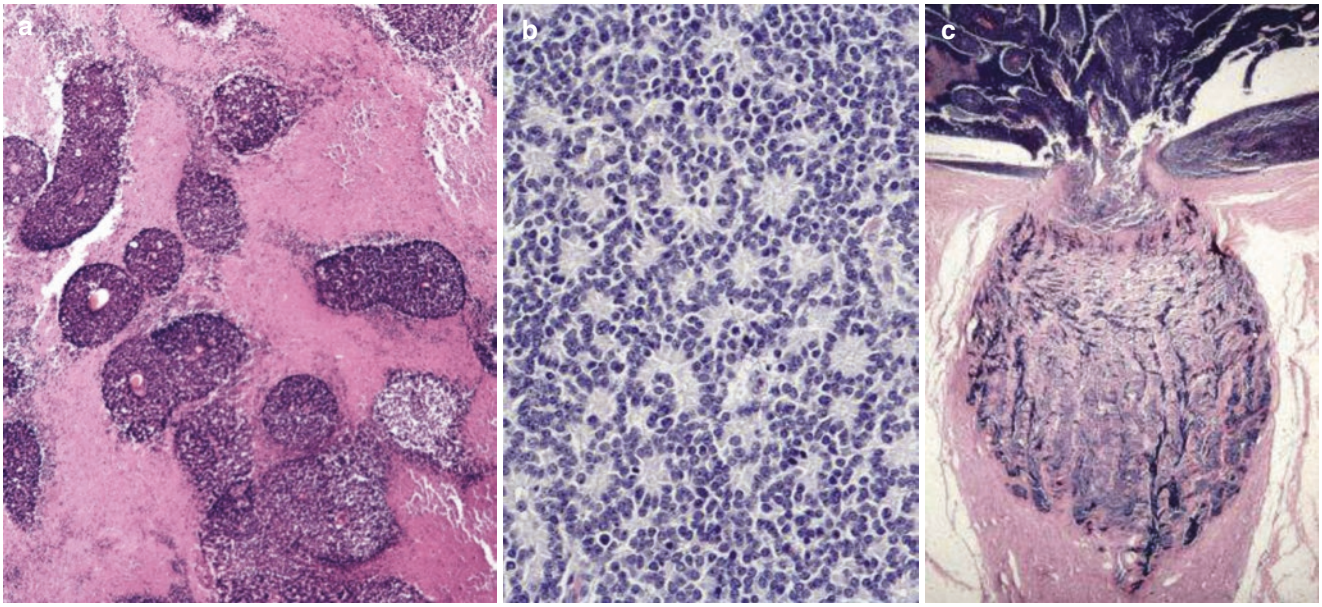

Fig. 10.39 (**a**) Low power view of a retinoblastoma showing the typical 'pink and purple' pattern, with the purple areas representing the viable tumor cells with their basophilic nuclei, and the pink being due to the eosinophilic necrotic debris. The tumor cells form the characteristic sleeve and cuff growth pattern around the blood vessels. (**b**) High power shows some characteristic features of retinoblastomas, including Homer Wright rosettes and Flexner-Wintersteiner rosettes. (**c**) Low power view of a retinoblastoma extending past the lamina cribrosa (LC) into the optic nerve, a feature associated with a poorer prognosis

specialised centres in Western countries. However, survival is dependent very much on the stage of presentation, which can occur late in areas such as remote India and Africa.

10.5.5.4 Glial-Derived Lesions

Retinal and optic disc astrocytomas and astrocytic hamartomas arise in the inner retinal layer usually within the nerve fibre layer before the non-myelinated nerve fibres penetrate the lamina cribrosa and become myelinated. The cells of origin are believed to be in the vast majority of cases retinal astrocytes; however, occasionally Muller cells and oligodendrocytes can give rise to these tumors.

Many isolated cases are believed to be congenital and may be considered hamartomas. True astrocytic neoplasms (astrocytic hamartomas) in otherwise normal retina are rare and usually are associated with either tuberous sclerosis complex (also called Bourneville-Pringle disease) or neurofibromatosis type 1, but they have been described independent of these syndromes. During their evolution, astrocytomas have been described to undergo calcification; hence they may be difficult to differentiate clinically from retinoblastoma.

Histologically, a well-circumscribed glial cell proliferation, sparing the outer layers of the retina, will be visible (Fig. 10.40). They express GFAP.

Optic pathway gliomas are frequently asymptomatic, and yet sometimes they demonstrate rapid growth, causing considerable visual dysfunction, neurologic deficits, and endocrine disturbances. Most optic pathway gliomas are diagnosed in patients with neurofibromatosis type 1 where multifocal lesions may be present. Histologically, optic nerve gliomas can be divided into pilocytic astrocytomas (low-grade) and glioblastomas (high-grade).

10.5.5.5 Vascular Lesions

Retinal vascular tumors can be classified into congenital, developmental, and acquired lesions, some of them having known aetiologies [171]. Retinal capillary hemangioblastoma or hemangioma can be isolated/sporadic but also syndromic and associated with von Hippel-Lindau disease. Retinal cavernous hemangioma has also been described as a solitary sporadic lesion and as bilateral disease associated with oculoneurocutaneous phacomatoses. Retinal arteriovenous communications are congenital non-neoplastic vascular malformations without known genetic risk factors or causative mutations. Vasoproliferative tumors of the retina are acquired vascular lesions that can be primary or reactive in nature.

Angiomatosis Retinae

Definition Retinal hemangioblastoma is a benign, highly vascularised tumor of the retina; 25 % of patients are associated with von Hippel-Lindau (VHL) disease. This disease is an autosomal dominantly inherited multi-system disorder characterised by hemangioblastic lesions of the central nervous system and visceral organs [171].

Clinical aspects Ophthalmoscopic examination shows a reddish endophytic or exophytic mass, which may be

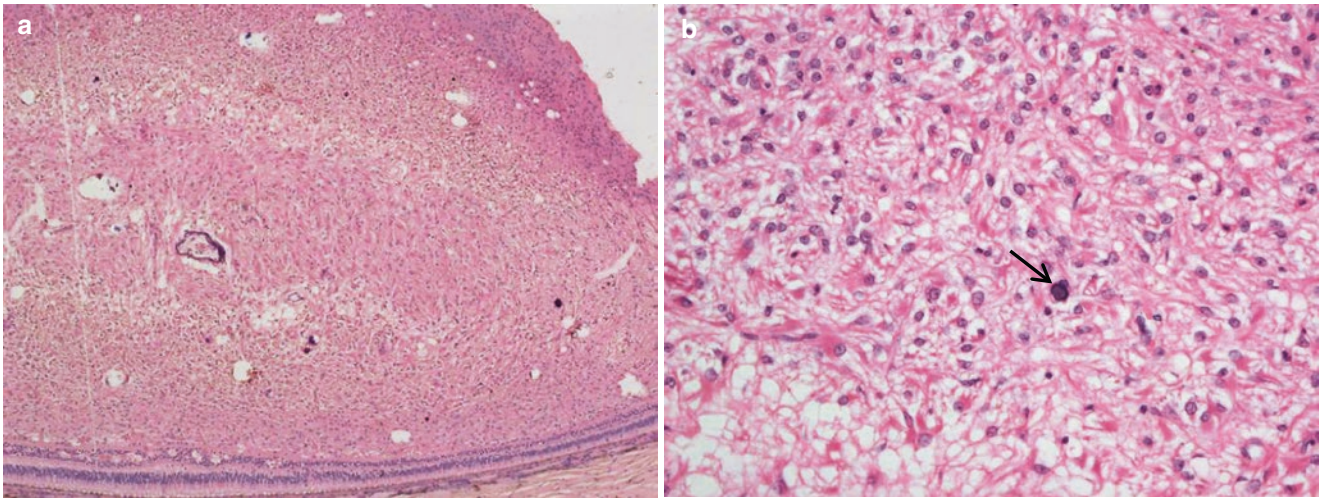

Fig. 10.40 (**a**) Enucleated eye with an astrocytic hamartoma arising from the inner layers of the retina. (**b**) Higher power of the same tumor showing the astrocyte-like cells as well as one small calcospherite (*arrow*). When the latter occur in aggregates, these tumors can mimic retinoblastoma

juxtapapillary or peripheral. Angiomatosis retinae (retinal hemangioblastoma) is often the first observable manifestation of VHL disease. In some patients with VHL disease or in the close relatives of such patients, unusual retinal vascular hamartomas other than retinal angiomas can be detected.

Microscopy Histology shows a proliferation of capillary endothelial cells and vacuolated stromal cells (Fig. 10.41). Reactive gliosis can be seen around the hemangioblastoma. In more advanced cases a proliferating fibrous tissue may lead to traction retinal detachment and, in combination with continuous exudation, to secondary glaucoma.

Immunohistochemistry The capillary proliferation can be demonstrated by endothelial markers (CD31, CD 34, ERG), whilst the reactive gliosis is positive for the GFAP staining.

Genetics *VHL*, a tumor-suppressor gene, resides on the short arm of the chromosome 3 and encodes two proteins VHL 30 and VHL 19. These products are involved in regulation of angiogenesis.

Treatment Various treatment options are used for angiomatosis retinae, such as observation, laser photocoagulation, cryotherapy, plaque, proton beam radiotherapy, and in complicated cases additional vitreoretinal surgery [171].

Cavernous and Capillary Hemangioma

Definition Diffuse hemangiomamatosis with facial skin involvement can be seen in the Sturge-Weber syndrome.

Clinical aspects Hemangiomas of the choroid can be diagnosed clinically by fluorescein angiography.

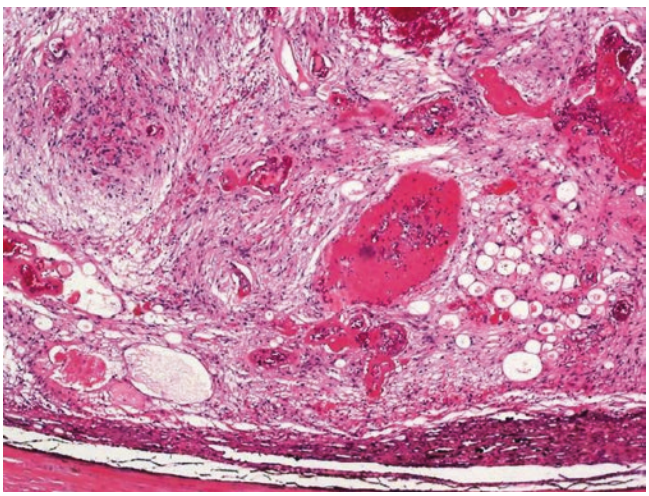

Fig. 10.41 Vasoproliferative mass seen in a patient with von Hippel-Lindau syndrome

Treatment The treatment of these vascular lesions is radiation (external beam or radioactive plaque); for that reason, pathologists do not see these lesions very often. Only when the hemangiomas lead to retinal attachment and blindness, enucleation will follow. The excised globe usually shows reactive changes due to radiotherapy [171].

10.5.5.6 Other Primary Tumors

Tumors other than melanocytic, lymphoid, retinoblastic, and vascular can be found in the intraocular structures, but they are extremely rare. The readers are referred to the recent textbook by Eagle with excellent illustrations of such tumors [172].

In the iris, the tumor-like and tumors reported are: *inclusion cysts of the pigment epithelium*, *juvenile xanthogranuloma*, *primary adenoma of the iris pigment epithelium* with very rare transformation to *an adenocarcinoma, leiomyoma*, *leiomyosarcoma*, *schwannoma* (also termed neurilemmoma), and *rhabdomyosarcoma*.

In the *ciliary body* can be found: *leiomyomas*, *leiomyosarcomas, schwannomas, adenomas,* and *medulloepitheliomas*. In the choroid are described*: osteomas, adenomas,* and *adenocarcinomas* of the RPE and hamartomas [172]. Choroidal osteomas can develop within a degenerated choroidal hemangioma or an inflammatory scar but can also be idiopathic.

10.5.5.7 Metastatic Tumors to the Eye

Clinical aspects Metastatic tumors to the eye have a predilection for the highly vascular choroid. The metastases can be discovered in a patient known with a malignancy, but they can also be the first presentation of the malignant disease.

Microscopy Histology most often shows an *adenocarcinoma* and the primary tumor is found in the breast in women or lung in males [173–175]. More rare are metastases from *thyroid carcinoma, carcinoid tumors, endometrial carcinoma, angiosarcoma,* and adenocarcinomas of the intestinal tract. Interestingly, metastatic *cutaneous melanoma* typically affects the vitreous and/or retina (Fig. 10.42) [176].

Treatment and prognosis The prognosis of patients with metastatic intraocular disease is poor, and most are treated with palliative radiotherapy.

10.6 Optic Nerve

10.6.1 Introduction

The optic nerve is subject to a wide range of pathologies of physiological, mechanical, and neoplastic causes. The reader is referred to a recent review by Cummings and van der Valk for details [177].

10.6.2 Papilloedema

Definition It means oedema of the optic disc without specification of its cause. Any condition in which the intracranial pressure is raised can cause papilloedema.

Clinical aspects The prelaminar part of the optic disc is swollen and the peripapillary photoreceptors are placed laterally. If the reason for the papilloedema is not identified, the oedema can present as a tumor.

Treatment This so-called 'pseudotumor cerebri' is treated by optic nerve fenestration to relieve the pressure in the subarachnoid space. The pathologist will receive the meninges surrounding the optic nerve, which are histologically completely normal. Occasional eyes with advanced papilloedema will be sent for histological examination (Fig. 10.43).

10.6.3 Optic Neuritis

Definition and etiology Inflammation of the optic nerve has a variety of causes, including infectious, *ischaemic, and (autoimmune) demyelinating, with the primary category in*

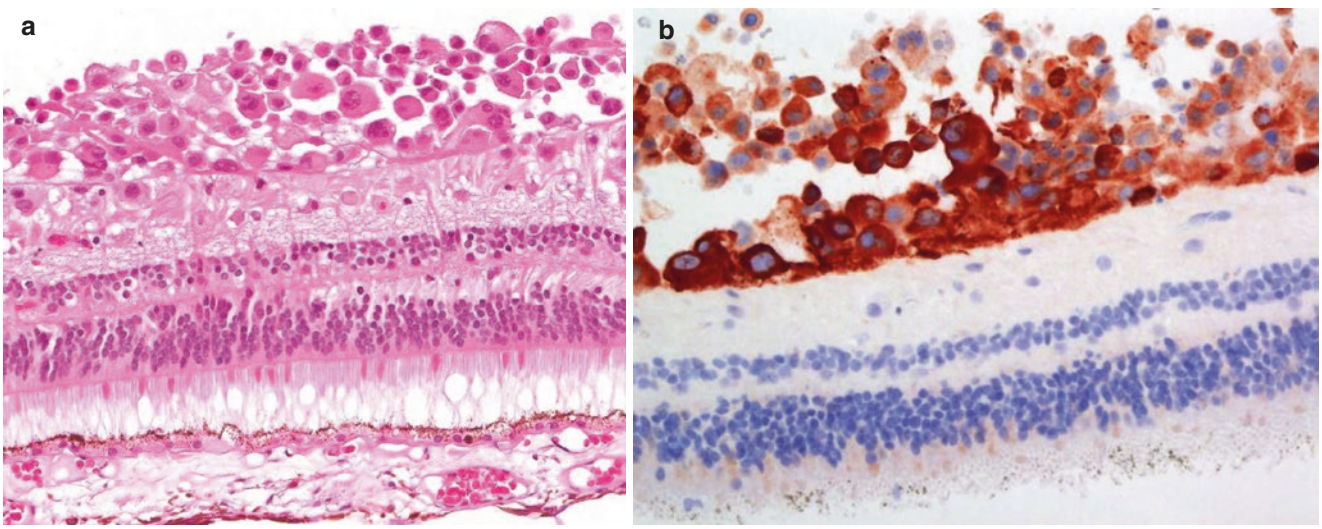

Fig. 10.42 (**a**) Metastatic cutaneous melanoma to the inner layer of the retina. (**b**) The pattern of distribution is highlighted in the MelanA stain

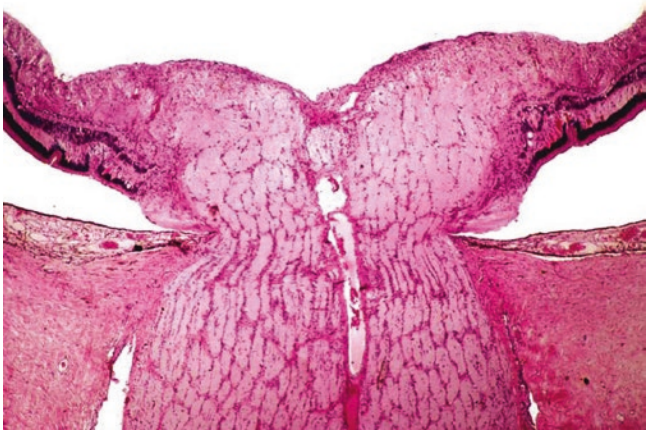

Fig. 10.43 Cross section of the optic nerve demonstrating clear papilloedema

the latter being multiple sclerosis. Most of these conditions represent secondary involvement of the optic nerve.

Clinical aspects Almost invariably, inflammation of the optic nerve causes painful visual loss. Magnetic resonance imaging is indicated to rule out a compressive optic neuropathy.

10.6.4 Optic Atrophy

In a normal optic disc, a large bulge of nerve fibres is formed. In enucleated glaucomatous eyes, the optic disc is obvious cupped and shrinks down to the lamina cribrosa, which becomes bowed posteriorly. Reactive fibrovascular tissue fills the cupped disc.

10.6.5 Tumors

10.6.5.1 Glioma

Definition Gliomas of the optic nerve arise from glial cells within the optic nerve and chiasma.

Clinical aspects Most glial tumors of the optic nerve are of the juvenile type. They present with slowly progressive proptosis, this is different from the more rare adult types, which are invariable lethal. Bilateral optic nerve gliomas can be found in patients with neurofibromatosis.

Microscopy Histologically the juvenile tumors are of the pilocytic type; the adult tumors resemble the high-grade glioblastoma multiforme. The histology is discussed in detail in the review by Cummings and van der Valk for details [177].

Prognosis The juvenile tumors have a good prognosis

10.6.5.2 Meningioma

Definition Meningiomas are tumors thought to arise from the meningothelial cells in the arachnoid mater of the optic nerve leptomeninges.

Clinical aspects As in the intracranial compartment, meningiomas of the orbit occur most commonly in middle-aged females. Optic nerve sheath meningiomas present with a slowly developing proptosis.

Microscopy Histologically, they are subtyped according to the WHO classification system, with most being WHO grade I and generally having a good prognosis [178].

10.7 Lacrimal Gland and Lacrimal Drainage System

10.7.1 Introduction

The pathology of the lacrimal gland and its associated drainage system including the sac have been described recently in detail with superb illustrations by Verdijk et al. [178]. As indicated in this review, lacrimal gland lesions are both inflammatory and neoplastic, with the neoplasms representing about half, and of these usually 50 % are malignant.

10.7.2 Inflammatory Processes

Definition and etiology Enlargement of the lacrimal gland is often caused by chronic inflammation (*dacroadenitis*), which can have many different causes. These include microorganisms (bacterial, viral, or fungal) as well as noninfectious causes, such as *sarcoidosis* [179] and systemic autoimmune diseases, e.g. *Sjogren syndrome*, *autoimmune thyroid disease*, *systemic lupus erythematosus,* as well as IgG4-related sclerosing disease (see below). The latter was formerly known as *Mikulicz' syndrome* when associated with enlargement of the salivary glands. *Acute dacryocystitis* is uncommon but may be due to dacryolithiasis and to lacrimal mucoceles [178].

10.7.3 Tumors and Tumor-Like Conditions

If a mass is found in the superolateral quadrant of the orbit, one should consider *dermoid cysts* and lacrimal gland neoplasms. Fifty percent of lacrimal gland tumors are benign with the majority being *pleomorphic adenomas* [5, 176, 178–180] (Fig. 10.44). Other benign tumors include *Warthin's tumor*, basal cell adenoma*, oncocytoma,* and *myoepithelioma* [5]. The malignant category of lacrimal gland

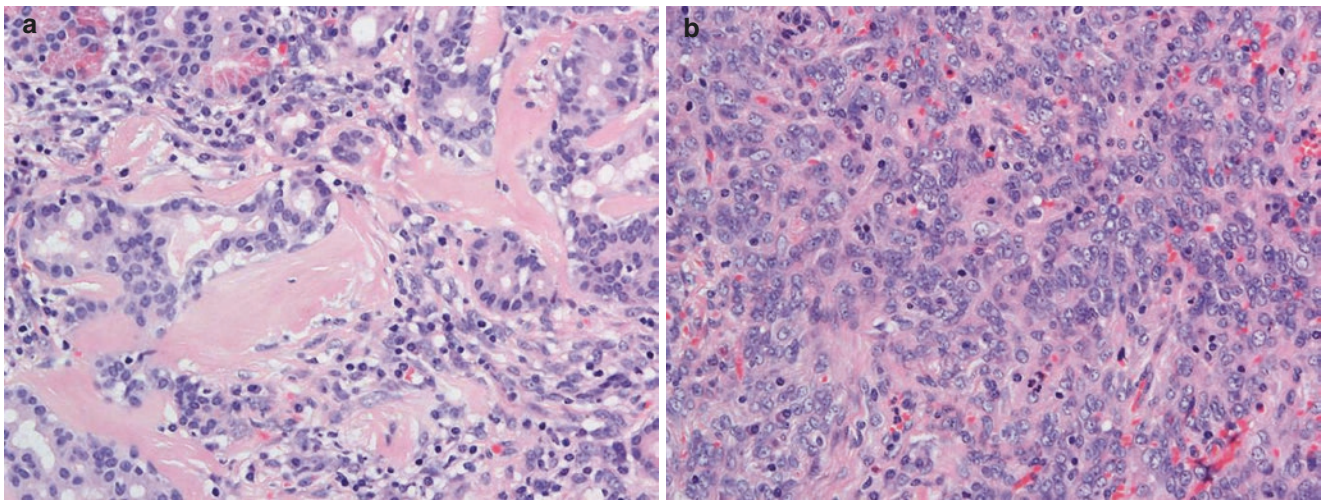

Fig. 10.44 (**a**) Pleomorphic adenoma of the lacrimal gland with areas of hyalinosis and sclerosis. (**b**) Other areas within the same tumor with dominance of the proliferation of myoepithelial cells

includes predominantly adenoid cystic carcinoma, carcinoma ex pleomorphic adenoma, *adenocarcinoma NOS*, and *mucoepidermoid carcinoma*. For rarer varieties, see Verdijk et al. [178]. Significant advances have been made in the understanding of the molecular alterations underlying the lacrimal gland neoplasms [179–182].

With respect to the lacrimal sac, *squamous cell (inverted) papillomas* and squamous cell carcinomas are the benign and malignant tumors most commonly seen. Secondary involvement of the lacrimal gland by conjunctival melanoma can occur through spread along the nasolacrimal duct, as well as some haematological malignancies.

The lacrimal gland and sac tumors have few distinguishing imaging features, showing mostly a homogeneous character and moderate contrast enhancement. Poorly defined tumor margins with bone destruction suggest a malignant tumor, but even the malignant lesions can be relatively well-defined.

Treatment is dependent on histological subtype and TNM stage [183], with the prognosis tending to be poor in the malignant tumors due to the perineural spread, high recurrence rates, and poor response to radiotherapy.

10.8 Eyelids

10.8.1 Introduction

An extensive review of eyelid pathology is provided by Salamao et al. [184]. Essentially, eyelid pathology can be divided into alterations caused by: congenital and developmental abnormalities, degeneration, inflammation, underlying systemic disease, cysts, and neoplasms. Only some of these conditions will be addressed here; others in Chap. 15.

10.8.2 Cysts

Because of the numerous adnexal glands present in the eyelids, cysts are very common at this localisation. The cysts can be of developmental origin (dermoid cysts) or can be caused by inclusion or retention.

10.8.2.1 Dermoid Cyst

The most common type of a cyst of the eyelids in children is the dermoid cyst, a developmental cyst caused by inclusion of ectodermal rests within the lines of closure of the branchial arches. Dermoid cysts are lined by stratified squamous epithelium with small pilosebaceous units attached to the wall. The lumen usually contains small hairs and keratin. The presence of pilosebaceous units differs this cyst from epidermal cysts.

10.8.2.2 Epidermal Cysts

Epidermal cysts (epidermoid cysts, keratinous cysts) are firm, often yellow-brown masses, diagnosed clinically as ‘sebaceous cyst’. However, real sebaceous cysts (steatocystomas) are very rare and most cysts will histologically show a lining with stratified squamous epithelium without pilosebaceous glands. The cysts are filled with strands of keratin. Epidermal cysts can be caused by dermal inclusion of epithelial cells after a microtrauma but also by occlusion of a pilosebaceous unit. If an epidermal cyst ruptures, keratin will be released between the collagen bundles of the dermis, causing a granulomatous foreign-body reaction.

10.8.2.3 Hidrocystoma

Definition and clinical aspects Cysts derived from the small sweat glands present in the eyelids present as bluish, round lesions and are clinically often misdiagnosed as

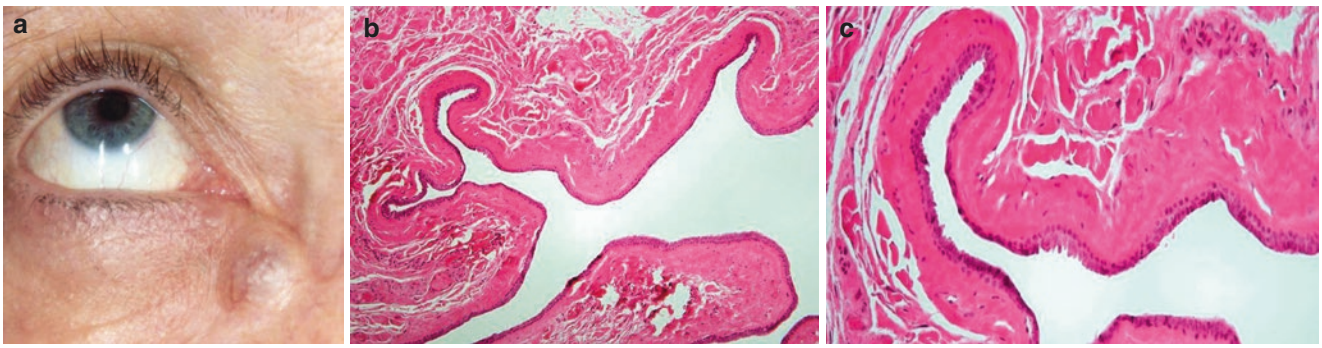

Fig. 10.45 (**a**–**c**) Clinical and histological images of a cyst of Moll (also termed sudoriferous cyst or hidrocystoma)

hemangiomas. The term hidrocystoma is preferred, but other names include cysts of Moll's glands and sudoriferous cysts. They can be multiloculated.

Microscopy The eccrine cysts are lined by cuboidal to flattened epithelium with a myoepithelial base (Fig. 10.45). Sometimes only one epithelial layer is visible, showing eosinophilic cytoplasm with snouts, characteristic of apocrine differentiation. It can be very hard to differentiate between eccrine or apocrine origin and sometimes both components can be found in the cysts.

10.8.3 Inflammatory Processes

A very common inflammatory condition of the eyelids is a chalazion (Fig. 10.46). Furthermore, many inflammatory skin diseases can involve the periorbital region. Periorbital eczema may be an expression of a constitutional disease or an irritant or allergic dermatitis. Other inflammatory dermatoses that can involve the eyelids are seborrheic dermatitis, psoriasis, acne rosacea, and dermatomyositis. Other causes of inflammation of the eyelids include bacterial, fungal, and viral infections.

10.8.3.1 Chalazion

Definition Chalazia are very common and are caused by the obstruction of the duct of a small (Zeis) or larger (meibomian) sebaceous gland.

Clinical aspects The clinical presentation usually is very typical with an acute swelling in the tarsal conjunctiva. In a few days the swelling becomes a firm nodule

Microscopy Typically, the small retention cyst is formed and subsequently ruptures causing the escape of fatty products into the surrounding tissues, triggering an acute inflammatory reaction, followed by a chronic granulomatous reaction (Fig. 10.46). In very late stages of chalazia, fibrosis and scarring can be seen. The presence of fatty cells or even larger optical empty spaces within a granulomatous reaction is characteristic for a chalazion. The only other conditions with similar lipogranulomatous reactions are leakage of implants (e.g. silicons) and dermatitis artefacta.

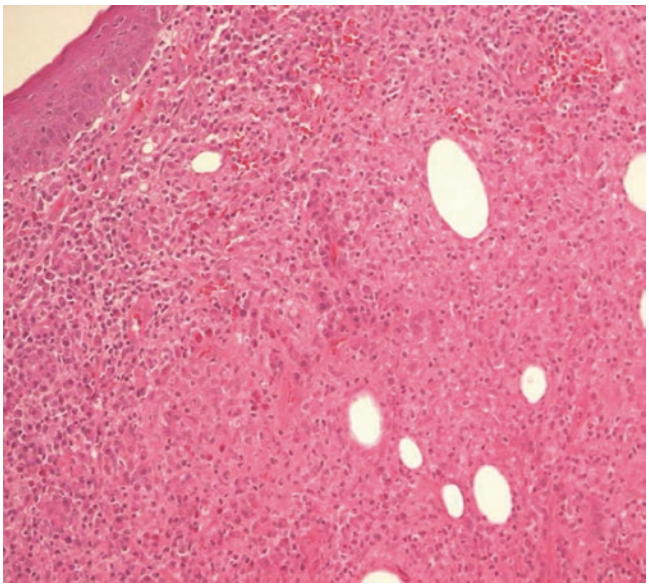

Fig. 10.46 Eyelid excision with typical features of a chalazion: i.e. acute-chronic inflammation, including multinucleate cells, surrounding empty lipid vacuoles, released from the meibomian and Zeiss glands. The cellular infiltrates include eosinophils, plasma cells, and lymphocytes

Differential diagnosis The major differential diagnosis of a persistent chalazion, particularly in an elderly patient, is a sebaceous carcinoma; it is to exclude this insidious lesion, which often mimics a chalazion, that clinicians are encouraged to send the surgical material for examination.

Treatment Excision or excochleation is the treatment of choice, but often the material is not sent for routine histological examination.

10.8.3.2 Deep Granuloma Annulare

Definition Granuloma annulare is characterised by raised lesions arranged in an annulare shape.

Clinical aspects It usually occurs on the dorsum of the hands and the lower arms and in adults can be associated with diabetes mellitus. However, in children the deep variant of granuloma annulare is a benign, relatively common dermatosis, not related to systemic disease. In granuloma annulare of childhood, lesions typically occur on the extremities and resolve spontaneously over a period of several months to years. Localised facial involvement with involvement of the eyelids can occur. This diagnosis should be considered for any acquired papules of the periorbital area, especially if there is a history of antecedent trauma.

Microscopy Histology shows deep foci of degeneration of collagen, surrounded by histiocytes, together with increased dermal mucin.

Treatment Unnecessary surgical excision can then be avoided.

10.8.3.3 Necrobiotic Xanthogranuloma

Definition and etiology Necrobiotic xanthogranuloma, distinctive dermal and subcutaneous xanthogranulomatous reaction, is a rare chronic and often progressive disorder with predilection for the periorbital skin [184]. It is often associated with monoclonal paraproteinaemia, with other more rare associations being hyperlipidaemia and leucopaenia. The prevalence of the disease is slightly higher in women than men, and onset is usually in the sixth decade of life.

Macroscopy Lesions present as sharply demarcated violaceus, partly xanthomatous nodules and plaques. Ulceration may develop. Scleritis, episcleritis, and keratitis are common ophthalmic complications.

Microscopy The histological changes are present in the dermis and in the subcutis. Large zones of necrobiotic collagen with hyaline and sometimes mucinous changes are present in the deep dermis. These areas are surrounded by histiocytes, partly with a foamy cytoplasm. Sometimes the xanthomatous changes are only minor. Multinucleated giant cells are easily found; they can be of the Touton type, but also of the foreign-body type with bizarre nuclei. In ulcerating lesions, transepidermal elimination of debris can be seen.

Treatment It should be directed to management of the underlying disease and control of the skin disease.

10.8.4 Amyloidosis

Definition and clinical aspects Amyloidosis is associated with a number of inherited and inflammatory diseases with extracellular deposits of fibrillary proteins in connective tissue of various organs. Solitary or multiple nodules of amyloid may occur both in the eyelid as a result of either primary systemic or localised amyloidosis. Typically the lesions are symmetrical, bilateral, single or multiple, confluent yellowish or waxy papules. Associated purpura is common due to the fragile vascular walls caused by the amyloid deposition.

Microscopy Histology shows amorphous, eosinophilic, Congo red-positive masses in the stroma. The walls of blood vessels often also contain amyloid. Subtyping on amyloid using tandem mass spectrometry analysis on formalin-fixed samples can be undertaken to determine the underlying systemic condition [183, 184, 186].

10.8.5 Tumors and Tumor-Like Conditions

Tumors of the eyelids are very similar to tumors occurring in the conjunctiva and the skin. The most important malignant tumors of the eyelids are basal cell carcinomas, squamous cell carcinomas, sebaceous carcinomas, and melanomas. These tumors are discussed in Chap. 15 on the skin and so will not be addressed or illustrated here. However, there are two conditions with predilection for the eyelids and the surrounding skin are xanthelasma and Merkel cell carcinoma: they will be discussed below.

10.8.5.1 Xanthelasma

Definition Xanthelasma is a form of cutaneous xanthoma, a group of lesions characterised by the accumulation of lipid-rich macrophages, also known as ‘foamy cells’ (Fig. 10.47).

Etiology and pathogenesis These lesions are usually associated with disorders of lipoprotein metabolism, although only a minority of individuals with such disorders develop xanthogranulomas. Consequently, this has led to the suggestion that there are an increased vascularity and permeability associated with the pathogenesis of these lesions.

Macroscopy The patients present with yellow papules and plaques, which most frequently occur in the medial upper eyelids and then spread laterally in a symmetrical fashion to the skin. They usually do not ulcerate. Most frequently they occur in populations in Asia and the Mediterranean.

Treatment It is not usually required unless desired by the patient for cosmetic reasons.

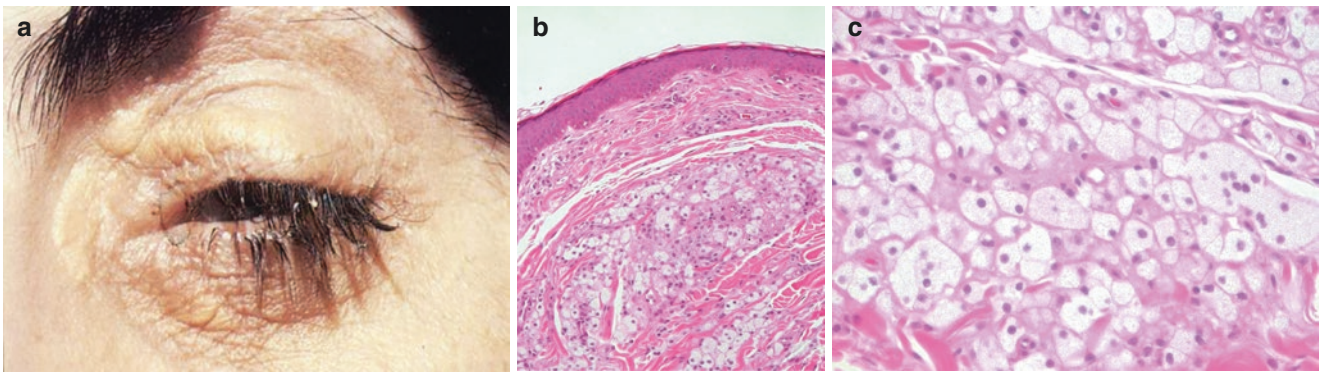

Fig. 10.47 (**a**–**c**) Clinical and histological images of a patient with xanthelasma. The *yellow* plaques in the skin of the medial eyelids contain numerous foamy macrophages

10.8.5.2 Merkel Cell Carcinoma

Definition Merkel cell carcinoma is a rare primary malignant cutaneous neoplasm with epithelial and neuroendocrine differentiation. It was first described by Cyril Toker in 1972 as 'trabecular carcinoma' [187]. It was thought to arise from Merkel cells of the skin based on electron microscopy; however, the current theory is that they arise from pluripotent stem cells similar in appearance to Merkel cells.

Etiology A new *polyomavirus* has been proposed to be involved in the development of the tumor [188, 189].

Clinical aspects Merkel cell carcinomas typically affect elderly fair-skinned female adults, with an increased frequency in those who are immunosuppressed.

Microscopy The tumors are 'small blue cell' neoplasms, composed of cells of relatively uniform size, with round nuclei, finely dispersed chromatin, and a small cytoplasmic rim. Mitotic figures are numerous, and apoptotic bodies are also frequent.

Immunohistochemistry There is a classic profile of positivity for CK 20, with co-expression of neuroendocrine markers (synaptophysin and chromogranin) in a paranuclear dot-like fashion (Fig. 10.48). The Ki-67 growth fraction is high.

Genetics With respect to genetic alterations, deletions of chromosome 1p and trisomy 6 are the most frequent alterations.

Treatment and prognosis Merkel cell carcinoma has an aggressive clinical course with metastatic spread to lymph nodes (60 % have lymph node involvement at presentation). There is no agreement on treatment strategies, which involves surgical resection, sentinel lymph node biopsies, radiotherapy, and, potentially, chemotherapy [185].

10.9 Orbit

10.9.1 Introduction

The orbit is the smallest region of the body to contain all conceivable tissue types, which can be affected by all pathologies. An excellent review is provided by Verdijk et al. [178].

10.9.2 Inflammatory Processes

The most common inflammatory diseases of the orbit include *Graves' disease* (also termed *dysthyroid ophthalmopathy*), orbital cellulitis, and 'idiopathic orbital inflammatory disease' (also known as inflammatory 'pseudotumors').

10.9.2.1 Dysthyroid Ophthalmopathy

Definition The most common cause of bilateral proptosis is Graves' disease, as an autoimmune inflammatory disease, characterised by hyperthyroidism.

Clinical aspects and macroscopy 70 % of cases are bilateral and symmetrical. In cases of unilateral involvement, other diseases must be considered. Females are more frequently affected than males. The disease is characterised by symmetrical swelling of the extraocular muscles. The inferior and medial rectus muscles are most often involved. The muscle enlargement characteristically involves the body of the muscle, sparing the tendinous attachment to the globe.

Microscopy Histologically, the fibrous tissue of the orbit and the swollen muscles show oedema and chronic inflammation in early stages and fibrosis in end-stage disease. The degenerated muscle fibres become hyalinised.

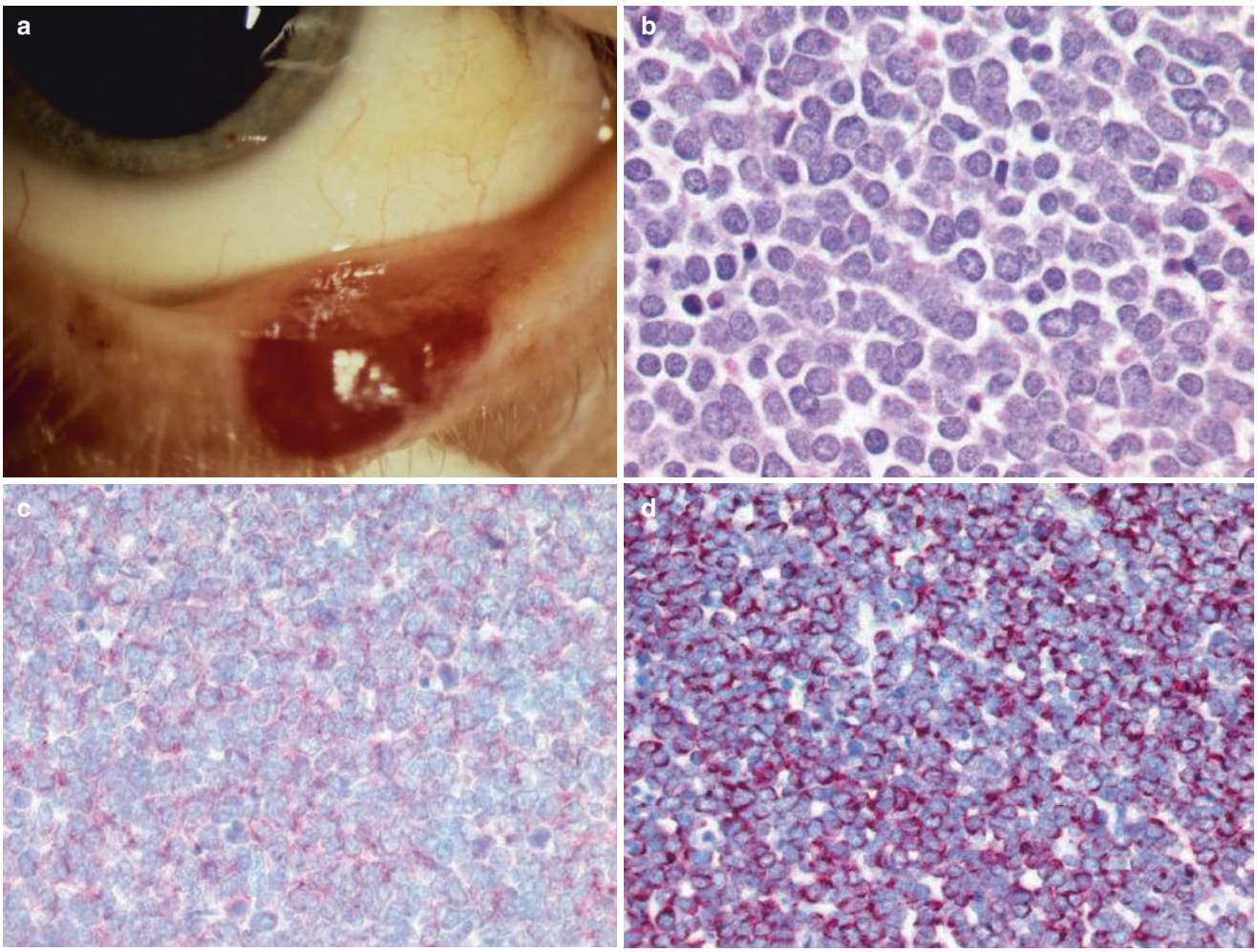

Fig. 10.48 (**a**) Clinical photograph of a red nodular lesion on the lower eyelid of a 70-year-old female patient with a Merkel cell carcinoma. (**b**) Histological examination reveals a small 'blue cell' tumor with scant cytoplasm, diffuse chromatin within the nucleus, and numerous mitoses. (**c**) The neoplastic cells stain for chromogranin and for (**d**) cytokeratin 20

10.9.2.2 Cellulitis

Definition and etiology Acute bacterial infection (cellulitis) of the orbit is uncommon. It is most frequently caused by direct spread of an infection from the paranasal sinuses or eyelids. It may also be of odontogenic origin and can be one of the presenting features of retinoblastoma or other tumors. Chronic intranasal cocaine abuse can result in extensive bony destruction of the orbital walls with associated orbital cellulitis. In patients with poorly controlled diabetes but also in immunocompromised patients, orbital cellulitis can also be caused by fungal agents, for example, by mucormycosis. Presenting symptoms most frequently include oedema of the upper eyelid, headache, and facial pain. Sometimes it can be asymptomatic.

Clinical aspects Orbital cellulitis is of great importance, as it is a severe disease with potentially disastrous consequences. Despite antifungal or antibacterial therapy, disease can progress. It may lead to optic neuritis, optic atrophy, blindness, cavernous sinus thrombosis, intracranial abscess formation, meningitis, subdural empyema, and even death. An incision biopsy of the process can be helpful in the diagnostic work-up.

Microscopy Histology will show an extensive neutrophilic infiltration of the orbital fibrous tissue and fat. The causative microorganisms can often be found with PAS, Gram, and silver stainings. It is important for the pathologist to exclude any underlying causes, like tumors, which may have presented in a 'masquerade' manner as inflammation.

10.9.2.3 Idiopathic Orbital Inflammatory Disease

Definition Non-specific inflammation of orbital tissues is known as 'idiopathic orbital inflammatory disease (IOI)' or previously 'orbital pseudotumor'. The latter term has fallen out of favour.

Clinical aspects It tends to be unilateral and accounts for 25% of all cases of unilateral exophthalmos. However, IOI can also be chronic and progressive. The diagnosis has to be confirmed by a tissue biopsy, especially in cases in which the IOI appears as a discrete mass and simulates a neoplastic lesion.

Microscopy Histology shows oedema of the orbital fibrous tissue and fat in the most early stages. This will be followed by lymphocytic infiltration and end with fibrous changes. In cases with massive infiltration by lymphocytes, extensive immunohistochemistry is necessary to rule out lymphoma. Another disease that can mimic 'pseudotumors' is sarcoidosis. Sarcoidosis is histologically characterised by typical non-caseating granulomatas.

Treatment and prognosis Spontaneously regression can occur and response to steroids is often seen.

10.9.2.4 IgG4-Related Orbital Disease

Definition IgG4-related disease is a recently identified inflammatory process characterised by tissue infiltration by dense IgG4+ plasma cells [190, 191].

Epidemiology This disease affects males and females in equal frequency. IgG4-related disease is considered to be a systemic condition and can involve not only the orbit but also the pancreas, hepatobiliary tract, retroperitoneum, salivary glands, and even lymph nodes. It appears to account for between 25 and 50% of all orbital inflammatory masses, although caution must be used to interpret these data, as IgG4+ plasma cells have been reported in many other distinct entities.

Pathogenesis Its pathogenesis is poorly understood, as it has features of both an autoimmune disease and an allergic reaction.

Clinical aspects Clinical presentation includes painless swelling of the eyelid swelling with our without proptosis and elevated levels of serum IgG4. Tissue biopsy is required to make the diagnosis and to exclude other conditions.

Microscopy Histological examination reveals orbital tissue with varying degrees of fibrosis and IgG4+-rich lymphoplasmacytic infiltration, which can be focal or diffuse (Fig. 10.49). Particular criteria have been recommended to enable establishment of the diagnosis, including 30–50 IgG4+ cells per high power field, with an IgG4+/IgG+ ratio >0.4 (Fig. 10.50) [192].

Treatment and prognosis Typically there is a good response to steroids; however, some cases have been reported to relapse, and others to progress to low-grade B-NHL [193, 194].

10.9.3 Tumors and Tumor-Like Conditions

A variety of tumors and 'pseudotumors' can involve the orbit. The orbital neoplasms vary from benign to high-grade malignant [178]. The percentage of malignant tumors increases with age, with 60% malignancies in patients over 60 years of age, because of the higher incidence of lymphoma and metastatic tumors in the elderly.

Orbital tumors of childhood are distinct from tumors that occur in adults. Many are congenital with early presentations. Most paediatric orbital tumors are benign (80%); developmental cysts comprise half of orbital cases, with capillary hemangiomas being the second most common orbital tumor in children. The most common orbital malignancy in children is rhabdomyosarcoma. Whereas the malignant tumors may be life-threatening, both malignant and benign tumors may be vision-threatening. Almost all lymphomas, soft tissue and bone tumors may involve the orbit. They are discussed under their appropriate chapters, including Chaps. 12 and 13.

10.9.3.1 Developmental Cysts

Epithelial rests found at sutural sites within the orbit can give rise to epithelial cysts. Cysts of surface epithelium are further divided into simple epithelial cyst (epidermal, conjunctival, respiratory, and apocrine gland) and dermoid cyst (epidermal and conjunctival). Epidermal *dermoid cyst* (dermoid) is by far the most common orbital cystic lesion in children, accounting for over 40% of all orbital lesions of childhood. Other developmental cysts are *teratomatous cysts*, *neural cysts* (*congenital cystic eye* and *colobomatous cyst*), and those associated with brain and meningeal tissue (encephalocele and optic nerve meningocele) [195]. Developmental cysts have to be differentiated from secondary cysts, like mucocele and inflammatory cysts. Mucocele can occur in children with cystic fibrosis. Inflammatory cysts are generally due to parasitic infestations and are more common in tropical areas of the world. Furthermore, non-cystic tumorous lesions with a cystic component (like rhabdomyosarcoma and lymphangioma) can present as a cyst.

10.9.3.2 Optic Nerve and Meningeal Tumors

Optic nerve and meningeal tumors can spread into the orbit. Together they present 8% of all orbital tumors.

10.9.3.3 Metastatic Tumors

The orbit can be affected by metastatic tumors, with 3–5% of all orbital tumor-like lesions being metastases [178]. Breast carcinoma is the most common metastatic tumor to the orbit in women followed by lung cancer (Fig. 10.50). In men the most common metastatic cancers to the orbit arise from the lung and prostate. The mean period of time between the onset of the primary disease and orbital mani-

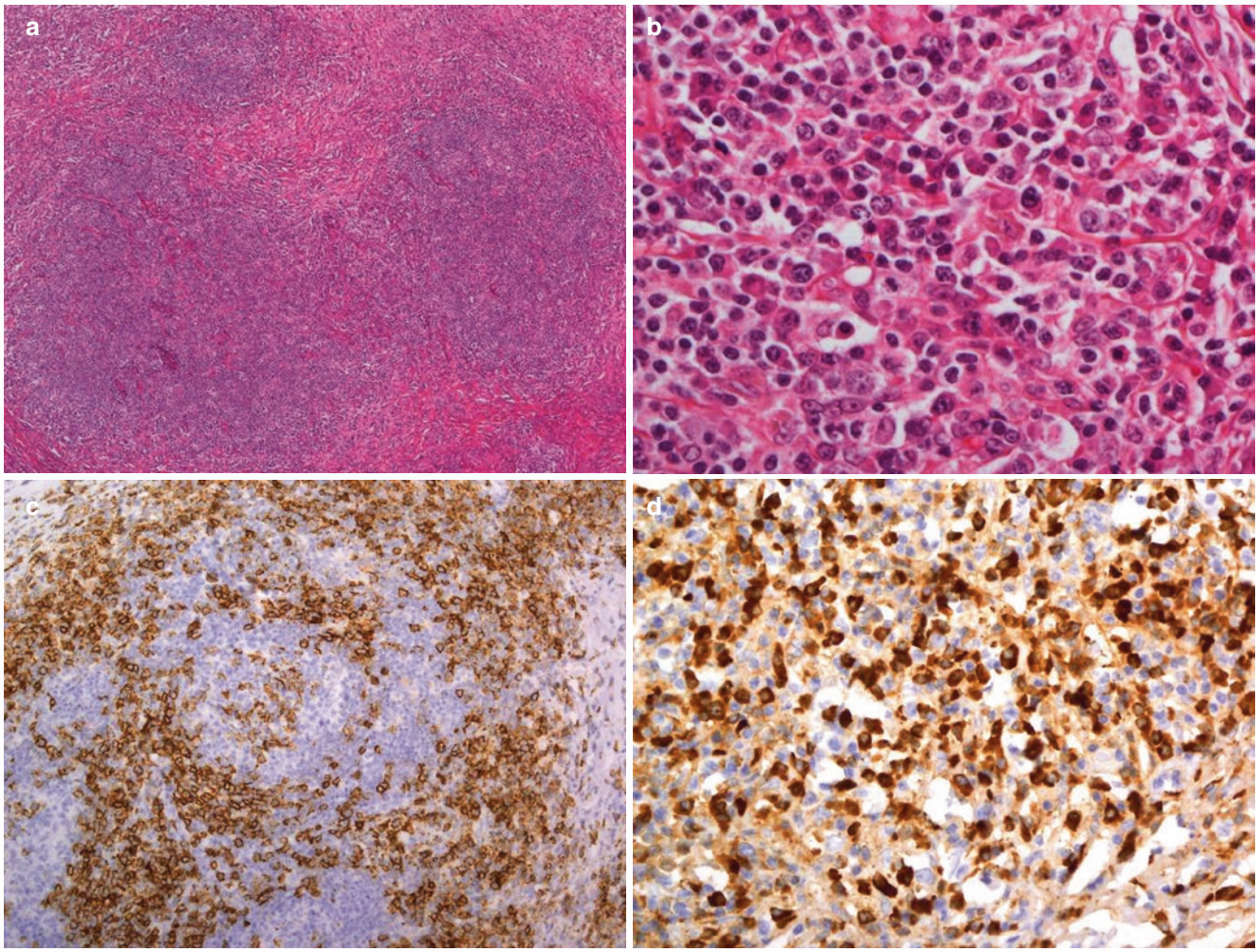

Fig. 10.49 (**a**) Orbital biopsy of a patient with an inflammatory orbital mass. (**b**) High power magnification revealed a dense inflammatory infiltrate with preponderance of plasma cells. (**c**) The latter are highlighted in the CD138 stain. (**d**) IgG4-positive cells were focally and sufficiently dense (35 IgG4+ cells per high power field), allowing for the diagnosis of IgG4+-related disease to be raised as a diagnosis. This was confirmed on serology

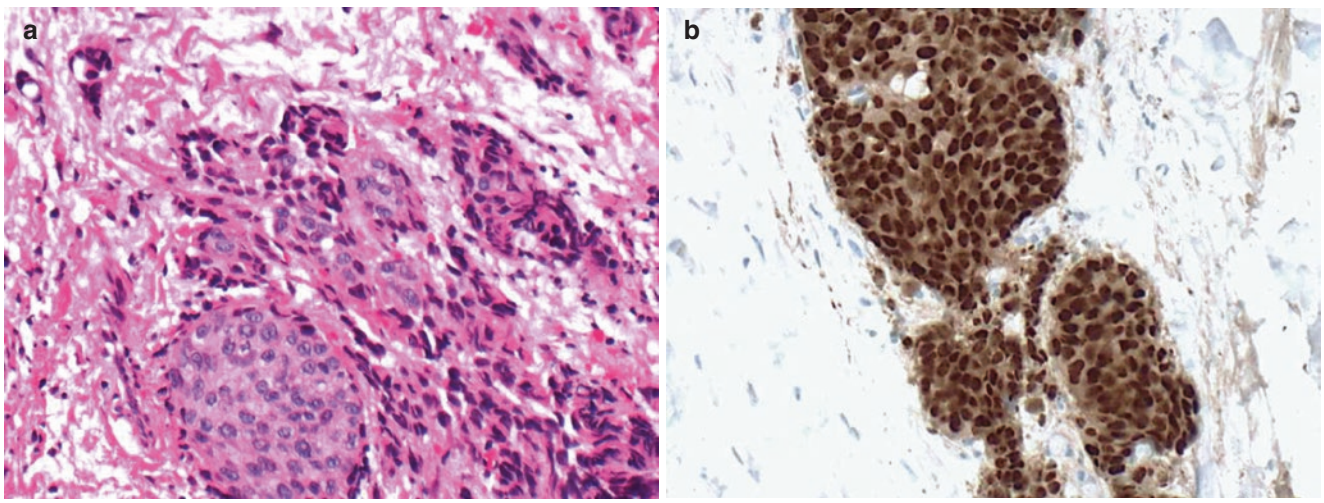

Fig. 10.50 (**a**) Metastatic breast (ductal) carcinoma to the orbit, with (**b**) clear nuclear positivity of the metastatic tumor cells for oestrogen receptor

festation is 5 years. The main primary symptoms are lid swelling, red eye, diplopia, and proptosis. Metastatic melanomas to the orbit are rare and generally occur in patients with disseminated metastases during the terminal stages of the disease and are hence associated with a short life expectancy. These can be either of uveal [196] or cutaneous origin [197].

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Neuroendocrine Neoplasms, Olfactory Neuroblastomas and Paragangliomas of the Head and Neck

11

Günter Klöppel, Alessandro Franchi, and Xavier Matias-Guiu

Contents

11.1 Introduction

11.1.1 Definition and Categorization

Endocrine tumors that secrete (glyco)peptide hormones and/or biogenic amines express the markers synaptophysin, neuron-specific enolase and chromogranin A and show typical membrane-bound granules at the ultrastructural level are called neuroendocrine neoplasms (NENs). The neural cell adhesion molecule CD56/N-CAM is also positive in many NENs, but is not specific for these tumors [1].

The cells that give rise to NENs in the head and neck area are thought to be either of epithelial or neural/neuroectodermal origin. While the neural/neuroectodermal cells colonize in neural tissues such as the paraganglia and olfactory membrane, the neuroendocrine cells with epithelial features have only been focally identified in the mucosal tissues of the head and neck area. In the larynx mucosa, argyrophilic cells comparable to the Kultschitzky cells in the bronchial epithelium have been described [2]. Other potential cells of origin are, like in the gastrointestinal tract, local pluripotent stem cells in the mucosal and glandular epithelium that are able to differentiate into neuroendocrine cells. The neoplasms of neural/neuroectodermal origin are olfactory neuroblastomas and paragangliomas; the other neuroendocrine neoplasms are those that have been described under the terms of carcinoid, neuroendocrine carcinoma (NEC) or poorly differentiated (small cell) neuroendocrine carcinoma. It is likely that the well-differentiated carcinoids and the poorly differentiated NENs derive from different types of stem cells.

The cells that give rise to paragangliomas reside in the paraganglia that are traditionally separated into sympathetic and parasympathetic paraganglia. The sympathetic paraganglia usually produce catecholamines and are associated with the peripheral sympathetic nervous system. They lie mainly in the thorax, abdomen and pelvis [3]; however there is also sympathetic paraganglionic tissue in the cervical region, corresponding to the distribution of sympathetic nervous system in the neck [4]. The most typical location is the superior cervical ganglion.

The parasympathetic paraganglia, which are associated with the parasympathetic nervous system, are predominantly located in the head and neck region, particularly along the cranial and thoracic branches of the glossopharyngeal

G. Klöppel (✉)
Department of Pathology, Technical University München, Ismaningerstr. 22, 81675 Munich, Germany
e-mail: guenter.kloeppel@alumni.uni-kiel.de

A. Franchi
Section of Anatomic Pathology, Department of Surgery and Translational Medicine, University of Florence, Largo Brambilla 3, 50134 Florence, Italy
e-mail: franchi@unifi.it

X. Matias-Guiu
Department of Pathology, Hospital Universitari Arnau de Vilanova, Av Rovira Roure, 80, 25198 Lleida, Spain
e-mail: fjmatiasguiu.lleida.ics@gencat.cat

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and vagus nerves. The most important parasympathetic paraganglia of the glossopharyngeal nerve are the carotid bodies and the tympanic paraganglia (in the wall of the middle ear). Carotid bodies are small ovoid structures in the carotid bifurcations on each side of the neck. Paraganglia of the vagus nerve include the jugular, superior and inferior laryngeal, and subclavial. Intravagal paraganglia may also be found within or close to the nerve trunk in relation to the nodose and jugular ganglia.

Microscopically, paraganglia have a lobular architecture ("zellballen") which is more typical of the carotid bodies and less evident in the other paraganglia. Lobules contain clusters and cords of neuroendocrine cells ("chief cells", "type I cells", "paraganglionic cells" or "chromaffin cells"), which show cytoplasmic argyrophilia and positive immunostaining for chromogranin A and synaptophysin. Neuroendocrine cells are polyhedral, with abundant cytoplasm and small spherical or ovoid, nuclei with coarse chromatin and a single, small, nucleolus. S100-positive sustentacular cells ("type II cells") and Schwann cell-like glial cells surround nests of chief cells. There is a variable fibrovascular component with pericytes and endothelial cells. Mast cells are also present.

Based upon morphological observations, Fernando de Castro was the first to demonstrate parasympathetic innervation of the carotid body and proposed a chemosensory role for this organ [5]. Later, paraganglia were found to synthesize and secrete catecholamines. Sympathetic paraganglia contain higher catecholamine concentrations than parasympathetic paraganglia. Paraganglia respond to neural and chemical stimuli by releasing a wide variety of hormones and regulatory neuropeptides. Hypoxia is one of the most important stimuli in sympathetic paraganglia. However, chemoreception is the best known role of parasympathetic paraganglia.

11.1.2 General Diagnostic Features

All neuroendocrine neoplasms, irrespective of their neural/neuroectodermal (olfactory neuroblastomas, paragangliomas) or epithelial origin, express neural markers and show neurosecretory granules. Crucial for the diagnosis is the demonstration of synaptophysin [6]. This is an integral membrane protein of small clear vesicles (diameter 40–80 nm) occurring in all normal and neoplastic neuroendocrine cells, where it usually shows a diffuse expression (Fig. 11.1a). Chromogranin A, which is a protein located in the matrix of large secretory granules (>80–200 nm), is, in contrast to synaptophysin, usually focally expressed in the cytoplasm of the tumor cell or is even lacking, since its expression depends on the number of neurosecretory granules present in the cells and on the cell type (Fig. 11.1). Poorly differentiated neuroendocrine carcinomas that contain only few and small secretory granules per cell may be chromogranin A negative (Fig. 11.1b). Staining for neuron-specific enolase (NSE) is often difficult to interpret because of the frequent unclear and unspecific reactions of the available antibodies. CD56 (i.e. N-CAM) and PGP9.5 stain many if not all NENs, but they are clearly non-specific markers for NENs and can therefore only be used in conjunction with synaptophysin. The electron microscopical demonstration of neurosecretory granules may be difficult, as some NEN cells contain only few and small "atypical" granules. In these cases the ultrastructural findings should be confirmed by a positive synaptophysin staining. CK18 (in contrast to CK7) staining is positive in all NENs of epithelial origin; olfactory neuroblastomas and paragangliomas which are of neural/neuroectodermal origin usually do not express CKs.

11.1.3 Preferred Sites in the Head and Neck Area

The upper part of the nasal cavity is the preferred localization of olfactory neuroblastomas. Paragangliomas are predominantly located in the area of the carotid bodies and are much less common at other sites such as the jugulotympanic and the vagal regions or the larynx. Carcinoids and poorly differentiated carcinomas occur most frequently in the larynx, followed by the salivary glands. Mixed adeno-neuroendocrine tumors arise predominantly in the middle ear.

11.2 Olfactory Neuroblastoma

Definition Olfactory neuroblastoma (ONB) is a malignant tumor composed of neuroblasts derived from the olfactory membrane [7–9]. Terms that are no longer in use are esthesioneuroepithelioma, esthesioneurocytoma and esthesioneuroblastoma.

Epidemiology ONB is a rare tumor, representing approximately 6 % of all sinonasal malignancies [10]. The incidence rate has been estimated at 0.4 cases/million inhabitants per year [11]. According to a recent analysis of the SEER database, the incidence has increased significantly over the period 1973–2006 [10], although these figures may also reflect an improvement in its histopathological diagnosis. ONB has a bimodal age distribution with peaks in the second and sixth decade [12]. This clearly differs from the age peak of adrenal neuroblastomas, which mostly affect children under 4 years of age. Both sexes are equally involved.

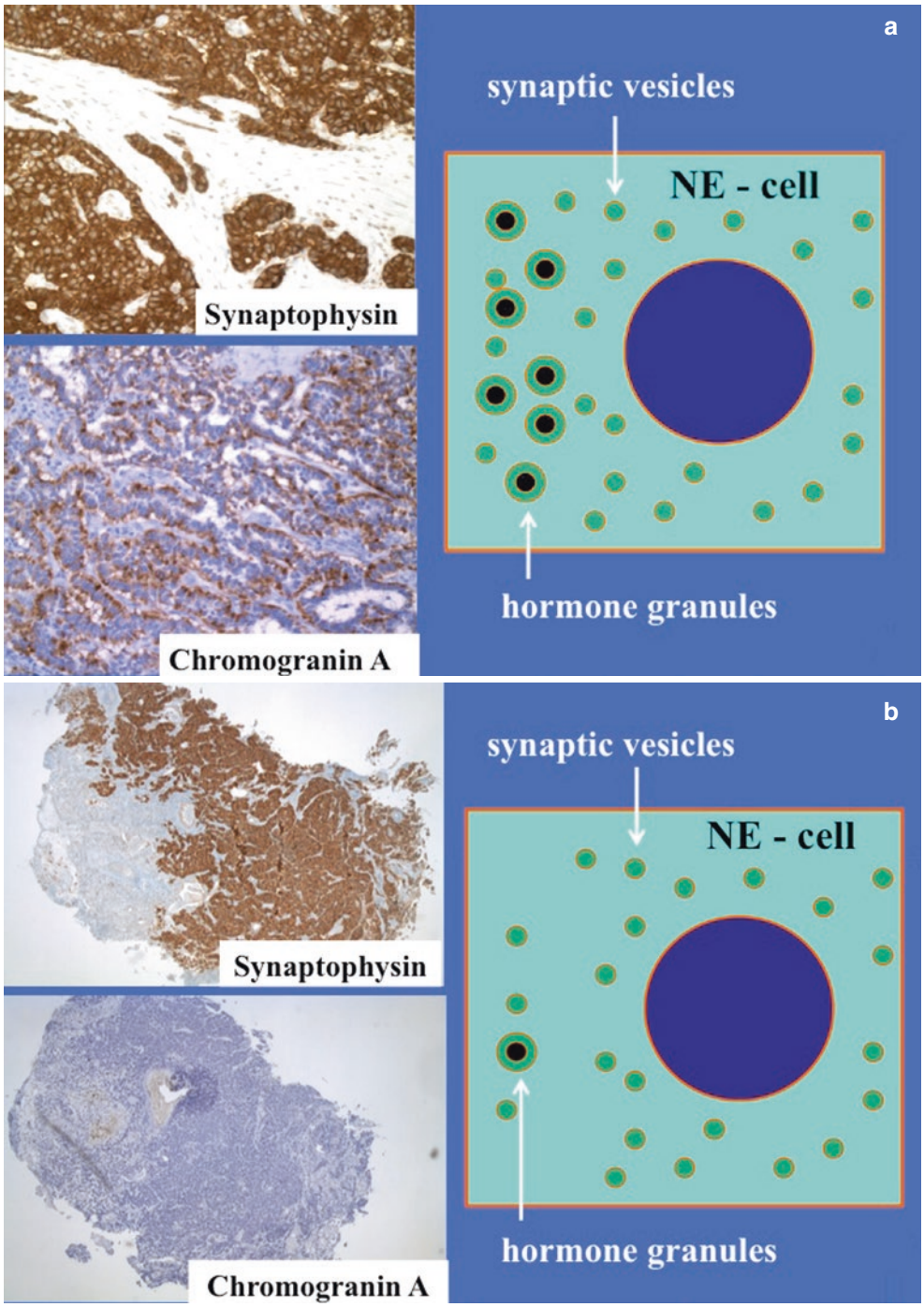

Fig. 11.1 Immunohistochemical expression patterns of synaptophysin and chromogranin A in neuroendocrine (*NE*) cells that (**a**) have many or (**b**) only single hormone granules. Synaptophysin and chromogranin A reside in the membranes of the small synaptic vesicles and the hormone granules, respectively

Localization The site of origin of ONB is confined to the olfactory mucosa that lines the upper part of the nasal cavity [13]. On rare occasions ONB involves predominantly the superior aspect of the cribriform plate and grows as an intracranial mass [14, 15]. The extremely rare ONBs that do not involve the olfactory membrane are known as "ectopic" olfactory neuroblastomas. This diagnosis, however, requires the careful exclusion of all other sinonasal small round cell tumors.

Clinical features The presenting symptoms are non-specific and include nasal obstruction, rhinorrhoea and epistaxis. Anosmia and hyposmia are related to the involvement of the olfactory membrane.

High-resolution CT scan is the radiological study of choice, which allows an evaluation of the local involvement and should also include the neck, for the evaluation of metastatic deposits [16].

Macroscopy: The tumors are often unilateral, presenting as smooth polypoid or fungating masses of fleshy consistency and yellow to pink colour.

Microscopy: At low magnification, ONB exhibits one of two main patterns of growth. Most often, it presents a lobular arrangement with well-defined groups of tumor cells separated by abundant oedematous stroma (Fig. 11.2). Less frequently, the tumor grows as diffuse sheets of cells with scanty but highly vascular stroma (Fig. 11.3). The neoplastic neuroblasts are typically small, showing round to oval nuclei with stippled chromatin, absent or small nucleoli and minimal cytoplasm. They are commonly separated by a neurofibrillary matrix formed by neuronal cell processes (Fig. 11.4), in which axons may be demonstrable by conventional silver stains. This background, seen in about 85 % of ONB, is the most helpful diagnostic feature. Homer Wright-type rosettes is quite characteristic of ONB; however they are less commonly seen (Fig. 11.5). They form when the tumor cells surround the neurofibrillary matrix in collar-like arrangements.

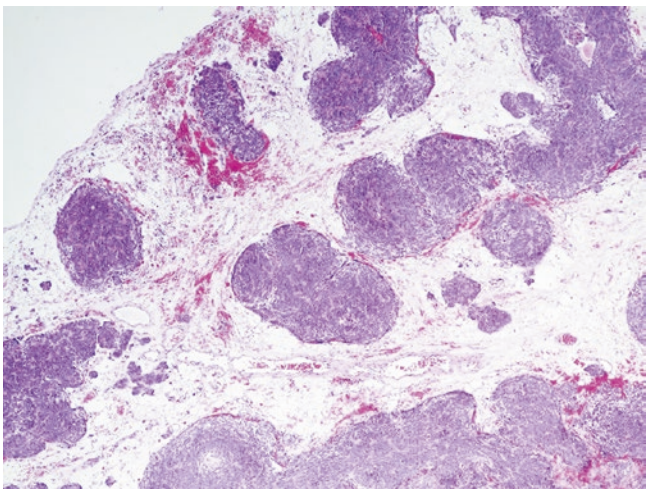

Fig. 11.2 Olfactory neuroblastoma. The tumor consists of lobules separated by oedematous stroma

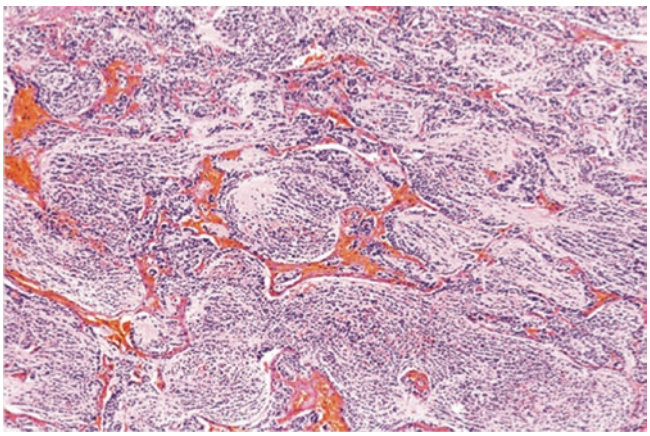

Fig. 11.3 Low power view of a low-grade olfactory neuroblastoma. The tumor has a lobular architecture, and neoplastic cells are set in abundant neurofibrillary stroma (Courtesy of Prof. Antonio Cardesa, Barcelona, Spain)

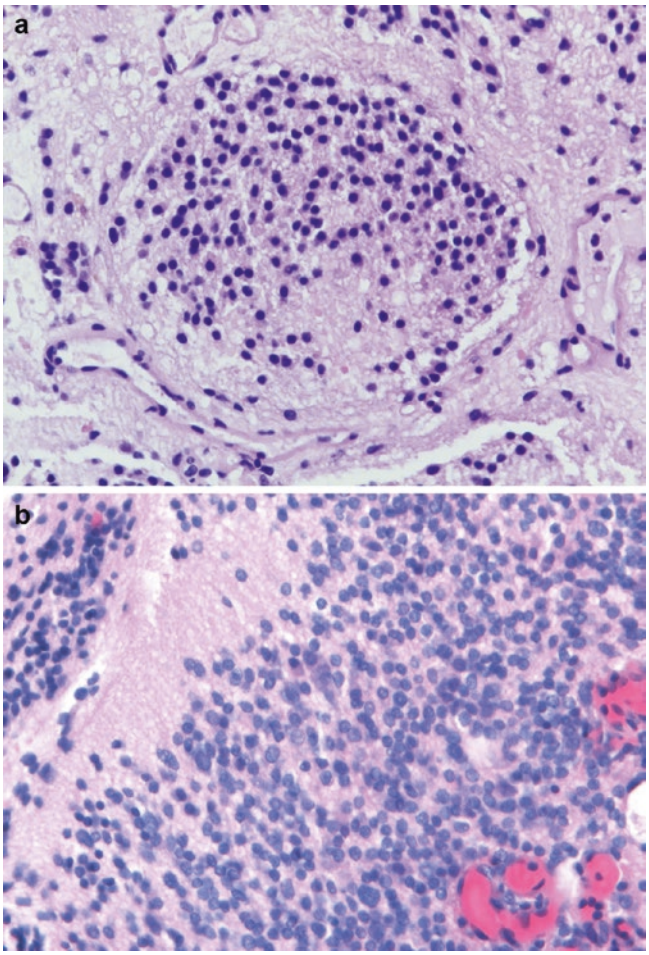

Fig. 11.4 (**a**, **b**) The presence of neoplastic neuroblasts set in neurofibrillary matrix is the most helpful diagnostic feature in olfactory neuroblastoma

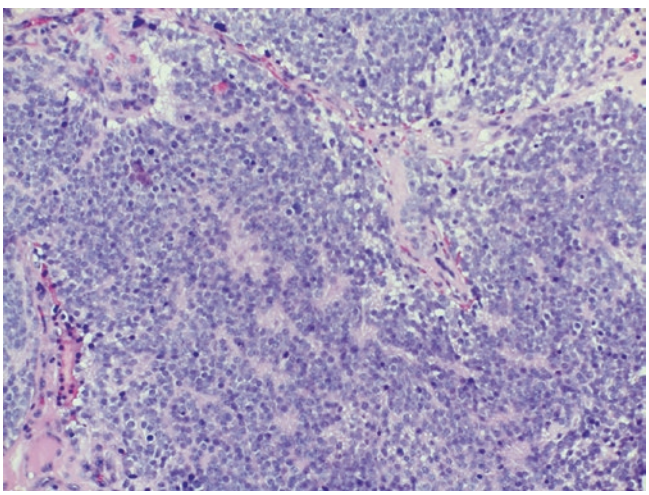

Fig. 11.5 Low power view showing several Homer Wright-type rosettes

Even more rare is true olfactory Flexner-Wintersteiner-type rosettes, which depict well-defined lumina lined by columnar cells resembling olfactory epithelium (Fig. 11.6). These cells generally have basally located nuclei and merge with the adjacent neuroblasts without any intervening basal lamina. Perivascular pseudorosettes, formed by tumor cells arranged around capillaries, are of no diagnostic value, for they may be found in several types of neoplasms. Presence of large ganglion-like cells is a rare finding (Fig. 11.7). Other uncommon features include stromal calcifications and pigment deposition.

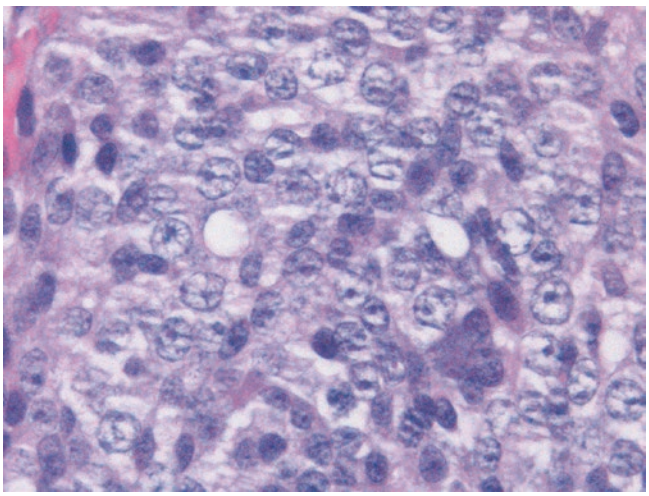

Fig. 11.6 Flexner-Wintersteiner-type rosette in olfactory neuroblastoma. Neoplastic cells surround a lumen-like structure

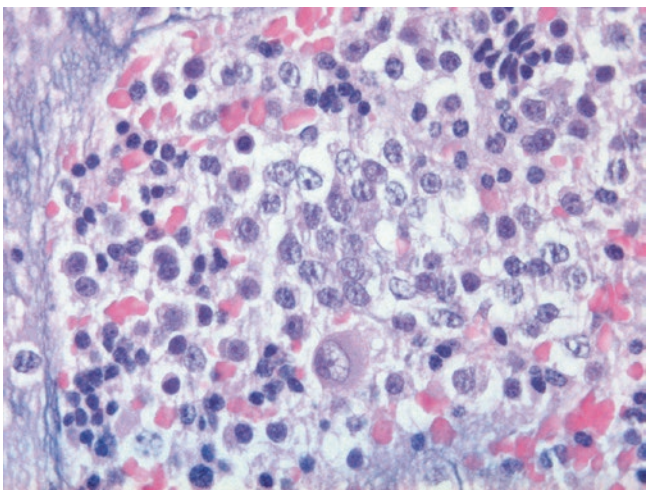

Fig. 11.7 Large ganglion-like cells can be occasionally seen

As the differentiation of ONBs varies from tumor to tumor, a four-tiered grading scheme has been proposed by Hyams [17], that is of prognostic value (Table 11.1). With increasing grade the tumors show loss of differentiation regarding the lobular architecture, neurofibrillary matrix and rosette formation. In addition, there are increasing mitotic activity and necrosis (Fig. 11.8).

Immunohistochemistry ONB shows diffuse positivity for synaptophysin and NSE, and also chromogranin A, neurofilaments and CD56 are often positive [18] (Fig. 11.9a). Neurofilament protein is more often expressed in tumors with diffuse, sheet-like pattern. The human protein analogue of achaete-scute gene *HASH1*, which is found in immature olfactory neurons, is also expressed in ONBs [19]. Conversely the olfactory marker protein [20] that occurs exclusively in mature olfactory neurons is not expressed. In tumors with a lobular nesting pattern, S100 protein is positive in sustentacular cells (Fig. 11.9b). CK is generally negative, although a few ONBs may exhibit focal staining for low-molecular-weight CK. P63 is negative or only focally positive, and EMA is negative [21]. The majority of ONBs shows diffuse staining for calretinin, a marker that is also expressed by olfactory neurons [22]. ONB lacks CD99 (MIC-2) expression.

Electron Microscopy As evidenced by neuroblastic differentiation, neuritic processes, neurotubules and membrane-bound dense-core granules can be demonstrated [23]. Sustentacular cells can be identified at the interface with the surrounding stroma and in close relationship with a well-formed external lamina [24].

Genetics Cytogenetic studies of ONBs have shown multiple chromosomal aberrations, predominantly located on 2q, 6q, 21q and 22q [25]. By array comparative genomic hybridization, the most frequently reported changes

Table 11.1 Hyams grading system for olfactory neuroblastoma

Feature	Grade I	Grade II	Grade III	Grade IV
Lobular architecture	Prominent	Present	Attenuated	Usually absent, but may be retained
Neurofibrillary matrix	Prominent	Present	Attenuated	Absent
Nuclear pleomorphism	Absent	Slight	Moderate	Prominent
Mitotic activity	Absent	Present	Moderate	Brisk
Necrosis	Absent	Absent	May be present	Present
Homer Wright pseudorosettes	Numerous	Present	Focal	Absent
Flexner rosettes	Absent	Absent	Present	Absent

From Ref. [17]

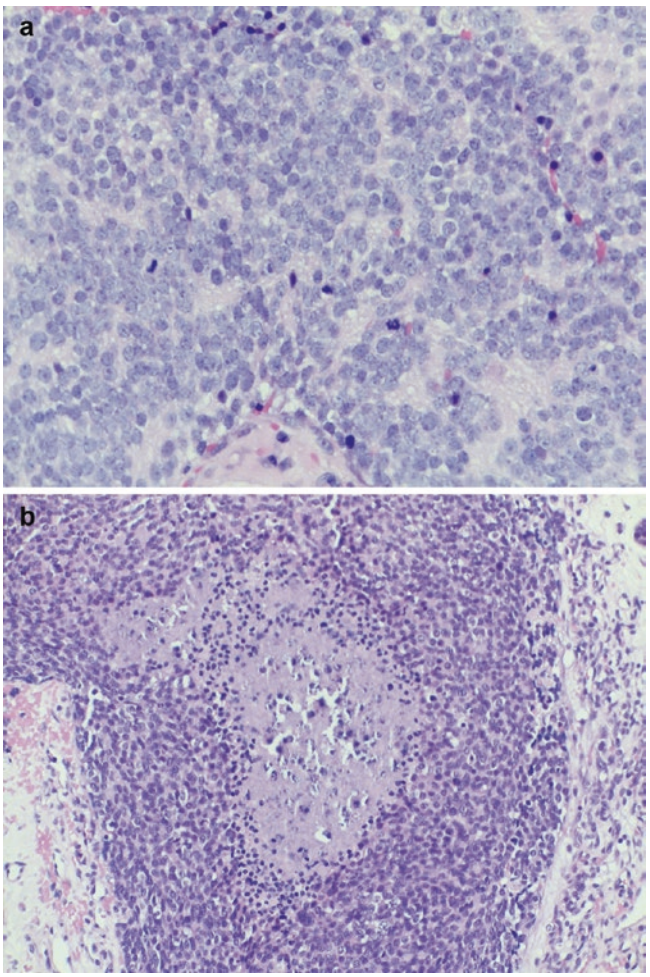

Fig. 11.8 High-grade olfactory neuroblastoma. Neoplastic cells are larger; the background fibrillary matrix is only focally evident. There are several mitotic figures (**a**) and areas of necrosis (**b**)

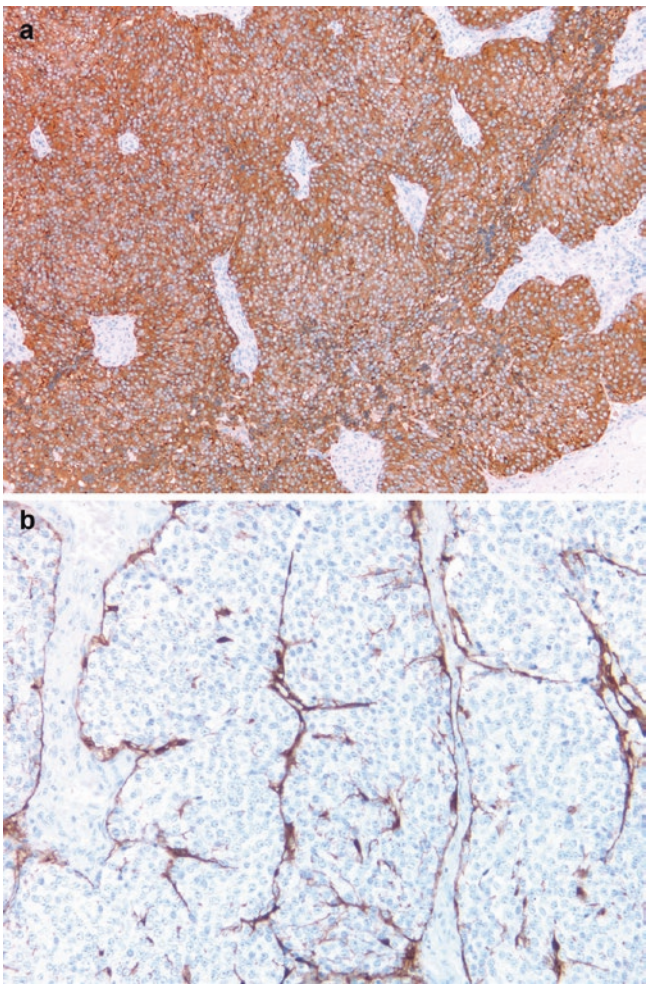

Fig. 11.9 (**a**) Diffuse positivity for synaptophysin in ONB. (**b**) S100-positive sustentacular cells are present at the periphery of neoplastic lobules

included gains at 7q11.22–q21.11, 9p13.3, 13q, 20p/q and Xp/q and losses at 2q31.1, 2q33.3, 2q37.1, 6q16.3, 6q21.33, 6q22.1, 22q11.23, 22q12.1 and Xp/q [26]. Genes relevant for ONB development appear to be located at 20q and 13q [26]. High-stage tumors show more alterations than low-stage ones [26].

ONB lacks the t(11;22) translocation characteristic of Ewing sarcoma/PNET and is therefore probably not related to this tumor group [27]. It also lacks the molecular genetic changes characterizing neuroblastoma in children that occasionally may metastasize to the sinonasal region.

Differential Diagnosis The most important differential diagnosis of ONB is with other small round cell tumors arising in the sinonasal region (Table 11.2). This distinction is important, because ONBs usually show a less aggressive growth than the other round cell malignancies [18, 28, 29]. Careful histologic evaluation, with search of the characteristic features of ONB, including the lobular architecture and the neurofibrillary background, integrated with imaging information on the site of the tumor, is the prerequisite for a correct diagnosis. In moderately and poorly differentiated lesions, a panel of immunohistochemical markers helps in separating ONB from morphological mimics.

Poorly differentiated carcinomas, including sinonasal undifferentiated carcinoma (SNUC), NEC, undifferentiated nasopharyngeal-type carcinoma, basaloid squamous cell carcinoma and NUT carcinoma, can be separated from ONB on the basis of diffuse positivity for cytokeratins of different molecular weights. Other useful markers to separate carcinomas from ONB include p63 and TTF1. Among non-epithelial neoplasms, the distinction of ONB from melanoma can be based on the diffuse positivity for S100 protein, HMB45, Melan-A and SOX-10 of the latter. Ewing's sarcoma/PNET may occur in the sinonasal tract and can be separated from

Table 11.2 Summary of the immunohistochemical and molecular features of selected sinonasal round cell tumors

Entity	CK	SYN	CHR	CD56	S100	HMB45	CD45	CD99	Desmin	P63	Calretinin	EBV	Molecular diagnostics
Olfactory neuroblastoma	–	+	+	+	sustentacular cells	–	–	–	–	–	+	–	–
Neuroendocrine carcinoma	Pan +, 5/6–	+	+	+	–	–	–	–	–	–	–	–	–
Sinonasal undifferentiated carcinoma	7+, 8 +, 5/6–, 13–	– (focal +)	– (focal +)	–	–	–	–	–	–	rarely +	–	–	–
Nasopharyngeal-type undifferentiated carcinoma	Pan +, 5/6 +, 13 +	–	–	–	–	–	–	–	–	+	–	+	–
Basaloid squamous cell carcinoma	Pan+, 5/6+	–	–	–	–	–	–	–	–	+	–	–	–
NUT carcinoma	Pan+, 7+	–	–	–	–	–	–	–	–	+	ND	–	t(15;19)
Ectopic pituitary adenoma	Pan+	+	+	+	–	–	–	+	–	–	+	–	–
Melanoma	– (rarely +)	– (rarely +)	– (rarely +)	– (rarely +)	+	+	–	–	– (rarely +)	–	– (rarely +)	–	–
Lymphoma	–	–	–	+ in NK/T cell	–	–	+	–[a]	–	–	–	+ in NK/T cell	–
Rhadomyosarcoma	– (rarely +)	– (rarely +)	– (rarely +)	– (rarely +)	–	–	–	– (rarely +)	+	–	ND	–	t(2;13) alveolar
Ewing's sarcoma	– (rarely +)	– (focal +)	–	– (focal +)	– (focal +)	–	–		–	–	–	–	t(11;22)
Metastatic neuroblastoma	–	+	+	+	–	–	–	–	–	–	–	–	N-MYC amplification

CK cytokeratin, *SYN* synaptophysin, *CHR* chromogranin, *ND* not determined

ONB, based on its positivity for CD99, which is absent in ONB, and if necessary, the EWS gene rearrangement can be searched for by FISH or PCR. Rhabdomyosarcoma, particularly the alveolar variant, may be included in the differential diagnosis of ONB and can be distinguished based on the positivity for desmin and myogenin. It should be remembered that alveolar rhabdomyosarcoma may be positive for chromogranin and synaptophysin, with significant implications for the differential diagnosis with ONB. Sinonasal lymphomas and plasmocytoma may be difficult to separate from ONB on pure morphological grounds in small biopsies, but their immunoprofiles do not overlap, and therefore the differential diagnosis is usually straightforward. Finally, teratocarcinosarcoma may be entered in the differential diagnosis of ONB for the presence of areas with neural differentiation, which may closely resemble the histological appearance of ONB. However, epithelial and mesenchymal elements can be recognized with a thorough sampling of the lesion.

Treatment and Prognosis In a meta-analysis of the literature, the 5-year overall survival for ONB was 45 %, although most larger series reported a survival of about 70 % [30]. Local recurrence occurs with a frequency of approximately 30 %, while regional spread is observed in 15–20 % and distant metastases in less than 10 % of patients [30]. Distant metastases mainly involve bone and lung [31]. Notably, recurrence may occur several years after treatment, and therefore long-term follow-up is recommended.

Staging of ONB is based on the Kadish system [32], in which stage A disease is confined to the nasal cavity, stage B is confined to the nasal cavity and paranasal sinuses, and stage C shows local or distant spread beyond the nasal cavity or sinuses. This correlates with prognosis [30].

Most relevant for the prognosis are the histopathological grading according to Hyams [33] and the presence of cervical lymph node metastases [30]. Necrosis is the single histological feature that seems to correlate with poor survival [8]. Complete surgical excision, followed by radiation therapy and/or chemotherapy, is the treatment of choice [34]. In advanced ONB, high-dose chemotherapy and autologous bone marrow transplantation have been used. Neoadjuvant chemotherapy and radiotherapy followed by surgery have also been employed in patients with locally advanced tumors [31, 35, 36].

11.3 Paraganglioma

Definition Paragangliomas (PGLs) are neuroendocrine tumors of the autonomic nervous system that arise from neural crest-derived cells of the paraganglia [37]. The use of the old term chemodectoma is discouraged. Tumors arising from the intra-adrenal paraganglia ("adrenal medulla") are known as pheochromocytoma (PCC), whereas similar but extra-adrenal tumors are named PGL. PGLs are further divided into sympathetic and parasympathetic tumors, depending on the type of paraganglia from which they originate (see below).

The concept of a unitary paraganglionic tissue was introduced by Alfred Kohn in 1900 [38]. He coined the term "chromaffin reaction" for the brown discoloration that takes place in the presence of chromate salts and called the stained cells "chromaffin cells". He identified chromaffin tissue in the retroperitoneum, outside the adrenal gland, but also in the carotid body. However, it is worth mentioning that chromaffin reaction is more characteristic of PCC and sympathetic PGL than of parasympathetic head and neck PGL, which have therefore also been called non-chromaffin PGLs.

Epidemiology PCCs and PGLs are rare tumors; their prevalence can be estimated between 1:6,500 and 1:2,500 in the United States. Both sympathetic and parasympathetic PGLs have been reported in all age group, with the greatest frequency in the fourth and fifth decades of live. Sex predilection varies with both tumor location and patient age. While there is no sex predilection for sympathetic PGL, parasympathetic tumors are more frequent in women.

Sporadic PCCs and PGLs are usually diagnosed in 40–50-year-old patients, whereas hereditary tumors are diagnosed earlier, most often before 40 years of age. PCCs and PGLs are rare in children, but when found, they are often extra-adrenal, multifocal and associated with hereditary syndromes.

Parasympathetic PGLs are multicentric in 3–5 % of sporadic cases but in 33 % of familial cases. Multiple sympathetic PGLs, often in association with PCCs, occur most frequently in children. Identification of multiple PGLs in an individual should raise the possibility of a familial disorder.

Hyperplasia Carotid body enlargement has been reported in people living in locations at high altitude [39]. Moreover, carotid body hypertrophy and hyperplasia have been detected in association with chronic obstructive pulmonary disease, systemic hypertension, cystic fibrosis and congenital heart disease [40, 41].

Clinical features Clinical presentation of PGLs can be highly variable [42]. Most often parasympathetic PGL presents as a mass. The rare sympathetic PGLs have symptoms caused by secreted catecholamines, the most common being hypertension, tachycardia, headache, sweating and anxiety. However, only about 1 % of parasympathetic PGLs are clinically functional.

Carotid Body PGL This is the most common head and neck parasympathetic PGL, accounting for 60–70 % of them [43, 44]. It occurs in individuals of either sex in the third to the

eight decade of life. It is a slow-growing, painless tumor, located near the angle of the mandible, deep to anterior border of sternocleidomastoid muscle in the upper or mid neck. Occasionally, patients have dysphagia, carotid sinus syndrome and hoarseness. Rarely, there is pain, with radiation into the head and shoulders as consequence of invasion or compression of the cervical and brachial plexuses. Symptoms due to catecholamine secretion are unusual. Some patients may have signs or symptoms of cranial nerve palsy and also erosion of the skull. Carotid angiography may be a valuable diagnostic aid. Malignancy is reported in around 10 %. Metastases are confined to regional nodes in 50 % of patients, but distant metastasis to bone and lungs may occur [45]. Carotid body PGLs are bilateral in 3–8 % of cases.

Jugulotympanic PGL This is the second most frequent parasympathetic PGL after carotid body tumor [46]. Women are more often affected than men [47, 48]. Most of the tumors arise in the paraganglia located on the tympanic canaliculus (40 %) or in the wall of the jugular bulb (28 %). However, they may also originate from paraganglia located near the middle ear surface of the promontory (20 %) but also from the petrosal ganglion (10 %). Distinction between jugular and tympanic PGLs can easily be made by using imaging methods. Tympanic PGL causes conductive hearing disturbances without cranial nerve involvement, while jugular PGL causes a jugular foramen syndrome, associated with cranial nerve palsy. However, symptoms may overlap in some patients. Some tumors may present as an aural polyp, filling the middle ear cavity and even extending into the external ear canal. In some rare cases, the tumor may grow intracranially, mimicking a meningioma or an acoustic neuroma. After surgical resection, local recurrences are common, but metastasis are very infrequent, representing less than 3 % of the cases, and are frequently restricted to lymph nodes. Tumoirs are bilateral in 1–2 % of cases.

Vagal Body PGL This tumor has also a predilection for women, particularly in the fourth and fifth decades of life [48, 49]. The vagal region is the third most frequent site of parasympathetic PGLs. It usually presents as a painless slowly growing tumor in the lateral neck. The mass is often located in the parapharyngeal space above the carotid bifurcation. Tumors may extend to the base of the skull. Cranial nerve involvement occurs in 30 % of the cases, with vocal cord paralysis, hoarseness or even dysphagia. Metastasis occurs in 10 % of cases, most often to regional lymph nodes but also to the lungs and bones. Multicentricity is seen in 15–20 % of cases.

Laryngeal PGL These PGLs are rare [50, 51]. They arise from superior and inferior laryngeal parasympathetic paraganglia, which are part of the branchiomeric paraganglionic system. Those arising from superior paraganglia are located in the false vocal cords, while those developing in the inferior ones occur at the vicinity of the cricoid cartilage. They occur more frequently in women, in the third and fourth decades of life. They are predominantly located in the supraglottic areas (82 %), followed by the subglottic (15 %) and the glottis area (3 %). Symptoms are related to the location of the tumor, but hoarseness and dysphagia are most frequent.

PGLs in Unusual Locations PGLs have been observed in the nasal cavity, nasopharynx, the orbit, pineal and sellar regions and the thyroid gland [52–54]. It is worth noting that PGL may develop in paraganglia located outside the well-established sympathetic and parasympathetic distributions, explaining presence of PGL in very unusual locations.

Cervical Sympathetic PGL There are very few examples of these tumors, which occur predominantly in the region of the superior cervical ganglion. There is a slight male predominance. All reported cases were benign.

Macroscopy PGLs are solid, well-circumscribed tumors, well demarcated, with expansile borders [55, 56]. They are partially or completely encapsulated with a thin fibrous pseudocapsule, composed of a compressed rim of connective tissue. The cut surface is soft and tan to red-brown (Fig. 11.10). Bands of fibrous tissue are common. The tumor is typically vascular. Congestion and haemorrhage are common. Areas of degeneration, with ischaemic and haemorrhagic necrosis and cystic change, are occasionally seen. Invasion of the lumen of large vessels, such as the carotid artery, may lead to total blood vessel occlusion.

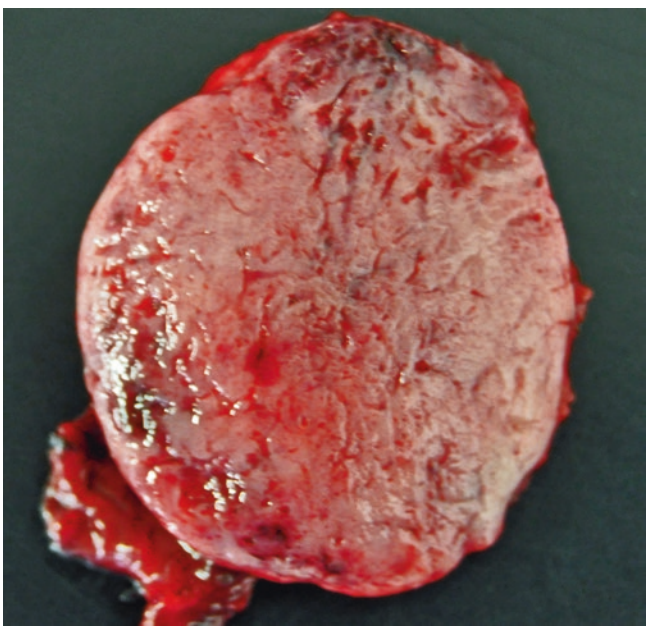

Fig. 11.10 Gross appearance of a carotid body paraganglioma

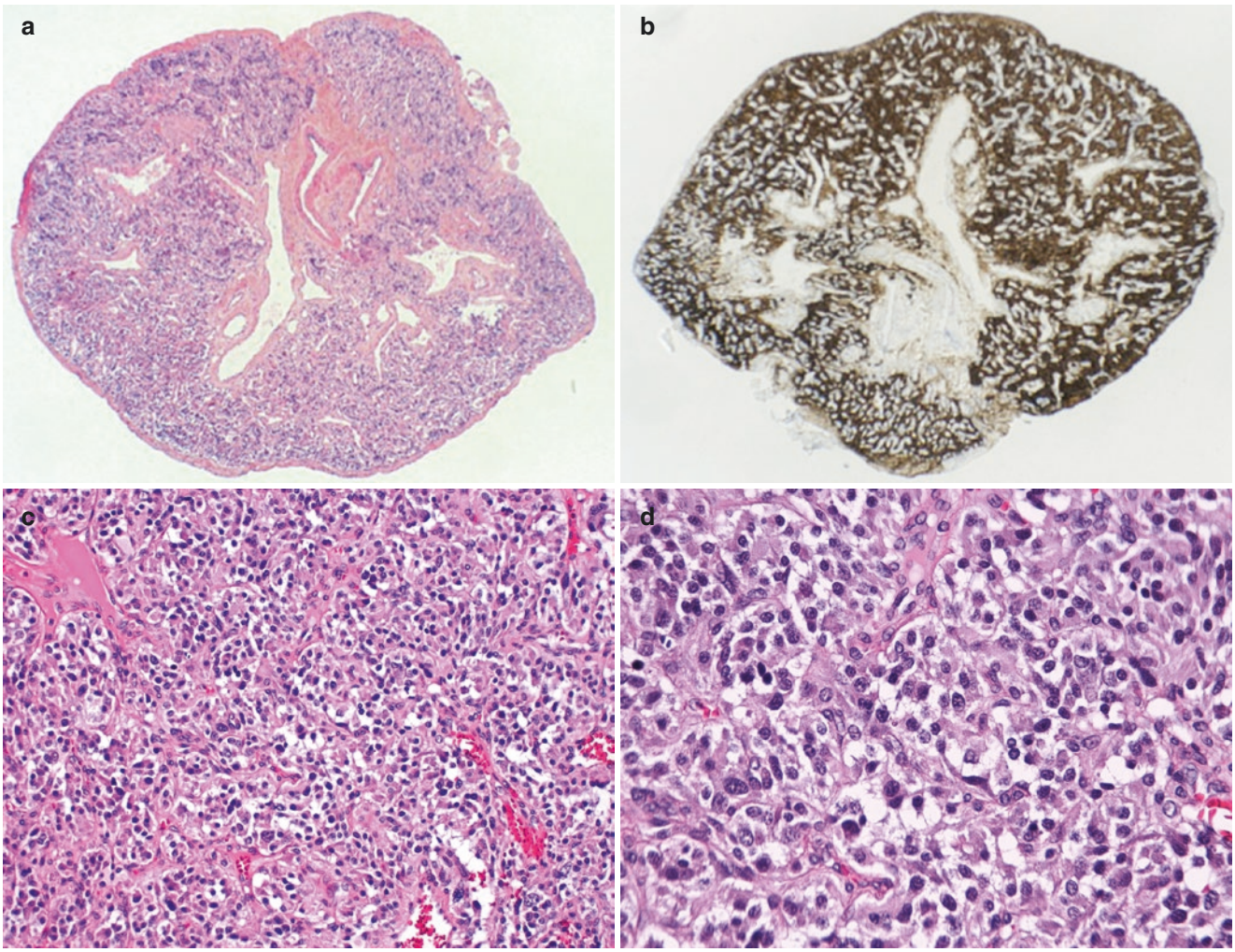

Fig. 11.11 Low power view of a head and neck paraganglioma (**a**, **c**), with diffuse positivity for synaptophysin (**b**). Notice the prominent "zellballen" pattern. High power view (**d**)

Microscopy Histologically, PGLs recapitulate the structure of the normal paraganglia, with the characteristic image of "zellballen" pattern, formed by nests or cords of large uniform polygonal cells with granular amphophilic or basophilic cytoplasm and round nuclei with prominent nucleoli [42, 57–60] (Fig. 11.11a–d). Tumor cells are surrounded by nonneoplastic sustentacular cells and a rich fibrovascular stroma, which may be highlighted by reticulin stains. PGLs from all regions of the head and neck are microscopically similar. However, the zellballen pattern is most often seen in parasympathetic PGL, while the sympathetic ones usually show cells arranged in cords. There is no cellular polarity within the nests.

PGL cells are usually cytologicaly monotonous. However, nuclear pleomorphism, with large bizarre, hyperchromatic cells, may be present, and it is not a criterion for malignancy. Nuclear pseudoinclusions are occasionally seen. Mitotic activity is low. Vascular invasion may occur.

Stromal changes can alter the microscopic appearance of the tumors. Extensive hyaline changes around vessels or sclerosis may be found, sometimes in association with old haemorrhage. Occasionally, perivascular sclerosis is prominent and may accentuate the organoid appearance of tumor cells. In other cases, sinusoidal sclerosis may compress nests of tumor cells, obscuring the neuroendocrine nature of the tumor [61] (Fig. 11.12). Some tumors have marked dilatation of vessels, giving rise to an angiomatoid appearance. Mast cells may also be abundant, as in normal paraganglia. Amyloid deposition has been rarely found.

Unusual microscopic patterns can be found, posing problems in differential diagnosis. One of them is the presence of epithelioid-like sheets of cells. In other cases, tumor cells have a prominent spindle cell arrangement, mimicking mesenchymal tumors. Cells with abundant cytoplasm, with morphological features of oncocytes, may be present. Necrosis is present when the patient had received preoperative embolization.

Immunohistochemistry The neuroendocrine (chief) cells are positive for chromogranin A and synaptophysin and usu-

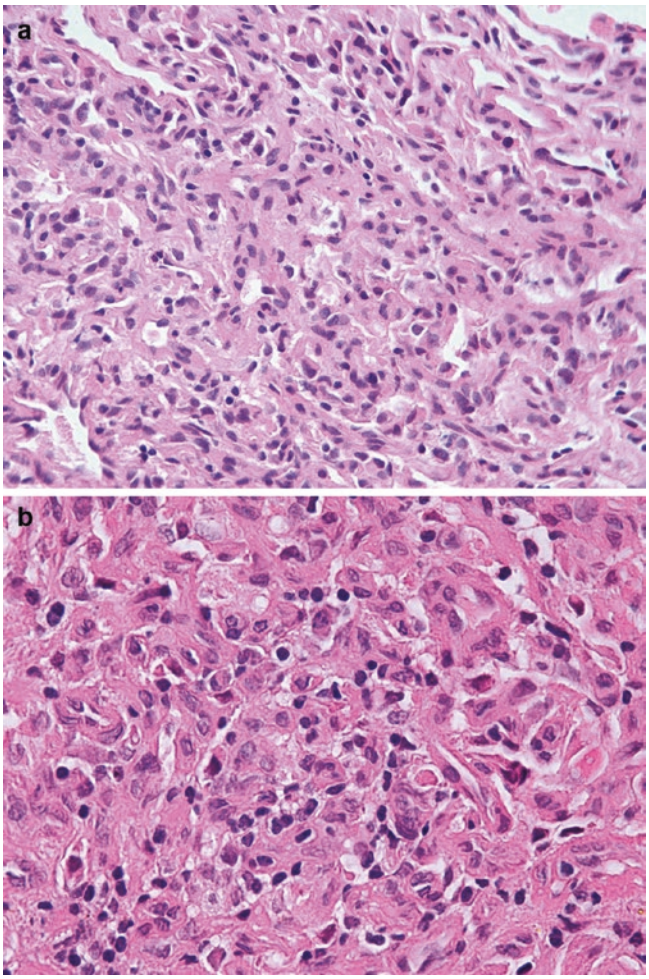

Fig. 11.12 (**a**) Sclerosis may obscure the neuroendocrine nature of head and neck paraganglioma. (**b**) Prominent fibrosis in a paraganglioma

ally negative for CKs. Only in occasional cases, they may be focally CK positive. Sustentacular cells stain for S100 protein and occasionally for glial fibrillary acidic protein. Immunoreactivity for tyrosine hydroxylase is helpful in distinguishing PGLs from other chromogranin A-positive tumors. However, tyrosine hydroxylase is often negative in head and neck PGLs.

Electron microscopy reveals tumor cells with cytoplasmic processes that surround neighbouring cells. The cytoplasm contains neurosecretory membrane-bound granules but also large mitochondria and inconspicuous Golgi apparatus and smooth and rough endoplasmic reticulum. Considerable heterogeneity regarding neurosecretory granules (granule size, shape and electron-density) may be seen between tumors but also between individual cells in one tumor.

Malignant PGL According to WHO 2003, malignant PGLs and PCCs are defined by the presence of metastasis, not by local invasion. Metastasis must be to a site where paraganglionic tissue is normally not found, as in the liver and bone. For lymph node metastasis, a clearly identifiable lymph node area infiltrated by tumor cells is required. As mentioned before, the risk of metastasis depends on the location of the tumor. For carotid body and vagal PGL, the estimated frequency is around 10 %. Metastases tend to occur in regional lymph nodes but occasionally in lung and bone.

There are no accepted microscopic criteria for malignancy. A number of studies have proposed that some morphologic features and also some clinical characteristics could be predictive of malignant behaviour, but they have been focused on PCCs or sympathetic PGLs, rather than parasympathetic PGLs. For that reason, their applicability to head and neck PGLs is questionable [62–64].

In one study, more than 70 % of PCC/PGLs could be classified correctly as malignant with more than 95 % probability on the basis of four criteria: extra-adrenal location, coarse nodularity, confluent necrosis and absence of hyaline globules [65]. The PASS system (phaeochromocytoma and adrenal scaled score) was proposed to assess the value of 12 different histopathological features in PCC (cellularity, necrosis, mitosis, nuclear pleomorphism, tumor cell spindling, growth pattern). All tumors that metastasized had a score greater than 4, but 17 of 50 with score greater than 4 had not metastasized [66]. An independent series of cases validated the significance of PASS scores higher than 4 in cases including PGLs. In a different study, vascular architecture, after CD-34 immunostaining, was characteristic of malignant behaviour in PCC [67]. A different scoring system addressed to both PCC and extraadrenal sympathetic PGL, combining histologic, immunohistochemical and biochemical features, was proposed by Kimura [68]. Several biomarkers have been proposed, as molecular markers of malignant PCC, including HSP90, human telomerase reverse transcriptase, VEGF, VEGFR2, HIF-2a, COX-2, tenascin C, N-cadherin and secretogranin II-derived peptide EM66. SDHB has also been suggested as a marker of metastatic potential [69]. Finally, Ki67 (with a threshold of 5 %) has been proposed as marker of metastatic potential, but there is still a need for standardization of methodology and scoring.

Differential Diagnosis Tumors that should be distinguished from PGLs are carcinoids, medullary thyroid carcinoma, hyalinizing trabecular tumors of the thyroid, hemangiopericytomas and glomangiomas. Less commonly, alveolar soft part sarcoma, melanoma, granular cell tumor and metastatic renal cell carcinoma may pose problems in differential diagnosis.

PGLs do neither express CK nor show cellular polarity within the nests. Together with a reticular staining pattern for S100, these are helpful findings in the differential diagnosis with carcinoids. The hyalinizing trabecular tumor of the thyroid is positive for CK and TTF-1.

Differential diagnosis with hemangiopericytomas and glomangiomas is easy with the help of reticulin stains but also immunostaining for vascular and neuroendocrine markers.

Probably, one of the most traditional problems is the differential diagnosis between PGL and medullary thyroid carcinoma (MTC) in two scenarios: first, a lymph node metastasis of MTC is mimicking a PGLand, second, a thyroidal PGL mimics a primary MTC. Usually, this differential diagnosis is solved with the help of some markers which are frequently expressed in MTC (TTF-1, calcitonin, CEA). However, some of these markers are not absolutely specific for MTC and may be expressed in other tumors as well. Recently, a panel of seven antibodies was proposed. In this study, the authors performed a comparative analysis of the expression profile of head and neck PGLs and MTCs, based on cDNA arrays from two series of fresh-frozen samples of PGLs and MTCs, respectively. Seven biomarkers, which showed differential expression, were selected, NDUFA4L2, COXIV2, VMAT2, CGRP/calcitonin, CEA, TTF-1 and immunohistochemically tested on two tissue microarrays constructed from two different series of paraffin-embedded samples of PGL and MTC. It was possible by combining the negativity or low staining of CGRP (Hscore <10) or calcitonin (Hscore <40) together with the positivity of any of the three markers NDUFA4L2, COXIV2 or VMAT2 to predict PGL in all cases [54].

Familial Syndromes with High Frequency of PGLs PCC and PGL can occur sporadically or as part of hereditary tumor syndromes such as neurofibromatosis type 1 (NF-1), von Hippel-Lindau disease (VHL), multiple endocrine neoplasia type 2 (MEN-2) and the succinate dehydrogenase (SDH) syndromes. NF-1, VHL and MEN-2 are usually associated with PCCs and PGLs outside the head and neck region, while patients with SDH syndromes have an increased risk of head and neck PGLs [70–76]. Recently, an international consortium registered patients with head and neck PGLs and performed mutation analysis of the *VHL* and *RET* genes. Among 809 patients, 11 had germline mutations in the *VHL* gene and 1 in the *RET* gene. A literature review revealed head and neck PGLs in five additional VHL carriers, one patient with MEN-2 and one patient with NF-1.

SDH syndromes have been classified genetically into four groups (PGL1, PGL2, PGL3 and PGL4) associated with germline mutations in the *SDHD*, *SDHAF2*, *SDHC* and *SDHB* genes, respectively. These *SDH* genes encode the subunits of succinate dehydrogenase, an enzyme with a key function in the Krebs cycle and the respiratory chain. *SDH* genes act as classical tumor suppressor genes. The mutations can also cause inactivation of SDH protein function. The neoplastic transformation occurs when in the somatic cells the remaining wild-type allele is lost or inactivated.

SDHA, SDHB, SDHC and *SDHD* encode for the four subunits of the SDH complex, also known as the mitochondrial complex II of the mitochondrial respiratory chain. This is a highly conserved enzyme, also involved in the Krebs cycle. Complex II is composed of a catalytic domain consisting of a flavoprotein (SDHA) and an iron protein (SDHB). The SDHC subunit functions, together with the SDHD subunit, as a membrane anchor of complex II that attach the catalytic domains to the inner mitochondrial membranes.

After the identification of additional PCC/PGL genes, about 40 % of the patients with apparently sporadic PCC and PGL elicited a germline mutation in a known susceptibility gene. Two additional susceptibility genes *KIF1Bβ* and *EGLN1/PHD2* have been reported but one in three patients only. This frequency can be as high as 79 % in cases with a family history of the disease and can reach values up to 54 % in patients with PGL in the head and neck region. *SDHB* and *SDHD* are the most commonly altered *SDH* genes and there are only two reports in the literature of somatic mutations (on each in *SDHB* and *SDHD*) in the absence of germline mutations [77–83].

PGL1 results from mutations of *SDHD* (11q23). The disease is inherited from the paternal line, probably as a result of maternal imprinting. It manifests normally with head and neck PGLs but also with sympathetic PGLs and PCCs. It has a high penetrance and multifocal tumors are common, but malignancy is rare.

PGL2 is a rare cause of head and neck PGLs, and no metastases have been reported. The gene responsible for PGL2, *SDHAF2* (11q13.1), is rarely mutated.

PGL3 is also rare and occurs together with mutations of *SDHC* (1q21–23). Patients develop tumors that are unifocal, usually benign, and typically located in the head and neck region, usually in the carotid body area. They also have an increased higher risk for the occurrence of gastrointestinal stromal tumors.

PGL4 is caused by *SDHB* mutations (1p36) and generally manifests as catecholamine-secreting sympathetic PGL occurring in the abdomen and pelvis or, more rarely, in the thorax (adrenal PCCs and head and neck PGLs are uncommon) and are characterized by a high risk of malignancy (35–50 %). *SDHB* mutations are associated with a dysregulated hypoxia pathway, as well as with the overexpression of HIFα and hypoxia-inducible gene products such as VEGF. *SDHB*-related extra-adrenal PGL can develop into highly aggressive tumors with an estimated frequency among observed populations that range from 31 % to 71 % and that are associated with poor prognosis. They can occur at very young age and tumor recurrence may also occur even up to 20 years after primary tumor diagnosis. *SDHB* mutations have been suggested also to be associated with malignant tumors of the extra-paraganglial system such as renal cell carcinoma, gastrointestinal stromal tumors and thyroid carcinomas.

Mannelli found *SDH* germline mutations in 31 % of 106 patients with head and neck PGLs [84]. *SDHD* mutations were more common (26 %) in comparison with those in *SDHB* (4 %) and *SDHC* (3 %) and were also more frequently associated with multifocal disease. Results have been confirmed in other series.

For correct diagnosis of these syndromes, clinical parameters including family history, multifocal disease, young age at onset and malignant tumor are insufficient. Furthermore, the familial basis of PGL and PCC may not be recognized clinically due to incomplete penetrance or phenotypic heterogeneity. Therefore, it is of great clinical relevance to determine if any of the features of PGL/PCC syndromes are associated with a particular gene mutation.

Early genetic testing is essential for patients with PGL because the identification of a *SDHD* mutation is suggestive of multiple disease, and *SDHB* mutations are strongly associated with malignancy and poor prognosis. Moreover, many SDHB patients with no family history present with a thoracic-abdominal or a pelvic disease, which in some cases is malignant. At present, and due to the costly techniques that have to be applied, it is controversial whether complete mutation testing should be performed in all PCCs/PGLs or should be limited to those tumors that are associated with risk factors such as young age, multiplicity, malignant behaviour and/or extra-adrenal location. As a cheap surrogate test, a negative immunostaining for SDHB has found to be a good indicator of SDHB, SDHC or SDHB mutations and probably also of other abnormalities in the mitochondrial complex II [85]. Obviously, the mutations in any of the different *SDH* genes cause blockage of SDHB protein expression as well as a loss of SDH enzyme activity regardless of whether its gene product has catalytic or anchorage function. In vitro studies have supported these assumptions. If the tumor exhibits negative or weakly positive SDHB staining, the patient should be tested for the presence of mutations in any of the *SDH* genes. SDHB-IHC [86–88] is therefore a cost-effective alternative to genetic testing for *SDHB, SDHC* or *SDHD* mutations and, probably also, for mutations in other genes that interact with the mitochondrial complex II (Fig. 11.13). Interestingly, negative SDH staining may be occasionally observed in patients without a mutation in any of the *SDH* genes. Two possible explanations can be offered. First, the patients have mutations that are not detected by molecular analysis (mutations in untranslated, intronic or promoter region of the genes) or, second, they have epigenetic alterations. It is worth mentioning that immunostaining for SDHA is also available (*SDHA*-mutated tumors do not react with both SDHB and SDHA antibodies), but the clinical relevance of this test is low, since mutations of the *SDHA* gene are very rare [88] (Fig. 11.14).

Microarray-gene expression profiling revealed distinct differences in gene expression among hereditary PGLs and

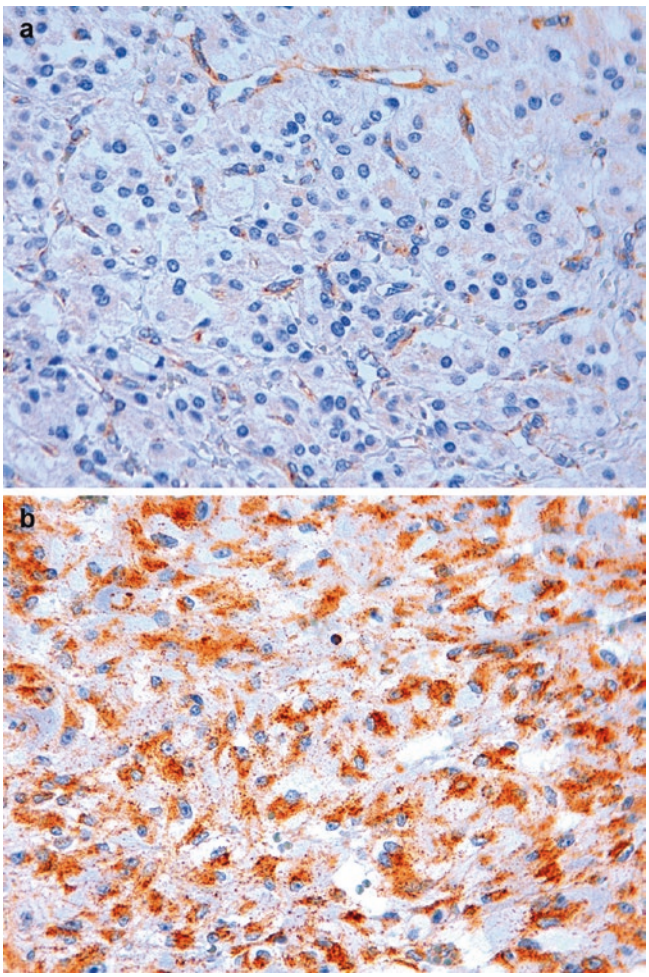

Fig. 11.13 Negative SDHB immunostaining in a cervical paraganglioma associated with a SDHB mutation (**a**). Positive SDHB staining in a cervical paraganglioma (**b**)

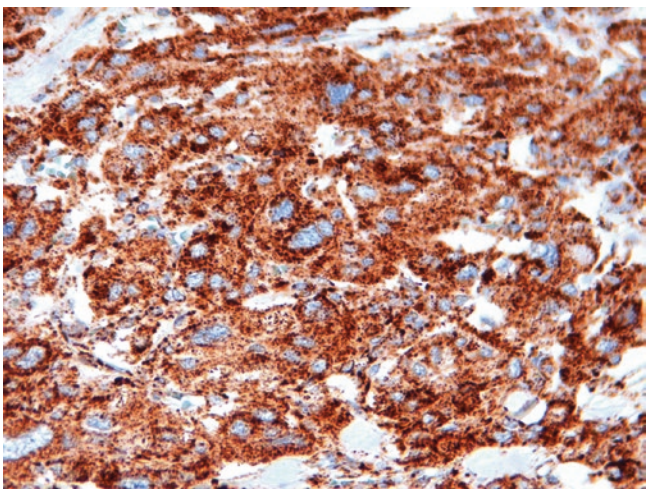

Fig. 11.14 Positive SDHA staining in a paraganglioma

PCCs. The first cluster comprised tumors caused by mutations in *VHL* and in *SDH* genes, while the second cluster encompassed tumors caused by mutations in *RET*, *NF-1* and *TMEM127*. In fact, these two main clusters discriminated the tumors by the absence or presence of epinephrine production.

11.4 Neuroendocrine Neoplasms: Carcinoids and Poorly Differentiated Tumors

Classification The classification of head and neck NENs of epithelial origin is still debated, but a recent proposal related it more or less to the classification of lung NENs that divides these neoplasms into four types [89]: (1) typical carcinoid ("well-differentiated neuroendocrine carcinoma"), (2) atypical carcinoid ("moderately differentiated neuroendocrine carcinoma"), (3) poorly differentiated neuroendocrine carcinoma of small cell type, and (4) poorly differentiated neuroendocrine carcinoma of large cell type. As an additional category the "mixed" neoplasms may be added, which show neuroendocrine tumor tissue in combination with a nonneuroendocrine carcinoma component. These tumors have been called combined small cell neuroendocrine carcinoma (because they are usually poorly differentiated) or mixed adeno- or squamous neuroendocrine carcinoma.

If the classification of the gastroenteropancreatic NENs is used as template for the categorization of the head and neck NENs, then again four types can be distinguished: (1) neuroendocrine tumor (NET) G1 (Ki67 index <2 %), (2) NET G2 (Ki67 index 2–20 %), NEC of the small cell type G3 (Ki67 index >20 %) and NEC of the large cell type G3 (Ki67 index >20 %). In this classification NET G1 is regarded as equivalent to typical carcinoid; NET G2 corresponds largely to the atypical carcinoid category, and NEC G3 meets the criteria of poorly differentiated small cell- or large cell-type neuroendocrine carcinoma. In a recent study in lung NENs, this classification has been validated regarding its biological relevance, using Ki67% cutoffs of <4 for G1, 4–<25 for G2 and >25 for G3 [90].

All the above-discussed classifications correlate well with the biological behaviour of NENs. The best prognosis and the slowest growth are associated with the typical carcinoid/NET G1 and the worst prognosis and fastest growth with the poorly differentiated neuroendocrine carcinoma/NEC G3. An intermediate prognosis is associated with the atypical carcinoid [91].

Used Nomenclature In this contribution three types of pure NENs will be distinguished and discussed: (1) typical carcinoid/NET G1 ("well-differentiated neuroendocrine carcinoma "), (2) atypical carcinoid/NET G2 ("moderately differentiated neuroendocrine carcinoma") and (3) poorly differentiated NEC G3, small cell type and large cell type. In addition, the combined/mixed neuroendocrine-nonneuroendocrine carcinoma will be introduced.

Distribution and Relative Frequency Epithelial NENs can occur almost everywhere in the head and neck region. However, they are not equally distributed. They are infrequent in the oral cavity, the tonsils, the ears and the orbit, but are common in the larynx and, in descending order, also in the salivary glands and the sinonasal tract. If they are separated according to their differentiation, the poorly differentiated tumors outnumber their well-differentiated counterparts, particularly in regions such as the salivary glands and the sinonasal tract.

Pathology The histological appearance of most NENs of epithelial origin, i.e. carcinoids/NETs and poorly differentiated carcinomas/NECs, is generally similar, irrespective of their site of origin. Therefore a summarizing account of their pathology is given below. Where there are special characteristics, these are mentioned in the sections devoted to the clinicopathological features of the various tumor entities discussed below.

Typical Carcinoid/NETs G1 *Macroscopically*, these tumors present as submucosal nodules or polypoid lesions measuring up to 2 cm in diameter. *Microscopically*, they are composed of small uniform cells growing in islands, ribbons and cords (Fig. 11.15a), occasionally forming gland-like structures. Mucin may occasionally be present. The nuclei are round, with finely dispersed chromatin and inconspicuous nucleoli; the cytoplasm is scant, clear or eosinophilic. Mitoses are sparse or absent, and there is no necrosis or cellular pleomorphism.

Immunohistochemically, they express synaptophysin, chromogranin A, neuron-specific enolase, CK18 and SSTR2A (Fig. 11.15b) [92]. Electron microscopy reveals typical dense-core neurosecretory granules [1, 93].

Differential diagnosis includes atypical carcinoid, paraganglioma and adenocarcinoma and is discussed at the end of the next section.

Atypical Carcinoid/NET G2 *Macroscopically*, this tumor type presents as a well-defined mass, usually in submucosal position with or without surface ulceration (Fig. 11.16a), measuring up to 4 cm in diameter (average 1.6 cm). *Microscopically*, the tumor grows in trabeculae, cords, ribbons (Fig. 11.16b) and solid structures; the tumor cells are round, with round nuclei and a moderate amount of cytoplasm, which is slightly eosinophilic or occasionally oncocytic. Mucin production may be present [94]. In contrast to typical carcinoid, there is increased mitotic activity (two to

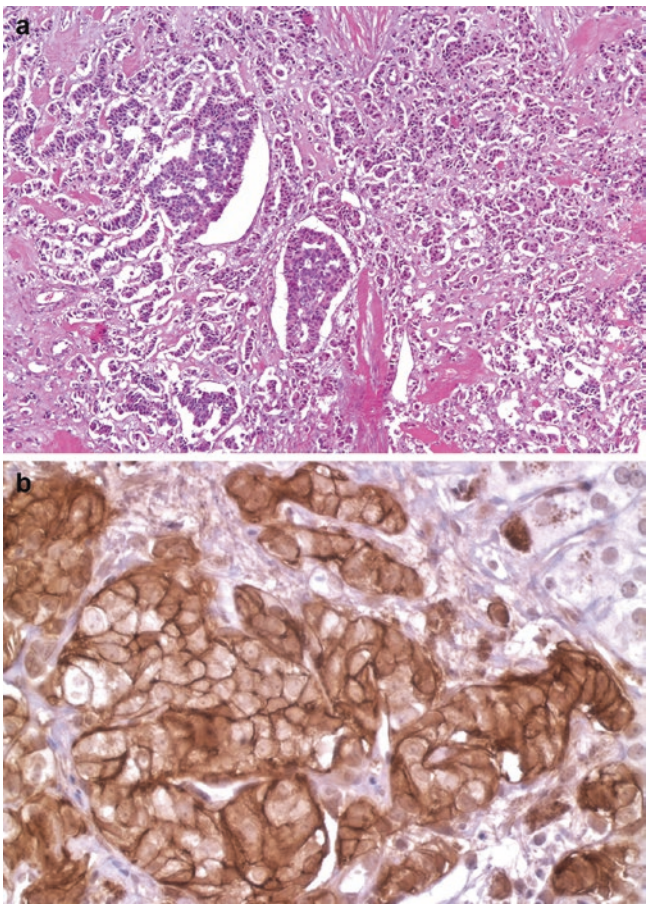

Fig. 11.15 Typical carcinoid of the larynx. (**a**) Trabecular and solid tumor formations embedded in dense stroma. (**b**) Membranous expression of the somatostatin receptor 2A (*SSTR2A*)

ten mitoses/2 mm^2 equaling approximately ten HPF), an increased Ki67/MIB1index (2–20 %), slight cellular pleomorphism and frequently necrosis. Vascular and perineural invasion may be present.

Immunohistochemically, synaptophysin, chromogranin A (Fig. 11.16c), CD56 and CK18 are positive. In the larynx, atypical carcinoids may also express calcitonin and CEA, but rarely somatostatin or serotonin [95, 96].

Differential diagnosis includes paraganglioma, adenocarcinoma, poorly differentiated NENs and, in the larynx region, thyroid medullary carcinoma. The differentiation between paraganglioma and atypical carcinoid is important because the former usually follows a benign course while the latter behaves as an aggressive tumor. The correct diagnosis relies on the demonstration of CK in atypical carcinoid, which is lacking in paraganglioma. Adenocarcinoma can be distinguished from carcinoid by the absence of neuroendocrine markers. Exceptions are some intestinal-type adenocarcinomas that show a neuroendocrine cell component that usually is less than 30 % of the tumor cell mass (see also below combined/mixed neuroendocrine and non-neuroendocrine carcinoma). The presence of cellular pleomorphism, increased mitotic activity and necrosis helps to distinguish atypical from typical carcinoids. Differentiation from thyroid medullary carcinoma may be difficult, especially when dealing with a cervical metastasis, as tumor cells in both medullary carcinoma and atypical carcinoid of the larynx express calcitonin. The most important distinguishing feature is then the different locations of the primary tumors. Additional useful information may be obtained by measuring the serum level of CEA, which is elevated in metastatic medullary carcinoma of the thyroid and usually normal in atypical carcinoid. Moreover, atypical carcinoids of the larynx, in contrast to medullary thyroid carcinoma, usually lack amyloid deposits. An elevated calcitonin serum level should not be considered as a reliable feature of medullary carcinoma, as it has been reported in patients with atypical carcinoids of the larynx [93, 96].

Poorly Differentiated Neuroendocrine Carcinoma/NEC G3 *Macroscopically*, these NENs usually form large masses, which, in a submucosal position, are frequently ulcerated and are indistinguishable from other carcinomas. *Microscopically*, the tumors are identical to their pulmonary small cell or large cell counterparts [97].

Small cell carcinomas are composed of closely packed (small- or intermediate-sized) cells with scant cytoplasm and hyperchromatic round, oval or spindle nuclei with dispersed ("salt and pepper") chromatin and without distinct nucleoli (Fig. 11.17). They show a diffuse solid growth. Mitoses are numerous and necroses, and vascular and perineural invasions are common.

In large cell carcinomas the cells show abundant cytoplasm and round nuclei with sparse heterochromatin and a prominent nucleolus. The tumor cells often still form an organoid carcinoid-like growth pattern but, in contrast to carcinoids, show extensive necrosis, many mitoses (greater than ten mitoses per 2 mm^2 equaling approximately ten HPF) (Fig. 11.18a) and an infiltration into the surrounding tissues [98]. The Ki67/MIB1 proliferation index exceeds 20 % and in many cases is greater than 50 % (Fig. 11.18b) [99].

Immunohistochemically, all tumor cells express synaptophysin (Fig. 11.18c), occasionally in a dot-like pattern, and usually also CD56. The stainings for chromogranin A and CKs (often dot-like) are variable (Fig. 11.18d). In small cell carcinomas, chromogranin A and CK may occasionally be negative. TP53 is commonly expressed [100]. By *electron microscopy*, neurosecretory granules (80–200 nm) are present, but can be so few that they are difficult to find.

In the *differential diagnosis*, the possibility of a metastasis from a poorly differentiated NEN of the lung must

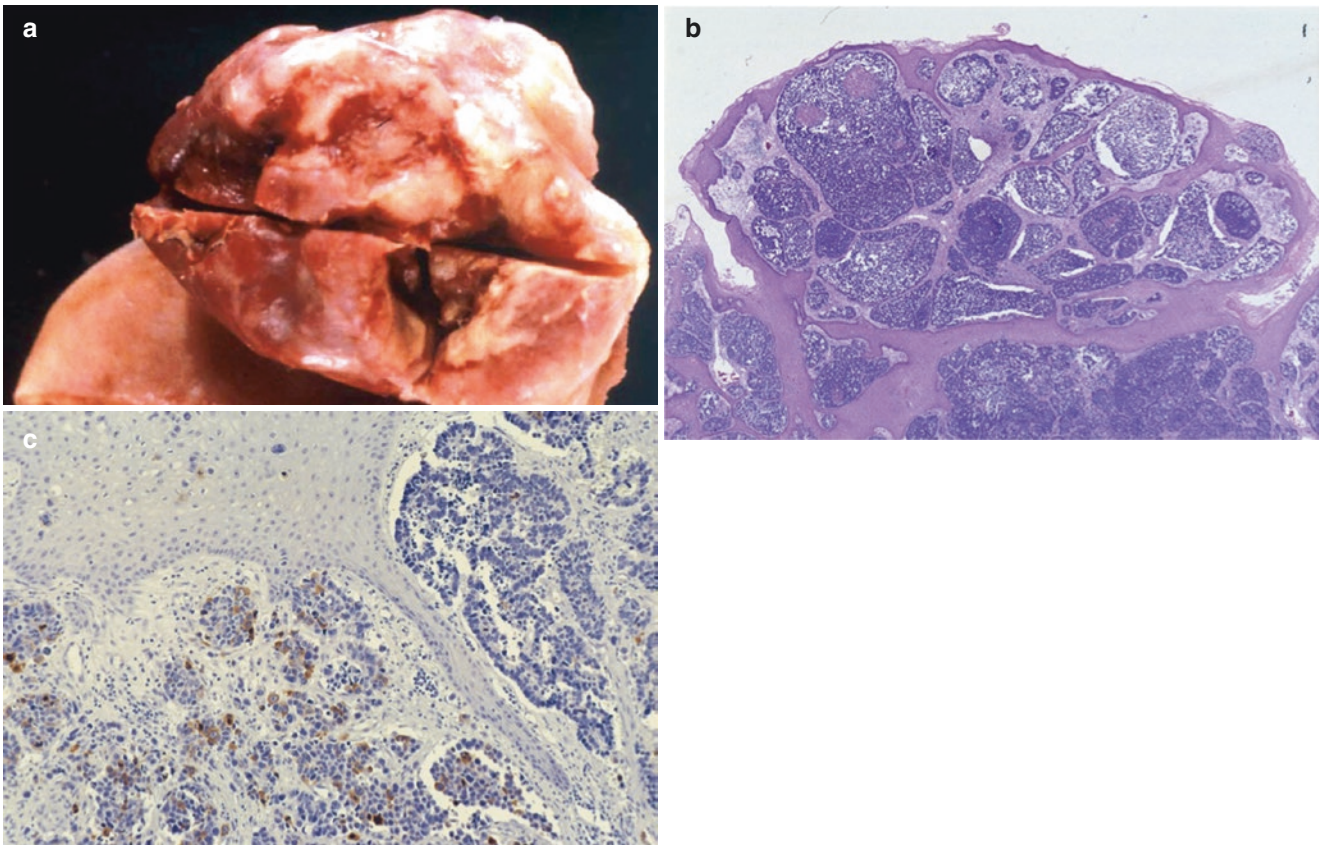

Fig. 11.16 Atypical carcinoid of the larynx. (**a**) Supraglottic polypoid tumor with ulcerations. (**b**) Subepithelial solid tumor cell formations with intratumoural necrosis. (**c**) Scattered tumor cells express chromogranin A

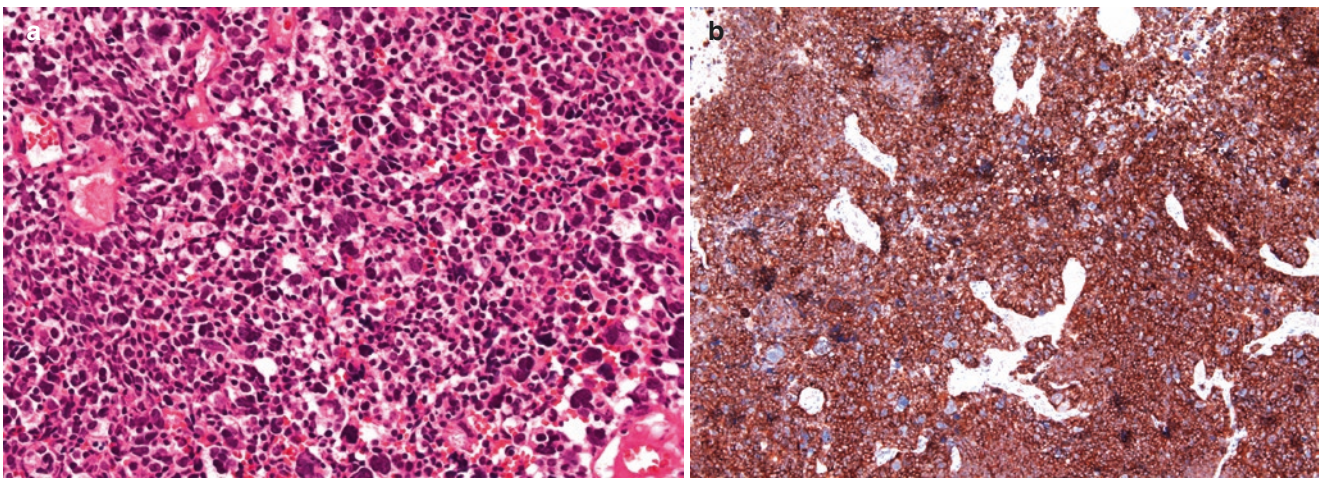

Fig. 11.17 Poorly differentiated neuroendocrine neoplasm of the larynx showing (**a**) pleomorphic cells and the nuclear features of small cell-type carcinoma and (**b**) synaptophysin positivity

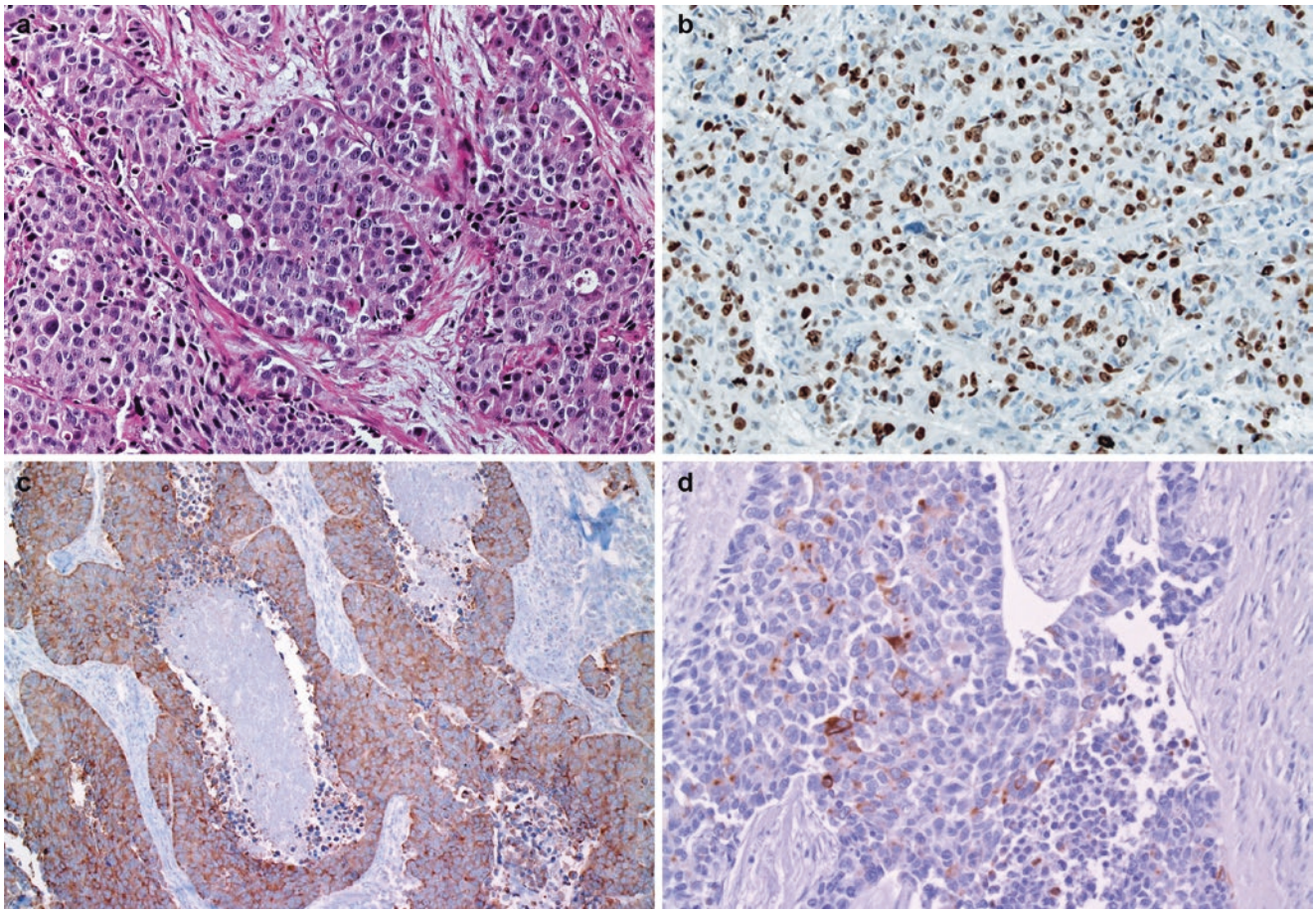

Fig. 11.18 Poorly differentiated neuroendocrine neoplasm of the larynx of large cell type. (**a**) The tumor cells carry a round nucleus with a distinct nucleolus and form solid and organoid structure. There are abundant mitoses. (**b**) MIB1 immunostaining reveals the high proliferative activity. (**c**) Diffusely positive immunostaining for synaptophysin. (**d**) Scattered immunostaining for chromogranin A

first be excluded. This has to be done clinically, since there is no good marker (including TTF1) which can be used for this distinction. Further, atypical carcinoid, basaloid squamous carcinoma, malignant lymphoma and malignant melanoma have to be considered. Atypical carcinoid is separated from poorly differentiated NEN, particularly of the large cell type, by the number of mitoses, the extent of necrosis and the common expression of TP53 [91]. Basaloid squamous carcinoma is composed of larger cells, contains areas of squamous differentiation, stains for p63 and CK5/6, and is frequently associated with atypia of the overlying squamous epithelium. Malignant lymphomas characteristically express leukocyte common antigen (CD45) and B- or T-cell markers. Malignant melanoma occasionally consists of small undifferentiated cells, thus resembling poorly differentiated NEN, but, in contrast to poorly differentiated NEN, it typically expresses S100 protein, melan A and/or HMB45.

Combined/Mixed Neuroendocrine and Non-neuroendocrine Carcinoma These are mixed carcinomas which combine a poorly differentiated NEN (either of the small or large cell type) with a squamous cell or an adenocarcinoma (Fig. 11.19). Both components may be either separated (collision tumor) or intermingled. The immunoprofile of the different components recapitulate the individual tumor-specific features. The proliferative activity should be separately assessed [101].

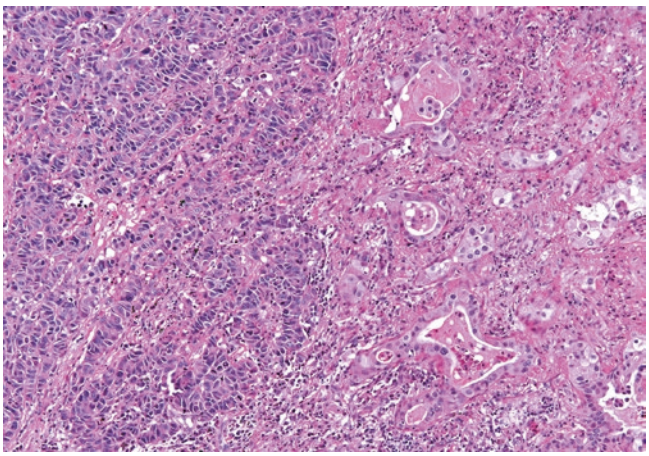

Fig. 11.19 Poorly differentiated neuroendocrine neoplasm of small cell type combined with a poorly differentiated adenocarcinoma

Larynx NENs of the larynx are uncommon, accounting for less than 1 % of all laryngeal tumors. Among the laryngeal NENs, atypical carcinoids/NETs G2 are most common, accounting for more than 50 % of all laryngeal neuroendocrine neoplasms [92, 95]. Second in frequency are the poorly differentiated small cell neuroendocrine carcinomas/NECs G3. They account for less than 0.5 % of all laryngeal carcinomas [102]. Extremely rare is the typical carcinoid/NET G1.

Typical carcinoids usually present in the supraglottic (arytenoid/aryepiglottic) area as polypoid lesions (<3 cm) in males (average age 58 years) with symptoms of hoarseness, dyspnoea and/or sore throat. The treatment of choice is complete surgical resection. Radiotherapy and chemotherapy have not proven to be effective. The prognosis is favourable (with a 5-year survival of almost 100 %), although metastases to the lymph nodes, liver, bones and skin may occur [103].

Atypical carcinoids, like typical carcinoids, mostly arise in the supraglottic region, where they present as nodular-polypoid masses (<4 cm), causing hoarseness, dysphagia and in 20–30 % of patients severe pain. They are more common in males, with a wide age range from 20 to 83 years. The majority of patients are heavy smokers. Associated hormonal syndromes are rare (see poorly differentiated NENs), but patients may have elevated serum calcitonin levels [96]. The mainstay of treatment is surgical resection, combined with neck dissection. The 5-year survival rates range from 60 % to 54 % [103].

Poorly differentiated NECs (small and large cell type) arise, like the other laryngeal NENs, most often in the supraglottis but also occur in other parts of the larynx [99]. They affect men more frequently than women. The patients are mostly heavy smokers between 50 and 70 years of age, who present with hoarseness and dysphagia, frequently associated with painless enlarged cervical lymph nodes due to metastases. They may occasionally show a paraneoplastic syndrome (Cushing, Schwartz-Bartter, Eaton-Lambert myasthenia syndrome) [101, 103]. The clinical course is highly aggressive, characterized by early metastasis to the regional lymph nodes and distant sites, especially to the lungs, bones and liver. In contrast to lung NECs, laryngeal NECs rarely metastasize to the brain. Radiation with chemotherapy is the treatment of choice. Surgical therapy is not indicated, because most patients have already disseminated disease at the time of diagnosis. The prognosis is poor, and the 5-year survival rates are 19 % and 15 %, respectively [104].

Recently high-risk HPV (HPV16 and HPV18) was detected in larynx and oropharynx NENs, most of which were classified as poorly differentiated small cell NEC [104]. In addition there were also one large cell NEC and an atypical carcinoid. Despite high-grade histologic features, two of four HPV-positive tumors showed a good response to therapy [105].

Oral Cavity and Tonsil NENs in these locations are extremely rare. They are usually poorly differentiated NENs (small or large cell type) and have been reported in the tongue and cheek mucosa [106–108] and the tonsil [109]. Some of them correspond to Merkel cell carcinomas, showing immunostaining for CK20 [110, 111]. All these tumors carry a poor prognosis and need appropriate treatment (see larynx).

Sinonasal Tract and Nasopharynx The NENs described in this area are usually poorly differentiated and of small or large cell type. Carcinoids are singularities [112].

The sinonasal NENs may derive from NE cells occasionally found in seromucous glands of the lamina propria. So far, many of them have not been well characterized, creating problems to separate them clearly from other round and small cell non-neuroendocrine neoplasms occurring in this region [113]. Table 11.2 provides current criteria for their proper recognition. Recently tumors combining intestinal-type adenocarcinoma and neuroendocrine elements have been reported [101]. The prognosis appears to be stage dependent [114].

Salivary Glands NENs of the salivary glands are usually poorly differentiated tumors [115]. They are second in frequency of all NENs of the head and NEC region and account for less than 1 % of all salivary gland tumors. The patients are mainly males, with a peak age range from 50 to 60. They present with a fast-growing mass, often with facial nerve palsy, and occasionally pain. Local recurrence and distant metastasis occur in more than 50 % of the patients. The sur-

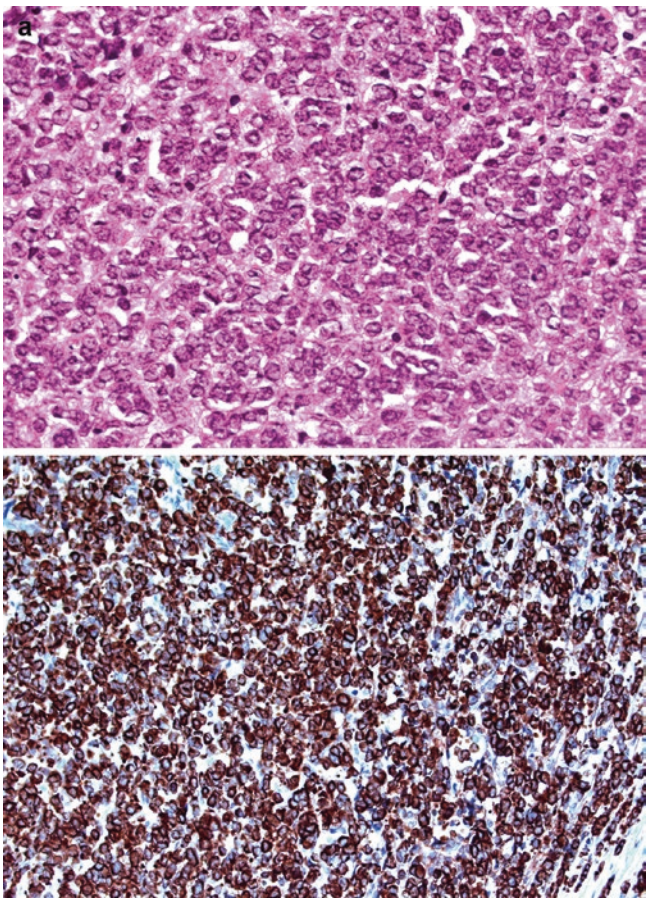

Fig. 11.20 Poorly differentiated neuroendocrine neoplasm of the parotid gland. (**a**) The small tumor cells show densely packed "washed-out" nuclei. (**b**) Dot-like immunostaining for CK20

vival rates range between 50 % and 60 % and are stage dependent [114]. Prognosis is reduced in NENs larger than 3 cm and associated with decreased immunoreactivity for neuroendocrine markers.

Salivary gland NENs present as firm, poorly demarcated tumors. *Microscopically* most of them are small cell carcinomas, few are large cell carcinomas, and very rare are atypical carcinoids [115]. Small cell-type NENs occur most frequently in the parotid gland and, less commonly, in the submandibular and sublingual glands. They can be divided in a Merkel cell subtype and a pulmonary subtype. While the Merkel carcinoma-like salivary NENs have round nuclei with pale "washed-out" chromatin and stain for CK20 (Fig. 11.20), the pulmonary-type NENs have elongated, chromatin-rich nuclei and are mostly CK20 negative (see Fig K4). It seems that the Merkel cell carcinoma subtype is not affected by the polyoma virus that is found in most of the cutaneous counterparts [116]. The Merkel cell carcinoma subtype appears to have a better prognosis than the pulmonary subtype. The differential diagnosis includes solid-type adenoid-cystic carcinoma (CEA, calponin, and often p63 positive), malignant lymphoma (CD45 positive) and metastasis from pulmonary small cell and Merkel cell carcinoma.

Large cell NENs are mainly observed in the parotid gland. The epidemiology and clinical features are similar to those of the small cell NENs. Occasionally they may show focal glandular or squamous differentiation.

Ear NENs are the most common tumors in the middle ear (see also Chapter 8). These tumors are also known under the terms "middle ear adenoma" or "carcinoid tumor of middle ear". They probably arise from the lining epithelium of the middle ear and show a varying mixed exocrine and endocrine differentiation [117]. Therefore, the name "mixed adeno-neuroendocrine tumor" (MANET) seems to be the most appropriate one.

MANETs of the middle ear are infrequent, but at this site they are the most common neoplasms. They show an equal sex distribution and a large age range (20–80 years, mean 45 years). The symptoms include a reduced hearing with pressure sensations, tinnitus and, occasionally, otitis in the affected ear. *Macroscopically*, the tumors are small (~1 cm), white, yellow, grey or reddish brown and not encapsulated. However, they are usually easy to remove from the walls of the middle ear cavity and only occasionally extend into the mastoid and Eustachian tube or spread through the tympanic membrane. A concurrent cholesteatoma may be also present. *Microscopically*, the tumor typically shows a mixture of well-differentiated trabecular and glandular structures in "a back to back" position (Fig. 11.21), focally with solid structures embedded in dense stroma [117, 118]. The cells are cuboidal or columnar (sometimes also "plasmacytoid") and when lining the glandular lumina may stain for PAS and Alcian blue that also decorate the mucoprotein secretions in the glands. The nuclei are round and condensed and show almost no mitotic activity. Myoepithelial cells are lacking. Immunohistochemically and electronmicroscopically, a bidirectional mucinous and also neuroendocrine differentiation (Fig. 11.21) can be demonstrated in the glandular structures, with apically located cells containing mucous granules and basally situated cells positive for neuroendocrine markers [117, 119]. Because of the neuroendocrine differentiation, the tumors may be detected by DOTATOC-PET/CT [120]. MANETs of the middle ear have to be distinguished from jugulotympanic paraganglioma (CK negative), ceruminous adenoma and meningioma (both synaptophysin negative). Complete local surgical excision, including the ossicular chain, is curative in most cases [121, 122]. The risk for metastasis seems to be very low [123].

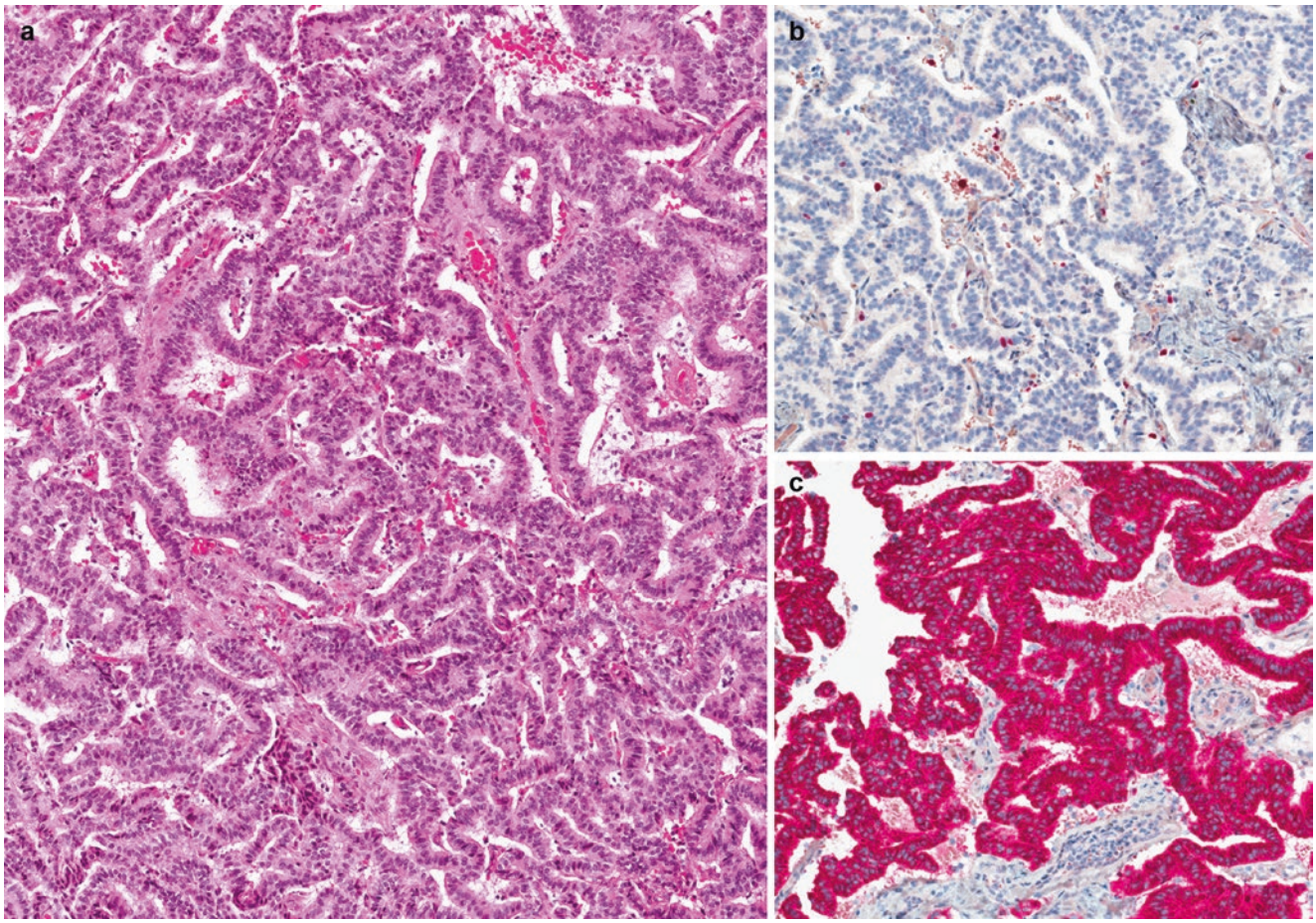

Fig. 11.21 Well-differentiated neuroendocrine neoplasm of the middle ear ("carcinoid tumor of middle ear"), showing trabecular-glandular structures in back to back position (**a** *left panel*), low proliferative activity (MIB1 immunostaining; **b** *upper right panel*) and immunostaining for synaptophysin (**c** *lower right panel*) (Courtesy of Professor Aurel Perren, Bern, Switzerland)

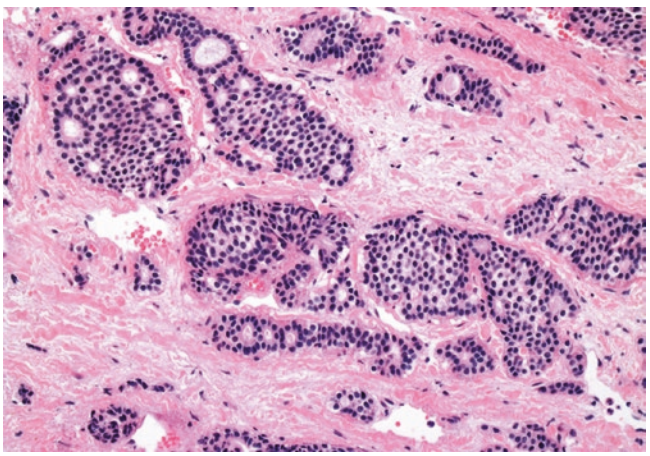

Fig. 11.22 Well-differentiated neuroendocrine tumor of the ileum (carcinoid) metastatic to the orbit. Note the typical palisading of the peripheral cells forming solid nests including small glands

Orbit The NENs occurring in the orbit, sclera or iris are usually metastases from bronchial or ileal carcinoids/NETs (Fig. 11.22). Primary NENs of the orbit, if they really exist, seem to be extremely rare [124, 125].

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Soft Tissue Lesions

12

Uta Flucke and Pieter J. Slootweg

Contents

U. Flucke, MD, PhD (✉) • P.J. Slootweg, MD, DMD, PhD
Department of Pathology, Radboud University Nijmegen Medical Center, Nijmegen 6500 HB, The Netherlands
e-mail: Uta.Flucke@radboudumc.nl; Piet.Slootweg@radboudumc.nl

12.1 Introduction

This chapter covers the broad spectrum of soft tissue lesions occurring in the head and neck, based on the current WHO classification. Most of these lesions are very rare, and sarcomas make up less than 1 % of head and neck cancers [1]. There are many overlapping features. Hence, morphological, immunohistochemical, and genetic criteria for making the diagnosis and comprehensive differential diagnoses are discussed.

Criteria regarding dignity depend on the entity dealing with and are consequently heterogeneous. Furthermore, specific immunohistochemical markers are still limited, and expression profiles are often only interpretable in the right clinicopathologic context.

Also, there are overlapping genetic features (e.g., *EWSR* rearrangement). Furthermore, since new detection methods as next-generation sequencing are available, knowledge regarding the genetic background of lesions is rapidly expanding, and again, the right clinicopathologic context helps to interpret the results.

12.2 Benign Lesions

12.2.1 Lipomatous Tumors

12.2.1.1 Lipoma

Definition Lipomas are benign soft tissue lesions composed of mature adipocytes [2].

Epidemiology 15–20 % of lipomas involve the head and neck region [3]. Adults are commonly affected [2, 4].

Clinical aspects Lipomas present as painless, slowly growing soft tissue masses and can arise superficially or deep. Deep-seated/intramuscular lipomas are often larger and less well defined than their superficial counterpart [2, 5]. They

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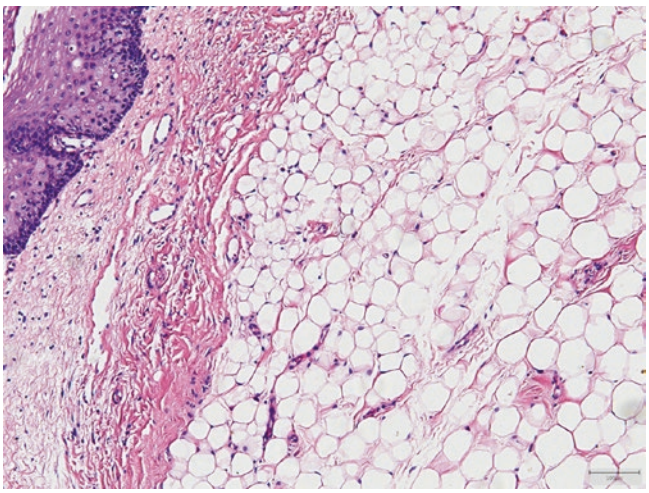

Fig. 12.1 Intraoral lipoma. The mature adipocytes show no atypia

are relatively uncommon in the oral and maxillofacial region with occurrence in the parotid region, buccal mucosa, lip, submandibular region, tongue, palate, floor of mouth, and vestibule [2–4].

Macroscopy Most tumors are circumscribed, encapsulated, yellow, and greasy. Deep-seated/intramuscular lesions can be poorly circumscribed (e.g., tongue) [2].

Microscopy Lesions are composed of variably sized adipocytes without cytologic atypia (Fig. 12.1). Secondary changes as necrosis, atrophy, hyalinization, or myxoid degeneration are visible in some cases. Dense collagen (fibrolipoma), metaplastic bone (osteolipoma) or cartilage (chondrolipoma) can be present, but the latter is very rare [2, 5].

Immunohistochemistry S100 is positive in the adipocytes and if present in the chondroid component [3, 6].

Genetics The 12q13-15 region is most commonly involved in ordinary lipomas followed by 6p21-23 and 13q. The most frequent translocation is t(3;12)(q27-28;q13-15) that fuses the *HMGA2* and *LPP* genes. Other fusion partners of *HMGA2* are *CXCR7*, *EBF1*, *NFIB*, and *LHFP. HMGA1*, a structural homologue of *HMGA2*, can alternatively be involved [5, 6].

Differential diagnosis Lipoma can be distinguished from atypical lipomatous tumor/well-differentiated liposarcoma, because of the absence of cellular atypia especially in septa and around vessels. Of note, reactive changes in lipoma possibly due to posttraumatic necrosis should not be confused with malignant features [2]. In this context, immunohistochemical staining to demonstrate macrophages (CD68, CD163) can be helpful. Maturated lipoblastoma is a differential diagnosis in the pediatric population and shows *PLAG1* rearrangement (see Sect. 12.2.1.4) [7].

Treatment and prognosis Simple excision is curative; recurrences rarely occur [4].

12.2.1.2 Spindle Cell Lipoma/Pleomorphic Lipoma

Definition Spindle cell lipoma forms a continuum with pleomorphic lipoma mainly arising in the superficial soft tissue of the neck [8].

Epidemiology Middle-aged men are commonly affected [2, 8].

Clinical aspects Besides the common occurrence in the neck, spindle cell/pleomorphic lipomas also originate in the head including the face, forehead, scalp, and, more rarely, the oral and maxillofacial region. The larynx can also be involved. Most patients present with an asymptomatic, circumscribed mass [2, 8].

Macroscopy Spindle cell/pleomorphic lipomas are well-circumscribed fatty nodules with variable grayish-white areas [2, 8].

Microscopy The characteristic components in varying proportions are mature fat, bland spindle cells with short stubby nuclei, bundles of ropey collagen, and scattered mast cells (Fig. 12.2). Myxoid changes are occasionally seen (Fig. 12.3). Pleomorphic lipoma shows additionally enlarged pleomorphic cells including floret-like giant cells (Fig. 12.4) [2, 8].

Immunohistochemistry CD34 is a consistent marker. Desmin and S100 are rarely positive in the spindle cell component [4, 8]. There is a loss of retinoblastoma protein expression [8].

Genetics A wide array of genetic changes including frequent rearrangements of 13q and/or 16q is present. Loss of genetic material from the 13q14 region results in the monoallelic deletion of *RB1* and *FOXO1* [5, 9–11].

Differential diagnosis The diagnosis is mostly straightforward. In cases with prominent myxoid changes, myxoid liposarcoma can be a diagnostic possibility, but there are no mucin pools or blood vessels in a chickenwire pattern in spindle cell lipoma. Ropey collagen is not a feature of myxoid liposarcoma. Pleomorphic cells in pleomorphic lipoma can be confused with cellular atypia as present in well-differentiated/dedifferentiated liposarcoma, but there is no *MDM2* amplification. However, expression of the corresponding protein is reported in some cases [12, 13]. Pleomorphic lipoma without a fatty component may be confused with sarcomas; the typical cells and the ropey collagen in addition with the strong expres-

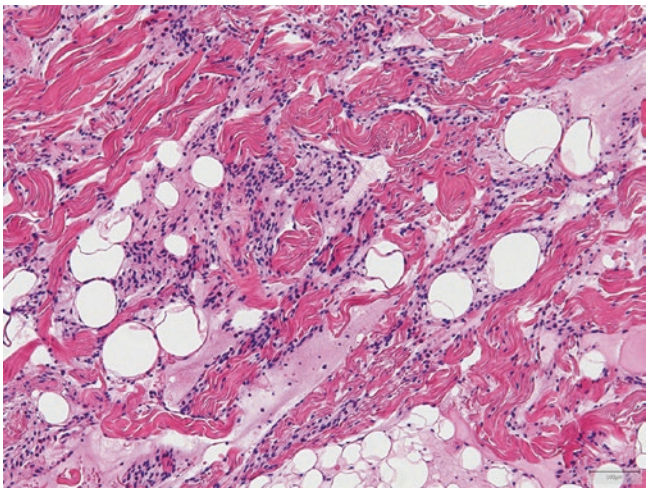

Fig. 12.2 Classical features of a spindle cell lipoma with short stubby nuclei and ropey collagen

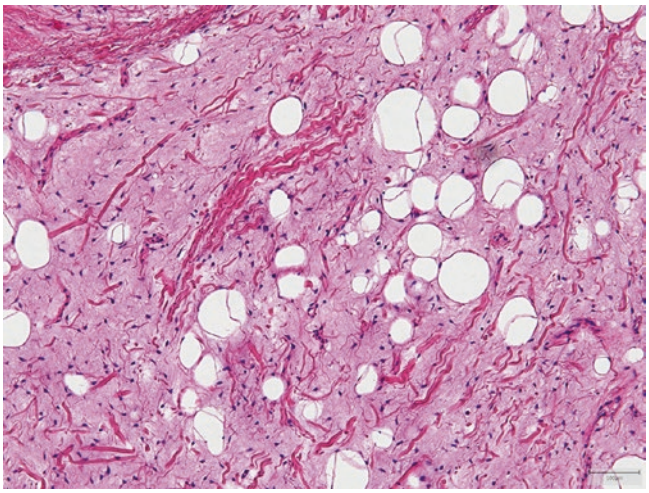

Fig. 12.3 Myxoid variant of a spindle cell lipoma. Note the typical collagen bundles

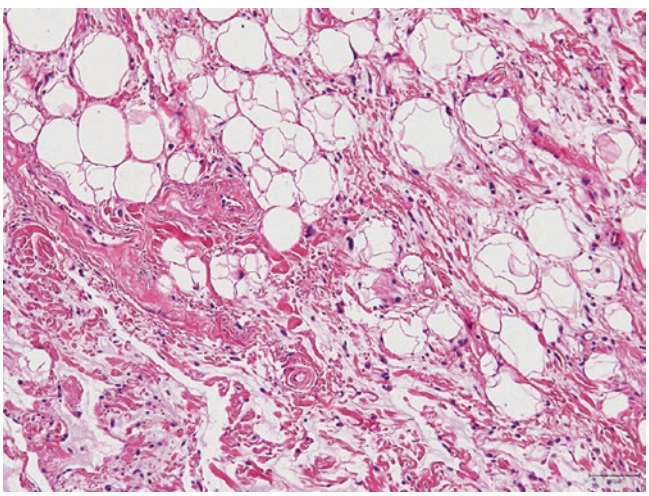

Fig. 12.4 Pleomorphic lipoma showing enlarged pleomorphic cells in a background of a spindle cell lipoma

sion of CD34 and the loss of retinoblastoma protein may help to make the diagnosis. Cellular examples of spindle cell lipoma can resemble solitary fibrous tumors, and CD34 expression is not discriminating. Nuclear staining of STAT6 however identifies solitary fibrous tumors and rules out spindle cell lipoma (see Sect. 12.4.1).

Treatment and prognosis Excision is the treatment of choice. Recurrences are rare [8].

12.2.1.3 Hibernoma

Definition Hibernoma represents an unusual tumor of brown fat which mainly occurs in the extremities and trunk. The head and neck region is more rarely involved.

Epidemiology Most patients are adults (mean age 38 years). Rarely children are affected [14].

Etiology and pathogenesis It is unclear whether hibernoma arises in residual brown fat or represents the outcome of an altered developmental program of the neoplastic fat cells toward brown fat differentiation [14].

Clinical aspects Most tumors present as a slowly growing painless mass. Occasionally, rapid growth has been reported [14].

Macroscopy Tumors are circumscribed, partially encapsulated, and lobulated. The cut surface varies from yellow to brown. Mucoid areas and hemorrhage are rare [14].

Microscopy Hibernomas are composed of multivacuolated brown fat cells with centrally placed small nuclei without atypia (Fig. 12.5). They can be intermixed with eosinophilic cells with granular cytoplasm and univacuolated white fat

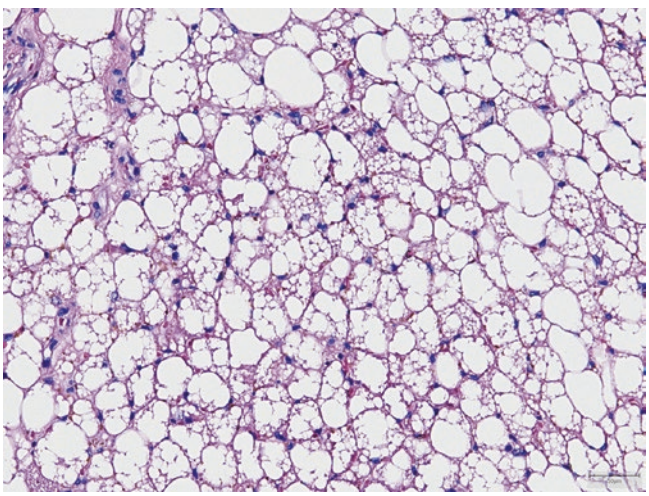

Fig. 12.5 Hibernoma: multivacuolated brown fat cells are intermixed with adipocytes

cells. A myxoid background is rarely seen. Combined/hybrid features with spindle cell lipoma are observed in the head and neck region [14].

Immunohistochemistry Most cases are positive for S100 ranging from focal to diffuse. CD34 may be expressed in the spindle cell component of the hybrid hibernoma/spindle cell lipoma [14].

Genetics Breakpoints in 11q21 and 11q13.5 near *GARP* and deletions of various genes of 11q13 were found [15].

Differential diagnosis Main differential diagnosis is atypical lipoma/well-differentiated liposarcoma. Myxoid changes in hibernoma may blur the distinction from myxoid liposarcoma. S100 expression should not lead to confusion with granular cell tumor, which may play a role in cases with eosinophilic granular cytoplasm [14].

Treatment and prognosis It is a benign tumor that does not recur after complete excision [14].

12.2.1.4 Lipoblastoma

Definition Lipoblastoma is a mainly immature adipose lesion of infancy and early childhood [7].

Epidemiology Nearly 90 % of cases occur before 3 years of age. It has been rarely reported in adolescents and young adults [7, 16].

Etiology and pathogenesis Lipoblastoma recapitulates the white fat development. *PLAG1* rearrangement is shown by FISH in cells of various stages of differentiation indicating that the aberration occurs in a progenitor cell that subsequently differentiates [7].

Clinical aspects Lipoblastomas can occur throughout the soft tissues in the head and neck. The mass grows slowly or rapidly. There are two forms of lipoblastoma, the superficial circumscribed form resembling lipoma and the diffuse lipoblastoma originating from the deep soft tissue. Lipoblastoma may compress or interfere mechanically with adjacent organs and tissues [7].

Macroscopy Most lipoblastomas are 3–5 cm in diameter, although tumors in excess of 10 cm have been reported. The soft, lobulated, and usually encapsulated mass has a yellow, creamy white, or tan cut surface with myxoid nodules, cystic spaces, or fine white trabeculae [7].

Microscopy These lobulated tumors are composed of fat cells with varying degrees of differentiation, including primitive cells or prelipoblasts, lipoblasts, and adipocytes. Hibernoma-like cells may be present. The fat lobules can exhibit a zonal maturation with mature areas in the center and immature areas at the periphery. The prominent fibrous septa contain numerous capillaries and venules, and the stroma may show myxoid changes (Fig. 12.6) [7, 16, 17].

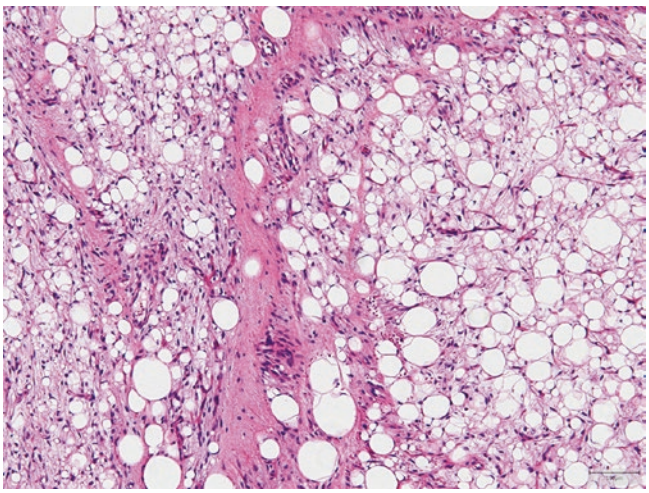

Fig. 12.6 Lipoblastoma is composed of fat cells with varying degrees of differentiation including prelipoblasts, lipoblasts, and adipocytes. Note the prominent fibrous septum containing numerous small vessels

Immunohistochemistry Lesions often express desmin in the primitive mesenchymal component and S100 in adipocytes [7].

Genetics Most lipoblastomas show *PLAG1* rearrangement [7, 16].

Differential diagnosis Main differential diagnostic considerations are lipoma and liposarcoma (well differentiated or myxoid). Lipoma may contain lipoblasts but the septa usually do not show a prominent vasculature. Cellular atypia of well-differentiated liposarcoma is absent in lipoblastoma. In myxoid liposarcoma, the lobulation is less distinct, and there are abundant mucinous pools and a homogeneous chicken-wire network of small vessels [7]. If overlapping features with a prominent myxoid matrix in lipoblastomas are present, molecular genetic analysis may help to confirm the diagnosis.

Treatment and prognosis Simple surgical treatment is commonly curative. Lipoblastoma may show maturation to lipoma. Recurrences are reported after incomplete excision [7].

12.2.2 Fibroblastic/Myofibroblastic Lesions

12.2.2.1 Nuchal-Type Fibroma

Definition Nuchal-type fibroma is a rare, tumorlike accumulation of collagen that predominantly occurs in the posterior neck of adults [18].

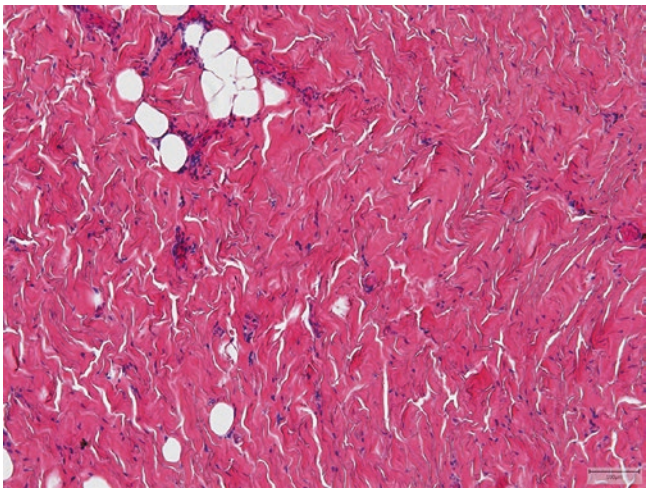

Fig. 12.7 Nuchal-type fibroma is a paucicellular lesion with broad collagen bundles and scattered inconspicuous fibroblasts

Epidemiology The age range is broad (3–74 years) with a mean of 40 years. Males outnumber by far females [18].

Etiology and pathogenesis There seems to be a strong association with diabetes and, to a lesser extent, a link to Gardner syndrome especially in children and adolescents [18–20].

Clinical aspects The nuchal region is mostly affected. Extranuchal sites of the head and neck are the face, temporal region, and the anterior neck [18]. Lesions are described as plaque-like [19].

Macroscopy Nuchal-type fibromas are poorly circumscribed masses with a firm consistency and an off-white color. The reported diameter ranges from 1 to 6 cm [18].

Microscopy The lesions are predominantly located in the subcutis with possible extension into the dermis (with encasement of adnexa) and, in some cases, involvement of the underlying skeletal muscle and periosteum of the skull. They consist of paucicellular, broad, haphazardly arranged collagen bundles with scattered inconspicuous fibroblasts (Fig. 12.7). A delicate network of thin, elastic fibers is often observed. Entrapped adipose tissue and neuroma-like nerve proliferations are typically present [18].

Immunohistochemistry The lesional cells are positive for CD34 and negative for smooth muscle actin, desmin, S100, and glial fibrillary acidic protein. The neural elements are positive for S100 [18–20]. Nuclear expression of β-catenin indicates a Gardner fibroma. However, this is not as consistent as in desmoid-type fibromatosis and therefore not always helpful [19].

Differential diagnosis Circumscribed storiform collagenoma can be part of Cowden's disease especially if multiple lesions are present, but this circumscribed dermal neoplasm shows a whorled or storiform collagen pattern with prominent mucin-filled clefts and absence of entrapped adipose tissue and neural proliferations. Other differential diagnostic possibilities are fibrolipoma, desmoid-type fibromatosis, and connective tissue nevus, which is a dermal nodule or plaque consisting of collagen and variable amounts of elastic tissue [18].

Treatment and prognosis The lesion is benign but has a potential for local recurrence [18].

12.2.2.2 Nodular/Cranial Fasciitis

Definition Nodular/cranial fasciitis is a self-limiting myofibroblastic condition, genetically characterized by a recurrent gene fusion [21].

Epidemiology The patients' age ranges from infants to the elderly. Cranial fasciitis (adjacent to the cranium) occurs predominantly in young children [22, 23].

Etiology and pathogenesis Rearrangement of *USP6* seems to play a major pathogenetic role [21].

Clinical aspects Most patients present with a several-week history of a solitary, rapidly growing, sometimes painful mass [23]. However, slow and progressive growth does not exclude this diagnosis [24]. Most lesions arise subcutaneously, but they also may originate in the dermis, (sub) mucosa, deep soft tissue, and salivary glands [23–25]. Mucosal tumefactions, especially with exophytic growth, can be secondarily ulcerated [24].

Macroscopy This is a nonencapsulated, nodular mass that is usually less than 3 cm in diameter. The cut surface can be soft and gelatinous or firm [23].

Microscopy Lesions are usually well delineated and unencapsulated. Infiltration into adjacent tissue and, occasionally, intravascular invasion are also found. They consist of plump, immature-appearing myofibroblasts arranged in irregular short fascicles. The cells vary in size and shape (spindle to stellate) and possess discrete nucleoli and (abundant) mitotic figures. Early lesions have a tissue culture-like appearance with cleft-like, sometimes cystic spaces containing erythrocytes and mucin (Fig. 12.8). In time, they become more collagenous with a fascicular or storiform appearance. Commonly, lymphocytes are scattered around and multinucleated giant cells can be present [23–26]. Osseous metaplasia is a feature of lesions adjacent to the bone (as seen in cranial fasciitis), and then, the term florid reactive periostitis is employed to denote a reactive periostal lesion [22].

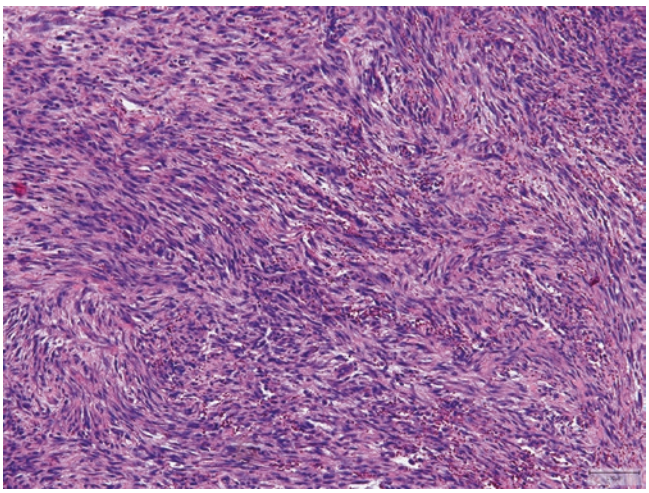

Fig. 12.8 Nodular fasciitis: plump, immature-appearing myofibroblasts are arranged in irregular short fascicles. Erythrocyte extravasation is very common

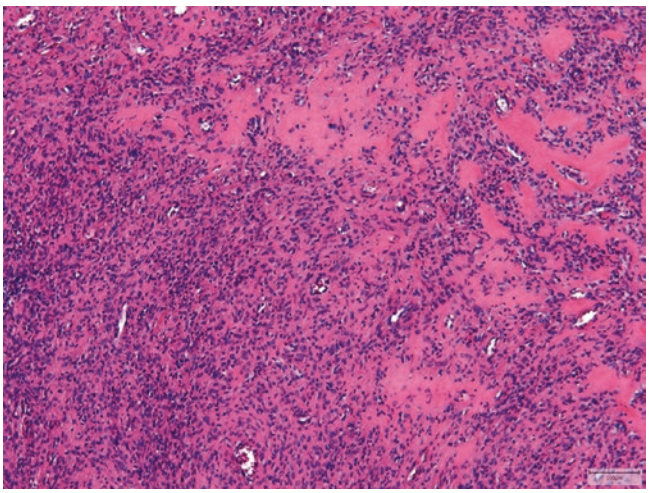

Fig. 12.9 Myofibroma shows a biphasic pattern in varying proportions with bundles of plump myofibroblastic spindle cells and a typical bluish matrix

Immunohistochemistry Cells stain with muscle-specific actin, smooth muscle-specific actin, and exceptionally with desmin [23, 25, 26].

Genetics A recurrent *USP6* (chromosome 17p13) rearrangement is reported in >90% of the cases with *MYH9* (chromosome 22q12.3) being the most common fusion partner [21, 27].

Differential diagnosis Other myofibroblastic lesions as desmoid-type fibromatosis, myofibroma, and low-grade myofibroblastic sarcoma enter the differential diagnosis. Myofibroma shows a biphasic pattern and a myxohyaline bluish matrix. Desmoid-type fibromatosis and low-grade myofibroblastic sarcoma are arranged in long fascicles with slight atypical cells defining the latter. Benign fibrous histiocytoma, another diagnostic possibility, exhibits storiform arrangement of fibrohistiocytic cells facultatively intermingled with foamy macrophages and Touton giant cells [24–26]. Occasionally, mandibular cases with extensive bone erosion and metaplastic bone formation may suggest peripherally located osteosarcoma [22].

Treatment and prognosis Simple excision is the treatment of choice. The risk of recurrence is low even in incompletely excised lesions [24–27].

12.2.2.3 Myofibroma

Definition Myofibroma, a benign myofibroblastic lesion, forms a spectrum along lesions with perivascular myoid differentiation, such as myopericytoma, glomangiopericytoma, and angioleiomyoma [28, 29].

Epidemiology Patients may have it at any age. Children are more frequently affected also regarding multifocal disease [28, 30].

Clinical aspects Myofibromas commonly arise in the head and neck region as small submucosal, subcutaneous, or soft tissue nodules [30]. The oral cavity, tongue, and gingival/jaw tissues are particularly involved [31]. These lesions can also originate in dermis or bone. Larger tumors may mimic malignancy because of the infiltrative growth and possible erosion of bone [30].

Macroscopy The tumor nodules are sharply and sometimes poorly demarcated with a firm tan or white-gray appearance, often with hemorrhagic discoloration [32].

Microscopy This lesion reveals a biphasic pattern in varying proportions with bundles of plump myofibroblastic spindle cells with bland nuclei closely resembling smooth muscle set in a distinctive bluish myxohyaline matrix. The second component is more cellular showing primitive round to spindle-shaped cells with scant cytoplasm and a branching hemangiopericytoma-like vasculature (Fig. 12.9). There is a propensity for intravascular extension. Mitotic figures are not something to be concerned about [28].

Immunohistochemistry There is expression of SMA and, in some cases, of desmin and CD34 [33].

Genetics *MIR143-NOTCH* fusions known to be present in glomus tumors are absent in myofibromas [34].

Differential diagnosis Other myofibroblastic lesions enter the differential diagnosis, but the biphasic pattern is distinctive for myofibroma. In infants, infantile fibrosarcoma should be excluded which is composed of sheets or fascicles of primitive myofibroblasts with enlarged nuclei. *ETV6-NTRK3* is the molecular key [30]. As mentioned above, there is a morphological continuum with myopericytoma [28, 29].

Treatment and prognosis There is a potential to either regression or recurrence. Simple surgical intervention is an adequate treatment especially in non-regressive and large lesions [28, 30].

12.2.3 So-Called Fibrohistiocytic Tumors

12.2.3.1 Deep Benign Fibrous Histiocytoma

Definition Deep benign fibrous histiocytoma is the subcutaneous or soft tissue counterpart of dermatofibroma.

Epidemiology Adults are mostly affected. The head and neck region is often involved [35].

Clinical aspects Most patients present with a painless, slowly enlarging mass. Deep-seated tumors are rare in comparison to subcutaneous lesions [35].

Macroscopy Tumors are well circumscribed with a firm yellow-tan cut surface. Cystic degeneration and necrosis are rarely described [35].

Microscopy The well-circumscribed lesions are often surrounded by a thick pseudocapsule. This is distinct from the skin counterpart that often shows infiltrative margins with entrapment of collagen. Storiform architecture dominates, and less commonly short fascicles are present (Fig. 12.10). The plump to ovoid spindle cells possess bland nuclei; however, pleomorphism is common. Atypical nuclei and mitotic figures may be seen. If present, Touton giant cells and foamy histiocytes are helpful characteristics. Lymphocytes are mostly admixed. A staghorn-like vascular pattern and stromal hyalinization are not uncommon [35].

Immunohistochemistry Lesions are facultative positive for CD34, SMA, and sometimes for desmin [35]. There is no key immunohistochemical marker so far.

Genetics Consistent molecular findings are not detected as yet.

Differential diagnosis When CD34 is positive and a branching vasculature exists, solitary fibrous tumor (SFT) is the main differential diagnosis. But the latter shows a "patternless pattern." A specific fusion gen, *NAB2-STAT6*, with consecutive nuclear expression of STAT6 has been detected in SFT [36–40].

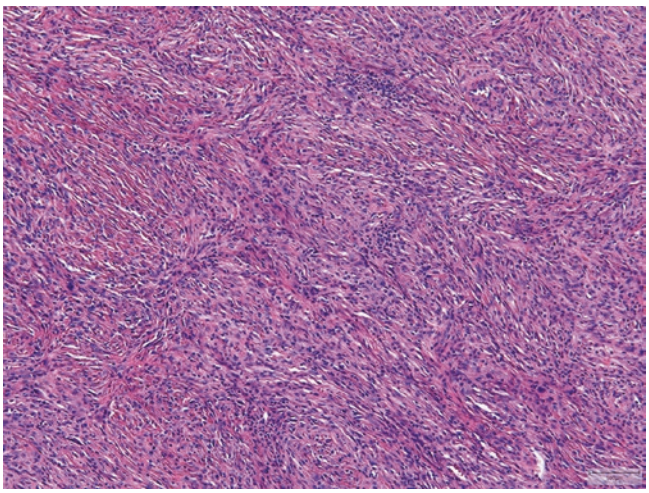

Fig. 12.10 Deep benign histiocytoma shows usually a storiform growth pattern of plump to ovoid fibrohistiocytic cells with bland-looking nuclei

Treatment and prognosis These tumors are treated by excision. As in dermatofibroma, occasionally metastatic potential may be shown, irrespective of morphological features [35, 41, 42].

12.2.4 Myoepithelial Tumors

12.2.4.1 Ectomesenchymal Chondromyxoid Tumor (Myoepithelioma)

Definition Ectomesenchymal chondromyxoid tumor is a rare benign myoepithelial neoplasm arising commonly in the (anterior dorsal) tongue [43–45].

Epidemiology As for other myoepithelial tumors, the age range is wide including children and older persons. There is no sex predilection [43].

Etiology and pathogenesis The occurrence of myoepithelial tumors at different sites at least outside salivary glands possibly reflects an aberrant gene expression pattern during oncogenesis rather than origin from a specific cell lineage [46].

Clinical aspects Usually, a slow-growing exophytic mass is visible on the dorsum of the tongue often without significant pain or discomfort [43, 47].

Macroscopy Lesions are in general small submucosal/intramuscular nodules with a rubbery to gelatinous consis-

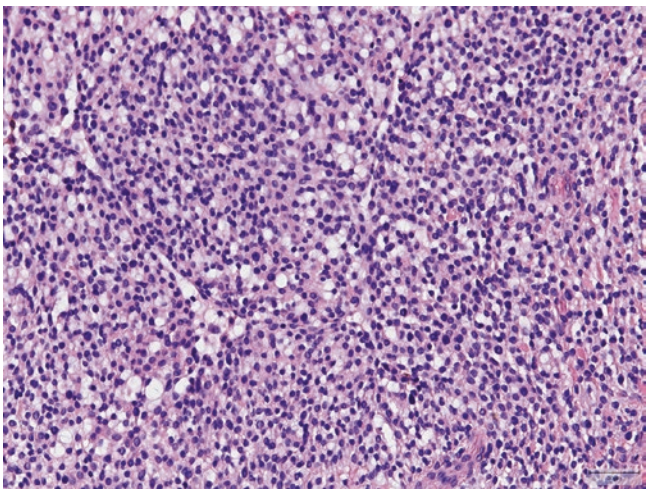

Fig. 12.11 Ectomesenchymal chondromyxoid tumor is a benign myoepithelial lesion composed of uniform round, fusiform, or polygonal cells with small nuclei and moderate amounts of faintly basophilic cytoplasm

tency and a pale gray to tan to yellow color [43]. Cystic changes are reported [45].

Microscopy The well-circumscribed, (multi)nodular lesions arise within the superficial musculature of the tongue. Large tumors show extension into deep muscle and fat and can abut on the epithelium sometimes with secondary ulceration. Entrapment of muscle fibers and an occasional nerve branch is not unusual. Tumors are composed of round, fusiform, or polygonal cells with uniform small nuclei and moderate amounts of faintly basophilic cytoplasm. The cells are arranged in cords, strands, and netlike sheets in a chondromyxoid background (Fig. 12.11). Clefts or pseudocystic spaces are often present [43–45, 47].

Immunohistochemistry There is a myoepithelial immunophenotype with variable expression of S100, GFAP, EMA, pankeratin, p63, and smooth muscle markers (SMA, MSA) [43–45, 47].

Genetics Within soft tissue myoepitheliomas, *EWSR1* and *PLAG1* rearrangement is described in a subset of cases [48]. For myoepithelial tumors of the salivary glands, see the chapter on salivary glands (Chap. 5). In ectomesenchymal chondromyxoid tumors, genetic aberrations are not reported as yet.

Differential diagnosis Myoepithelial tumors and ectomesenchymal chondromyxoid tumors show a similar morphology and immunophenotype. There is no objectifiable sign of discrimination between these two entities [44, 47]. Therefore, it seems logical to believe that they are identical [48]. Other differential diagnoses are nerve sheath myxomas (myxoid neurothekeomas) and chondroma when a chondroid matrix is present [47]. Both are S100 positive by immunohistochemistry [48].

Treatment and prognosis Excision is the treatment of choice and recurrence is rarely reported [43].

12.2.5 Benign Peripheral Nerve Sheath Tumors

Approximately 45 % of the benign peripheral nerve sheath tumors occur in the head and neck [49]. They are composed of various cell types contributing to the formation of the nerve sheath. Whereas schwannomas and perineurioma consist exclusively of Schwann cells and perineurial cells, respectively, neurofibromas contain a heterogeneous admixture of Schwann cells, fibroblasts, and perineurial-like cells, as well as axons [50].

12.2.5.1 Schwannoma

Definition Schwannomas are benign neurogenic tumors consisting of Schwann cells. They are often occurring in the head and neck region [51].

Epidemiology Adults are mostly affected. Schwannomas are unusual in the pediatric setting. Tumors are commonly solitary and sporadic [51].

Etiology and pathogenesis Schwannomas are neuroectodermal tumors arising from Schwann cells. Multiple tumors or plexiform schwannomas may be indicative of neurofibromatosis type 2 (NF2) or schwannomatosis [51]. Melanotic schwannomas may be associated with Carney complex [52].

Clinical aspects Patients presenting with a slowly growing mass, which can be painful. There are other nonspecific symptoms of compression, obstruction, or invasion of local structures with destruction and/or deformity. Unusual cases show intracranial extension [49, 51].

Macroscopy Schwannomas are often encapsulated rubbery nodules. Occasionally an associated nerve can be identified splayed over its peripheral aspect. The cut surface reveals variegated, firm, and glistening white tan or yellow with cysts and focal hemorrhage in long-standing lesions [51].

Microscopy These commonly encapsulated tumors are composed of variably cellular proliferations arranged in alternating cellular Antoni A and myxoid Antoni B areas. The elongated monomorphic spindle cells possess oval, tapered, or buckled nuclei and a poorly defined eosinophilic cytoplasm. Nuclear palisading is a frequent feature occasion-

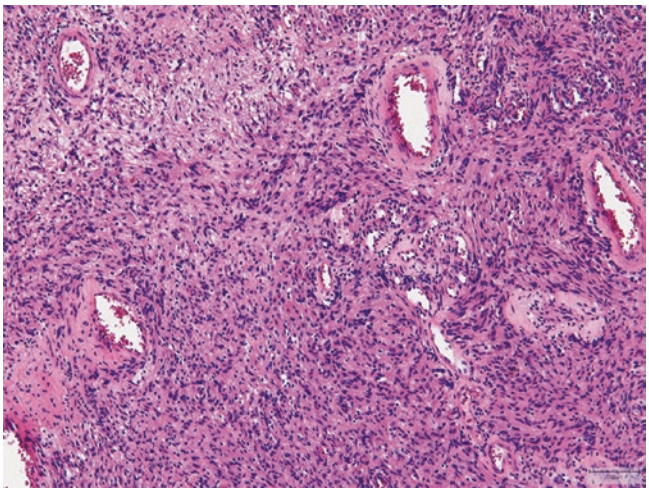

Fig. 12.12 Schwannoma with ancient features. Note the haphazardly arranged neuroid cells with hyperchromatic nuclei and the prominent hyalinized vessels

ally with Verocay body formations. Degenerative nuclear atypia and mitotic figures should not be interpreted as ominous signs. Hyalinized blood vessels are often prominent (Fig. 12.12). Hemorrhage, lymphocytes, and foamy macrophages may be present [49].

There are four subtypes: cellular schwannoma, plexiform schwannoma, epithelioid schwannoma, and melanotic schwannoma. The cellular and plexiform variants show a predominance of Antoni A fascicular growth pattern in the former and a plexiform architecture in the latter. Plexiform schwannoma may appear unencapsulated [49, 51]. Epithelioid schwannoma, a rare subtype, exhibits mainly epithelioid cells arranged in trabeculae, loosely aggregated clusters, and cohesive nests set in a finely collagenous, myxohyaline, or myxoid stroma [53]. Melanotic schwannomas are very rare lesions arising in the cranial nerve roots. They show syncytial-like nests of spindled or epithelioid Schwann cells containing melanosomes. Another exceedingly rare subtype is neuroblastoma-like schwannoma. Hybrid tumors with combined features of schwannoma/neurofibroma or schwannoma/perineurioma are also reported [51].

It should be noted that schwannomas of the sinonasal tract and nasopharynx lack encapsulation [49].

Immunohistochemistry There is strong and diffuse expression of S100 in a nuclear and cytoplasmic pattern [49, 51, 54]. SOX10 shows nuclear expression in almost all lesions [55]. Scattered CD34-positive cells may be seen, particularly in Antoni B areas [49]. Melanotic schwannomas are additionally positive for HMB45 and Melan-A [51].

Genetics Complete or partial loss of chromosome 22 is the most common cytogenetic abnormality in schwannoma. *NF2*-inactivating mutations have been detected in ca. 60 % of sporadic schwannomas with consecutive loss of schwannomin expression. Mutations of *SMARCB1* are associated with schwannomatosis, a condition characterized by the presence of multiple schwannomas [56]. Melanotic schwannomas show genetic alterations of the 2p16 region [56].

Differential diagnosis Other peripheral nerve sheath tumors should be considered in the differential diagnosis. Cellular schwannoma can be extremely difficult to distinguish from low-grade malignant peripheral nerve sheath tumor (MPNST). The latter is typically more mitotically active and may have atypical mitotic figures and characteristical perivascular hypercellularity. They lack encapsulation and the distinctive hyalinized blood vessels of benign schwannomas [49, 51]. MPNSTs are usually focal positive for S100 or devoid of S100 staining [57] (see also Sect. 12.5.9). Other differential diagnoses are juvenile angiofibroma, sinonasal glomangiopericytoma, desmoid-type fibromatosis, leiomyoma/leiomyosarcoma, solitary fibrous tumor, low-grade fibromyxoid sarcoma, and synovial sarcoma. They can be distinguished from schwannomas by the lack of S100 expression [49, 51, 54].

Treatment and prognosis Tumors follow a benign clinical course, but they can be locally aggressive. Therefore, excision with clear margins is the treatment of choice. Unlike neurofibromas, malignant transformation is exceedingly rare [49].

12.2.5.2 Neurofibroma

Definition Neurofibromas are benign nerve sheath tumors containing a heterogeneous admixture of Schwann cells, fibroblasts, and perineurial-like cells, as well as axons [50, 52].

Epidemiology Neurofibromas are the most common benign peripheral nerve sheath tumors affecting infants, children, adolescents, and adults [51].

Etiology and pathogenesis Most neurofibromas occur sporadically, although approximately 10 % ultimately prove to be associated with neurofibromatosis type 1 (NF1). Plexiform neurofibromas localized to a major nerve are almost always NF1 associated [52].

Clinical aspects Neurofibromas are relatively common, particularly at superficial cutaneous sites, where they present as nodular and/or pedunculated lesions. Other localizations are intraneural or soft tissue. Diffuse neurofibromas arise usually in the skin and/or soft tissue of the head and neck region [52]. A neurofibroma in a child less than 10 years of age should prompt clinical evaluation for NF1 [51].

Macroscopy The lesions are commonly nodular or fusiform/wormlike (intraneural subtype) with a tan-white, glistening cut surface [52]. Diffuse neurofibromas are plaque-like masses [52].

Microscopy The specific subtypes encompassing localized, diffuse, and plexiform are based on architectural growth patterns [52]. All lesions are characterized by random rearrangement of spindle cells in a collagenous to myxoid stroma. The nuclei are "wavy" [54]. The cytoplasm is inconspicuous. Mitotic figures are usually absent. The collagen bundles typically look like shredded carrots (Figs. 12.13 and 12.14). Diffuse neurofibromas show Wagner-Meissner bodies. Other subtypes are myxoid, hyalinized, epithelioid, granular, or pigmented [51].

Nuclear atypia, in the form of pleomorphism, hyperchromasia, smudgy chromatin, and nuclear pseudoinclusions, may be seen in some neurofibromas (Fig. 12.15). In the absence of other worrisome findings, such as mitotic activity, hypercellularity, and fascicular growth, these features likely represent degenerative changes and are not indicative of malignancy [51]. Cellular neurofibromas may show moderate cellularity and a more pronounced fascicular growth, but lack the monotonous cytological atypia, chromatin abnormalities, and mitotic activity as seen in MPNSTs [52].

Immunohistochemistry The lesions are positive for S100 and CD34 [54]. Nuclear expression of SOX10 is present in almost all cases [55].

Genetics Inactivation of both copies of *NF1* [51].

Differential diagnosis Other benign peripheral nerve sheath tumors enter the differential diagnoses, schwannoma and less likely perineurioma. Perineuriomas may also be positive for CD34, but the swirling architecture of perineurioma is discriminating as are the monotonous oval nuclei and the lack of S100 expression. The thick collagen bundles may suggest spindle cell lipoma. Also CD34 expression is an overlapping feature in this context, but S100 and SOX10 will not be expressed in the spindle cell component of spindle cell lipomas. Solitary dermal neurofibromas are sometimes difficult to distinguish from a spindle cell or neurotized melanocytic nevus, but most nevi are positive for HMB45 and tyrosinase [51]. The diffuse growing neurofibroma may resemble dermatofibrosarcoma protuberans (DFSP), but the latter usually has a storiform growth pattern and does not express S100. Of note, both lesions, neurofibroma and DFSP, can show melanin pigmentation [51]. Mucosal neuromas are most often seen in the oral cavity or around the mucocutaneous junctions of the face and are composed of an increased number of disorganized and hyperplastic nerve fibers surrounded by a thickened perineurium. Traumatic neuromas are composed of

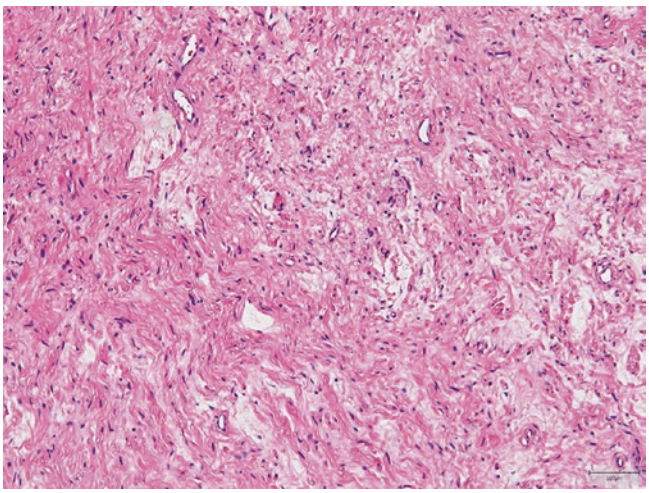

Fig. 12.13 Neurofibromas show a random rearrangement of spindle cells in a collagenous to myxoid stroma

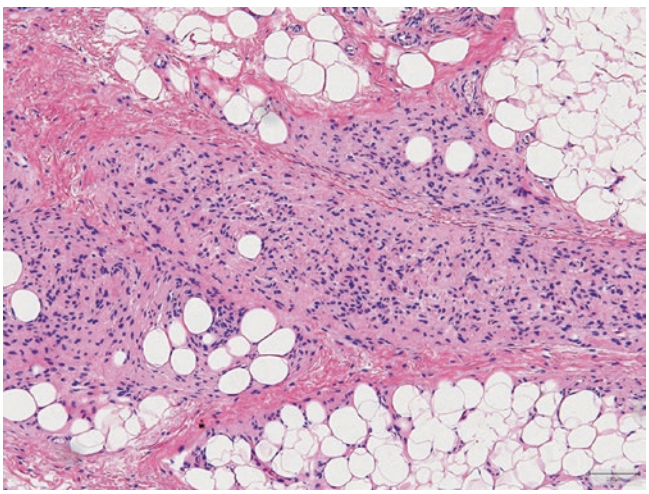

Fig. 12.14 Neurofibroma with a diffuse growth pattern

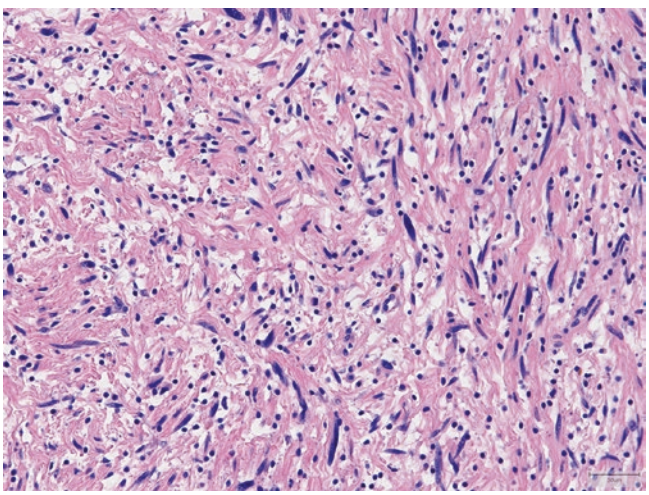

Fig. 12.15 Neurofibroma with atypical nuclei

reactive nerve fibers embedded within a fibrotic stroma [51]. Desmoplastic melanoma may show deceptively bland cells with wavy nuclei. Important clues to this diagnosis are the presence of sun damage, atypical junctional melanocytes or melanoma in situ, fibrosis, and deep nodular lymphoid aggregates. Melanoma-specific markers can be absent [52]. Low-grade fibromyxoid sarcoma is discriminated by the lack of S100 [54] and expression of MUC4 (see also Sect. 12.5.5).

Treatment and prognosis Excision is the treatment of choice. Especially in NF1 patients, neurofibromas have a potential for malignant degeneration, but only deeply located, plexiform and large intraneural neurofibromas have an increased risk for malignancy, with a lifetime risk of approximately 5–10 % [51, 52].

12.2.5.3 Soft Tissue Perineurioma

Definition Soft tissue perineurioma is a rare benign peripheral nerve sheath tumor consisting of perineurial cells [50].

Epidemiology Middle-aged adults are mainly affected. However, the age range is broad including children [50, 51].

Clinical aspects Most patients present with a painless mass. Pain and discomfort are also reported in a number of cases. The head and neck are rarely involved with neck, face, tongue, and retrotonsillar region being reported. Cases are often subcutaneously located but also in deep soft tissues [50].

Macroscopy Tumors are mostly well circumscribed. They are (multi)nodular, firm, or soft with a yellow to tan-white and occasionally gelatinous cut surface [50].

Microscopy The commonly well-circumscribed tumors are unencapsulated. There is a swirling and storiform growth pattern of bland spindled cells with wavy or ovoid nuclei and elongated bipolar cytoplasmic processes (Fig. 12.16). Some tumors exhibit a more fascicular appearance. Scattered pleomorphic cells are akin to those seen in ancient schwannoma. Perineurioma may show myxoid and fibrous zones [50].
Other perineurioma variants are intraneural, sclerosing, and reticular [50]. There are also schwannoma/perineurioma hybrid cases showing intermingled Schwann cells and perineurial cells with a swirling or storiform architecture (Fig. 12.17) [58].

Immunohistochemistry EMA is positive in the great majority of cases, although this can be weak and focal [50, 59, 60]. CD34 and SMA are expressed in up to 64 % and 20 % of the cases, respectively [50]. Claudin-1 and GLUT-1 are positive in a variable number of cases [50, 60, 61], and focal staining for S100 may be seen [50].

Genetics Alterations of chromosome 22 including *NF2* mutations are reported in a subset of cases [62].

Differential diagnosis A lot of differential diagnostic possibilities have to be considered. Low-grade fibromyxoid sarcoma shows a prominent curvilinear vasculature not seen in perineurioma (see also LGFMS; Sect. 12.5.5) [54, 63]. Cellular myxoma is distinguished by the absence of a storiform/whorled growth pattern. Other differential diagnoses are solitary fibrous tumor, dermatofibrosarcoma protuberans (see Chap. 15 on skin lesions), soft tissue meningioma, and perineurial MPNST, but this latter lesion shows cytologic atypia and frequent mitoses. It has to be emphasized that the simple presence of infiltrative margins or scattered atypical cells in an otherwise typical soft tissue perineuri-

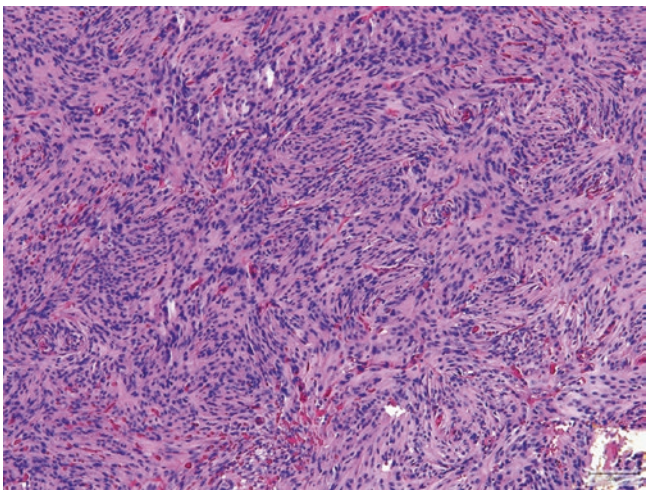

Fig. 12.16 Perineurioma with a whorling growth pattern of monomorphic cells with oval nuclei and long cytoplasmic processes

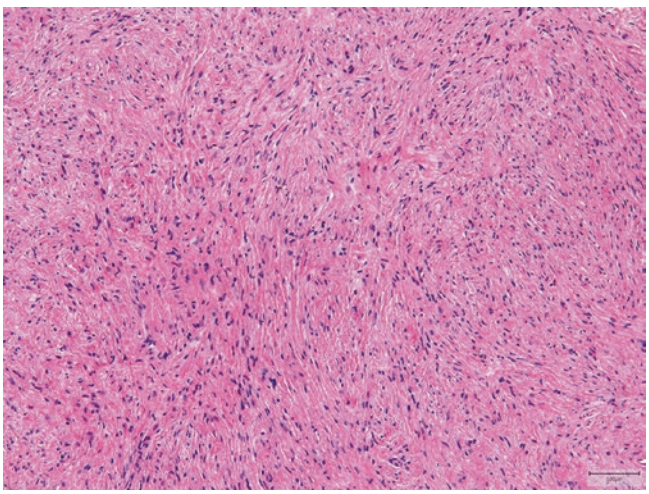

Fig. 12.17 Hybrid perineurioma/schwannoma showing an admixture of perineurial cells and slightly pleomorphic Schwann cells in a whorling architecture

oma should not lead to the diagnosis of MPNST [50]. One study showed that soft tissue meningiomas immunohistochemically express somatostatin receptor 2 and progesterone receptor in contrast with perineurioma and perineurioma in turn often shows a claudin-1/GLUT1 immunohistochemical phenotype [61].

Treatment and prognosis Excision is the adequate treatment. These lesions behave in a benign fashion. Rarely they may recur [49, 50, 64].

12.2.6 Smooth Muscle Tumors

12.2.6.1 Leiomyoma

Definition Leiomyomas are benign tumors with smooth muscle differentiation [65].

Epidemiology They are extremely rare in the head and neck region [65].

Clinical aspects Tumors often present as a long-standing mass. Adults are commonly affected [65].

Macroscopy Lesions are nodular and sharply demarcated with a trabecular cut surface [66].

Microscopy Spindled tumor cells are arranged in intersecting fascicles. The nuclei are oval to elongated and cigar shaped. There is eosinophil cytoplasm. Mitotic figures are absent (Fig. 12.18). This is in contrast with leiomyosarcoma (see below). Calcification/ossification or myxohyaline degeneration can occur as a sign of regression in long-standing lesions [66, 67].

Immunohistochemistry Lesions express smooth muscle markers (ASMA, MSA, desmin, caldesmon) [66].

Genetics No consistent genetic features are known so far, may be due to the rarity of soft tissue lesions.

Differential diagnosis The criterion for differentiation from leiomyosarcoma is at least one mitotic figure [66, 67]. Angioleiomyoma has a prominent vasculature [33].

Treatment and prognosis Marginal excision is the treatment of choice. The prognosis is excellent. Recurrences can occur [66].

12.2.6.2 Angioleiomyoma

Definition Angioleiomyoma is a perivascular smooth muscle tumor and forms a spectrum with lesions showing pericytic differentiation, including myofibroma and myopericytoma [68].

Epidemiology Adults are mainly affected [68].

Clinical aspects Angioleiomyomas are commonly superficial nodules which may be painful [33].

Macroscopy Angioleiomyoma usually forms a small 1–2 cm homogeneous and well-circumscribed rubbery nodule [33].

Microscopy The lesions are composed of eosinophilic smooth muscle cells intimately associated with the vein wall. The recognized variants are solid (very small lumina), venous (medium-sized lumina) (Fig. 12.19), and cavernous (large lumina and thin smooth muscle elements in-between). The latter two variants may overlap with myopericytoma show-

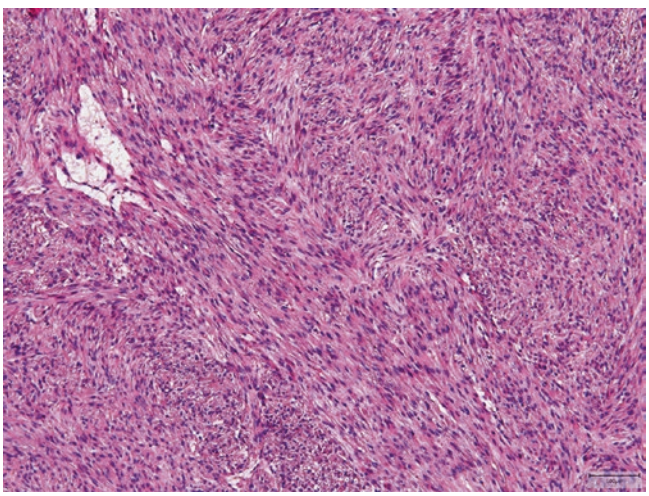

Fig. 12.18 Tumor cells of leiomyomas are arranged in intersecting fascicles. The nuclei are elongated. Mitotic figures are absent

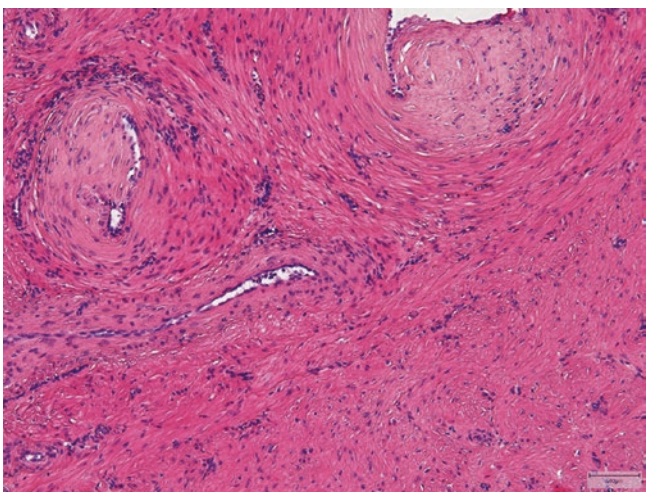

Fig. 12.19 Angioleiomyomas are composed of eosinophilic smooth muscle cells intimately associated with the vein wall

ing less complete smooth muscle differentiation. Unusual features are fatty degeneration and calcification [33].

Immunohistochemistry Lesions express smooth muscle markers (ASMA, MSA, desmin, caldesmon) [33, 68].

Genetics In one genomic array analysis, the most common loss involved 22q11.2, and the most common gain was low-level amplification of Xq [68].

Differential diagnosis Leiomyomas enter the differential diagnosis but do not show the prominent vasculature blending with intervascular smooth muscle cells. (Metastatic) Leiomyosarcoma shows atypia and mitotic figures [33, 68].

Treatment and prognosis Simple excision is curative. Recurrences are very rare [68].

12.2.7 Rhabdomyomatous Lesions

12.2.7.1 Adult Rhabdomyoma

Definition Adult rhabdomyomas are very rare benign lesions with predilection for the head and neck region (90 %) exhibiting skeletal muscle differentiation [69].

Epidemiology Adults are commonly affected with a peak incidence of 60 years and a male predominance [69].

Clinical aspects Lesions arise in soft tissue mostly of the neck or at mucosal sites in the upper aerodigestive tract (pharynx, oral cavity, larynx). Mucosal lesions can cause airway obstruction. Tumors present as a (multi)nodular mass. Multicentricity is rarely described [69].

Macroscopy The neoplasms are tan to red brown, circumscribed, and lobulated [69].

Microscopy The well-circumscribed lobulated and unencapsulated lesions consist of large polygonal cells with scant stroma containing thin-walled vessels. The tumor cells have small, round, and centrally or peripherally placed nuclei with prominent nucleoli and abundant eosinophilic, granular, or vacuolated cytoplasm. The latter evokes, when peripherally located, a "spider web" appearance. Cross-striation is commonly visible (Fig. 12.20). The glycogen-rich cytoplasm is PAS positive [69].

Immunohistochemistry There is positivity for desmin as well as a multifocal nuclear expression of myogenin [70].

Differential diagnoses The following alternatives have to be considered, granular cell tumor (S100 +), hibernoma (brown fat cells, S100 +), oncocytoma (keratin +, EMA +), and paraganglioma (S100 + in sustentacular cells, neuroendocrine markers +) [69]. In none of these tumor types, there is any desmin or myogenin positivity.

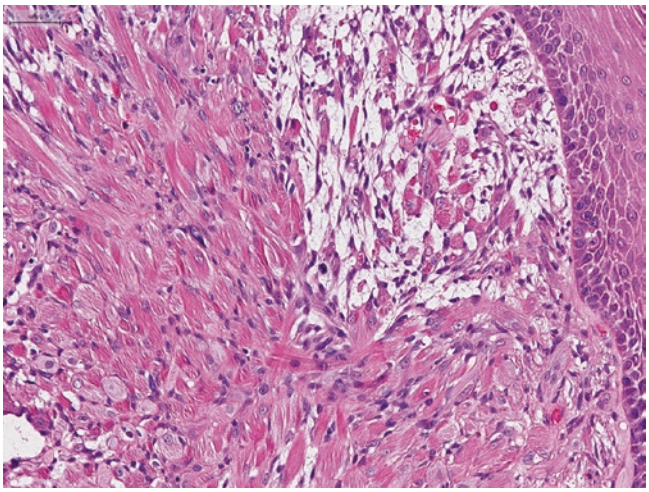

Fig. 12.20 The cells of adult rhabdomyomas have small, round, and centrally or peripherally placed nuclei with prominent nucleoli and abundant, eosinophilic, granular, or vacuolated cytoplasm which shows often cross-striation

Treatment and prognosis Surgery is the treatment of choice in these benign lesions. Recurrences occur in ca. 40 % of the cases irrespective of the margin status [69].

12.2.7.2 Fetal Rhabdomyoma

Definition Fetal rhabdomyomas are very rare benign lesions usually occurring in infants. They have a predilection for the head and neck region and show rhabdomyoblastic differentiation.

Epidemiology Fetal rhabdomyoma may occur at any age, although most are diagnosed within the first 3 years of life. About 25 % of cases are congenital. Males are more commonly affected [71].

Etiology and pathogenesis Fetal rhabdomyoma may be associated with the nevoid basal cell carcinoma (Gorlin-Goltz) syndrome [71].

Clinical aspects The great majority of cases arise in the head and neck, particularly the posterior auricular region. Symptoms are related to the site of involvement. Mucosal lesions are polypoid. Rare cases are multifocal [71, 72].

Macroscopy Fetal rhabdomyomas are circumscribed lesions with a glistening mucoid appearance on cut sections [71].

Microscopy Lesions are circumscribed and display skeletal muscle cells in varying stages of maturation. There are two

subtypes: the classic (or myxoid) subtype and the cellular (juvenile or intermediate) subtype. The former is mild to moderately cellular composed of oval-spindled undifferentiated mesenchymal cells and spindled rhabdomyoblasts with larger "strap cells" showing obvious cross-striation. Cells are haphazardly arranged within a loose myxoid matrix. Cellular fetal rhabdomyomas show a predominance of less immature-appearing spindle rhabdomyoblasts with variable degrees of differentiation, arranged in interlacing fascicles with minimal myxoid stroma (Fig. 12.21). Focally infiltrative growth, increased mitoses (up to 14/50 HPF), focal necrosis, and mild to moderate nuclear hyperchromatism and pleomorphism have been reported in both subtypes [71].

Immunohistochemistry There is diffuse reactivity for desmin and myogenin [71, 72].

Genetics Lesions associated with the Gorlin-Goltz syndrome show loss of function mutation in PTCH1 with consecutive activation of the hedgehog signaling. Non-syndromic lesions reveal activation of the same pathway by an as yet unknown mechanism [72].

Differential diagnosis Distinction from embryonal and spindle cell rhabdomyosarcoma can be challenging in more cellular and mitotic active lesions or when foci of necrosis are present. The circumscribed nature, superficial location, cellular maturation, and lack of striking nuclear atypia support the diagnosis of fetal rhabdomyoma [71].

Treatment and prognosis Complete excision is an effective treatment. Rare local recurrences are related to incomplete excision. The possibility of malignant transformation is questionable [71].

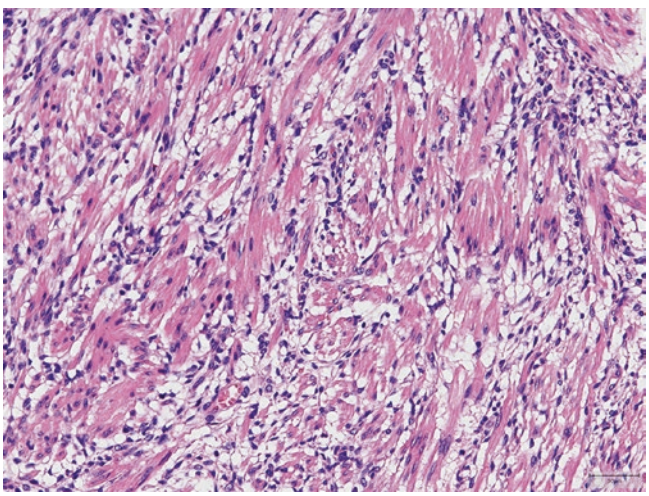

Fig. 12.21 This cellular fetal rhabdomyoma shows rhabdomyoblasts with variable degrees of differentiation, arranged in fascicles

12.2.8 Tumors of Uncertain Differentiation

12.2.8.1 Ectopic Hamartomatous Thymoma

Definition The term of this distinctive tumor reflects the initial belief that it derives from abnormal thymic tissue of the third branchial arch. Additional data support reclassification as a branchial anlage mixed tumor because of the absence of true thymic tissue [73].

Epidemiology Ectopic hamartomatous thymoma is a rare neoplasm with a peak incidence in the fourth and fifth decade and a striking male predominance [73].

Clinical aspects It presents as a slow-growing, well-marginated mass in the lower neck and sternoclavicular and presternal regions and arises deep to the platysma muscle and sometimes involves the sternocleidomastoid muscle, but does not affect the thyroid gland, large vessels, or mediastinum [73].

Macroscopy The lesions are well-circumscribed (multi) nodular masses with a firm or rubbery consistency and a grey-white to tan appearance with yellowish foci [73].

Microscopy The tumor is commonly circumscribed and composed, in varying amounts, of three components including plump and delicate (fibroblast-like) spindle cells, mature adipose tissue, and epithelial cells, comprising squamous and glandular elements with cysts showing an epithelial lining. The plump spindle cells show relatively abundant cytoplasm and are arranged in fascicles or in a storiform fashion. There are spindle cell areas merging imperceptibly with epithelial cells (Figs. 12.22 and 12.23). Mitotic figures can be

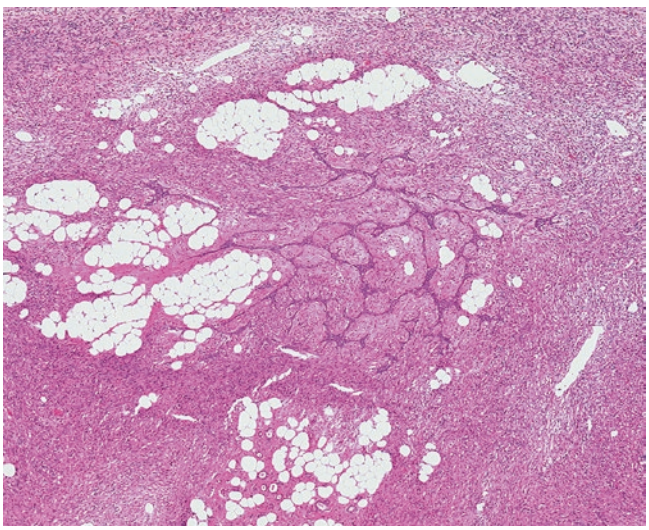

Fig. 12.22 Low magnification of ectopic hamartomatous thymoma shows a composition of spindle cells, adipocytes, and epithelial cells (By courtesy of Prof. Dr. Thomas Mentzel)

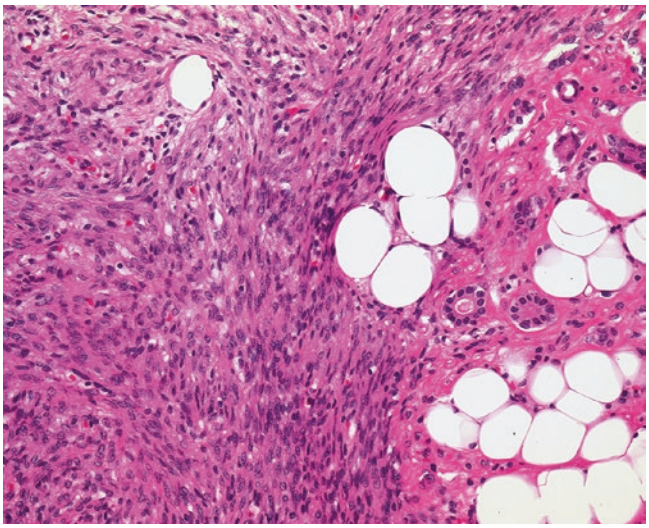

Fig. 12.23 High magnification of ectopic hamartomatous thymoma shows bland cytomorphological features and epithelial glands like a mixed tumor (By courtesy of Prof. Dr. Thomas Mentzel)

present. Facultative findings are variable amounts of collagen, intralesional nerve segments, and smooth muscle and the presence of lymphocytes [73].

Immunohistochemistry There is coexpression of keratins, SMA, calponin, and EMA in the spindle cell component in the absence of S100, GFAP, and desmin [73].

Differential diagnosis Conventional mixed tumor of the skin, soft tissue, or salivary gland origin showing varying proportions of myoepithelial cells with epithelioid, plasmocytoid, spindled, and clear cell appearance and glandular epithelial structures. Biphasic synovial sarcoma consists of fascicular arranged monomorphic spindle cells and glandular elements with scant cytoplasm. Peripheral nerve sheath tumor with glandular structures is also composed of fascicular arranged but atypical spindle cells classically with a perivascular cuff. Cystic teratoma possesses epithelial, mesenchymal, and neuroectodermal derivates [73].

Treatment and prognosis All examples to date have pursued a benign clinical course with local recurrences being uncommon but if present typically attributable to an incomplete excision [73].

12.2.8.2 Angiomatoid Fibrous Histiocytoma

Definition This is an uncommon, translocation-associated histiocytoid soft tissue neoplasm that behaves mainly in a benign fashion.

Epidemiology Although the age distribution is wide ranging from 2 months to 70 years, adolescents or young adults are classically affected [74].

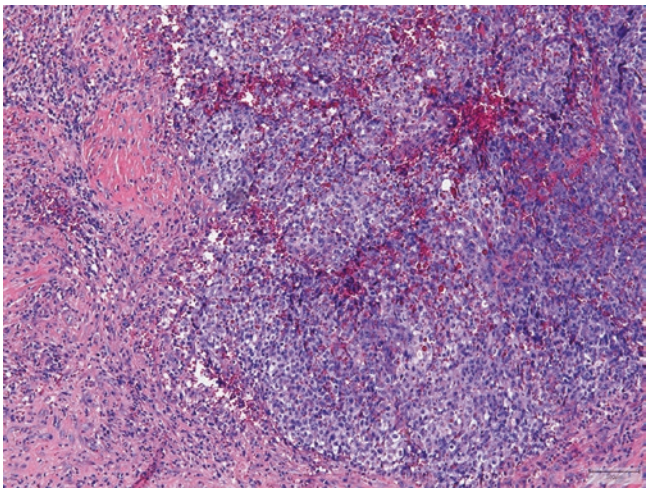

Fig. 12.24 The tumor nodules of angiomatoid fibrous histiocytoma are encapsulated and consist of uniform histiocytoid cells with syncytial growth. Note the blood-filled pseudovascular spaces and the lymphocytes surrounding the lesion

Clinical aspects Limbs are the most common sites of involvement; however, these lesions can also arise in the head and neck [74]. They are mostly superficially/subcutaneous localized. Origin in deep soft tissue occurs more rarely [75].

Macroscopy The tumor shows usually a (multi)nodular appearance with a tan, variably hemorrhagic cut surface [76].

Microscopy The tumor nodules are encapsulated and consist of uniform histiocytoid or spindle cells with compact syncytial growth. Small blue round cell morphology can be focally present and could be confused with small round cell sarcomas. Cellular atypia and increased mitotic activity may be noted but have not been shown to be associated with outcome. There are variably blood-filled pseudovascular spaces within the lesion, and outside the capsule, there is a lymphocytic cuff with occasional germinal center formation (Fig. 12.24) [74, 76].

Immunohistochemistry Desmin and EMA are expressed in ca. 50 % of the cases. CD99 and CD68 expression is frequently noted [74].

Genetics *EWSR1-CREB1* is the most commonly involved fusion gene. Others are *EWSR1-ATF1* or *FUS-ATF1* [74, 75].

Differential diagnosis The lesion can mimic a lymph node metastasis [74].

Therapy and prognosis These neoplasms are almost always benign and are curated by simple excision [74, 76].

12.2.8.3 Ossifying Fibromyxoid Tumor (OFMT)

Definition OFMT is a rare, mostly benign, translocation-associated mesenchymal neoplasm. Atypical and malignant cases are also reported.

Epidemiology This is a lesion of adulthood but has occasionally been diagnosed in adolescents as well [77–79].

Clinical aspects It arises in a variety of sites with a low frequency for the head and neck. Most lesions are located subcutaneously followed by deep soft tissue and rarely skin [77–79].

Macroscopy Neoplasms are (multi)nodular with a tan-white cut surface and a firm or rubbery consistency [79, 80].

Microscopy The lesions are lobulated and commonly sharply demarcated with an incomplete peripheral bone shell. The bone shell is lacking in about 20–40 % of the cases appropriately called non-ossifying fibromyxoid tumor [78, 79]. Some neoplasms show infiltrative growth and satellite nodules, which should not be interpreted as a sign of malignancy. The uniform, round to oval cells are arranged in a cord-like or nested fashion. They possess small vesicular nuclei with minute nucleoli and small amounts of pale to eosinophilic cytoplasm. The mitotic index varies but is usually low. The cells are dispersed in a fibromyxoid matrix (Figs. 12.25, 12.26 and 12.27) [77]. Atypical features are increased cellularity, nuclear atypia, increased mitotic activity (>2/50 HPF), and randomly distributed osteoid with possible malignant appearance (atypical osteoblastic nuclei) [77, 78, 81].

Immunohistochemistry Desmin and S100 are expressed in ca. 70 % and of the cases with malignant cases being less frequent positive [78, 80, 82]. Exceptionally, GFAP and keratins are positive [79].

Genetics Up to 85 % of the cases show a *PHF1* rearrangement irrespective of benign or malignant morphological features with *EP400* being the most common fusion partner [82, 83]. Other fusion partners are *MEAF6* and *EPC1* [82].

Differential diagnosis Myoepithelial tumors are the main differential diagnosis showing also arrangement in cords and nests of lesional cells in a fibromyxoid stroma. In contrast, cells of this latter tumor show more heterogeneity with epithelioid, spindle, clear, and plasmocytoid features. Ductular structures, if present, are also a discriminating sign. Expression of keratins and GFAP and rearrangement of *EWSR1* and *PLAG1* belong to the signature of myoepithelial tumors [48]. Other differential diagnoses are low-grade fibromyxoid sarcoma and sclerosing epithelioid fibrosarcoma (see Sects. 12.5.5 and 12.5.6).

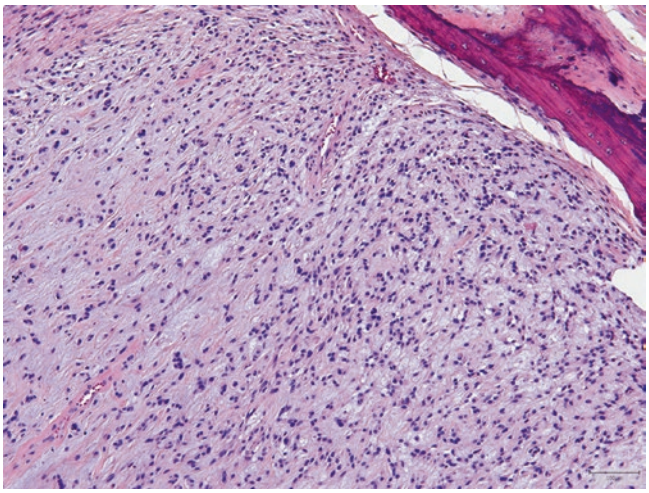

Fig. 12.25 Ossifying fibromyxoid tumor showing classically a peripheral bone rimming and a fibromyxoid stroma

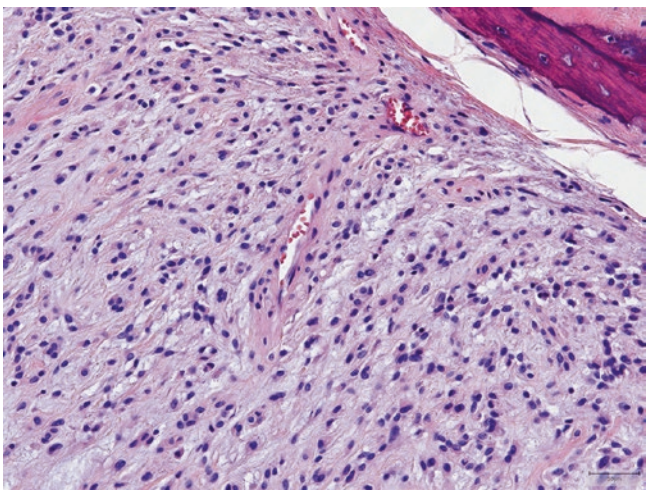

Fig. 12.26 Note the uniform epithelioid cells of ossifying fibromyxoid tumor

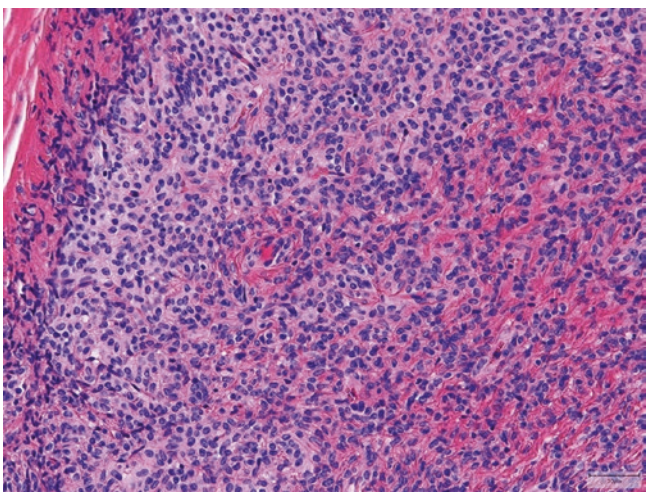

Fig. 12.27 Some cases of ossifying fibromyxoid tumor show no bone shell (non-ossifying variant)

Treatment and prognosis Complete excision and long-term follow-up are considered optimal treatment [79]. Most lesions behave in a benign fashion. Local recurrences can occur, and metastases develop rarely; both events are often associated with malignant morphological features. However, these features not always correlate with clinical behavior [77, 78, 81].

12.3 Intermediate/Locally Aggressive Lesions

12.3.1 Desmoid-Type Fibromatosis

Definition Desmoid-type fibromatosis is a locally aggressive (myo)fibroblastic lesion [84].

Epidemiology and clinical aspects The head and neck region is more often involved in children than in adults. Preferential anatomic sites are the neck and sinonasal cavities. Secondary bone involvement reflects invasive and destructive growth. Conversely, primary bone lesions (desmoplastic fibroma) with possible extension into soft tissue occur mainly in the mandible [85].

Macroscopy These infiltrative lesions are firm and white with a coarse trabeculation on the cut surface. Myxoid areas can be present [85].

Microscopy Neoplasms are composed of ill-defined long fascicles of uniform bland (myo)fibroblasts infiltrating adjacent structures. There are slender, tapering nuclei with open chromatin. Mitotic figures can occur. The background is usually collagenous sometimes with coarse bundles. Alternatively, the matrix can be, at least focally, myxoid. Vessels show parallel alignment to the fascicles, and a perivascular edema is typical (Fig. 12.28) [84, 85].

Immunohistochemistry Characteristically, there is nuclear accumulation/expression of β-catenin. SMA is often positive. (Co)expression of desmin is seen in individual cases. Some cases are reported to show traces of S100 [85].

Genetics Activating mutations in *CTNNB1* are found in approximately 85 % of all sporadic desmoids, with p.T41A, p.S45F, and p.S45P being the most frequent [86–90]. In familial adenomatous polyposis patients, desmoid develops on the basis of inactivating *APC* mutations, which, however, may also occur in sporadic cases [30, 85].

Differential diagnosis Other (myo)fibroblastic lesions, as cranial/nodular fasciitis, myofibroma, low-grade fibromyxoid sarcoma, low-grade myofibroblastic sarcoma, lipofibromatosis, inflammatory myofibroblastic tumor, infantile fibrosarcoma, and hypertrophic scar. Other differential diagnoses are low-grade malignant peripheral nerve sheath tumor and low-grade leiomyosarcoma [85]. Long fascicles of monomorphic myofibroblastic cells with slender nuclei paralleled by small vessels and nuclear immunohistochemical expression of β-catenin are most useful characteristics to make the difference to the mentioned other entities as is the demonstration of the typical genetic change mentioned above.

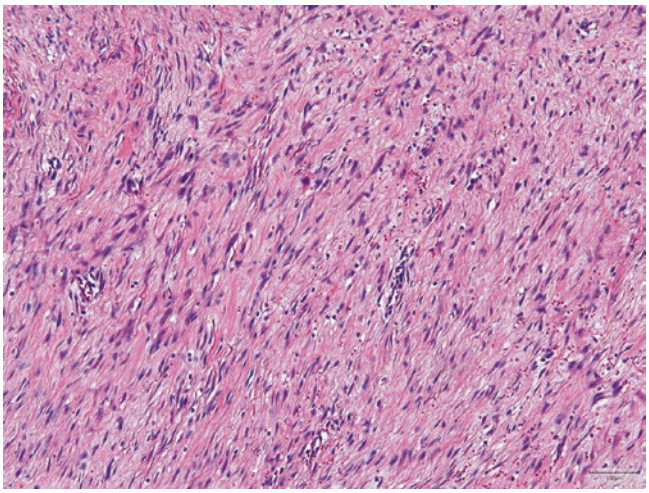

Fig. 12.28 Desmoids are composed of ill-defined long fascicles of uniform bland (myo)fibroblasts infiltrating adjacent structures

Treatment and prognosis One of the main problems in managing desmoids is their high propensity to recur after surgery, but this is unpredictable and stable disease and even regression is possible. Therefore, surgery as the mainstay is under debate, and a wait-and-see policy as initial treatment in a non-life-threatening site and in the absence of marked progression is being adopted. Furthermore, function-sparing intervention should be preferred to aggressive surgery aiming at negative margins [84, 85].

12.4 Intermediate/Rarely Metastasizing Lesions

12.4.1 Solitary Fibrous Tumor (SFT)

Definition These fusion gene-associated tumors are of fibroblastic type. They can originate at any anatomic location with the head and neck region being a relatively rare site [91]. SFT variants are giant cell angiofibroma, fat-forming SFT, and dedifferentiated SFT. The behavior is unpredictable on morphological grounds [92].

Epidemiology Adults are affected [92].

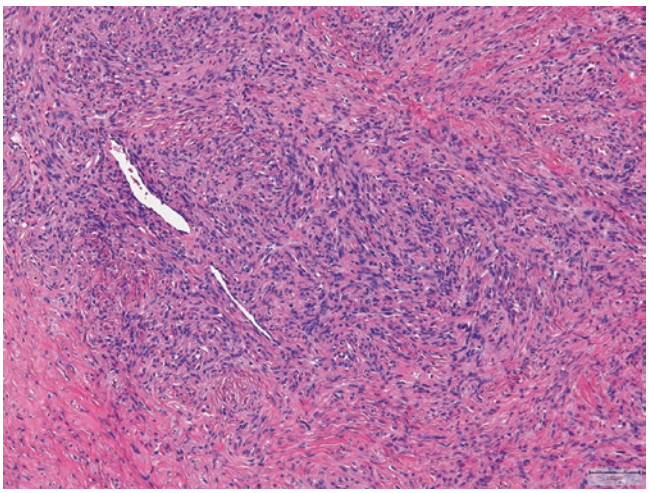

Fig. 12.29 Solitary fibrous tumor shows a patternless architecture with variably hypo- and hypercellular areas composed of spindled to ovoid fibroblastic cells

Clinical aspects In the head and neck region, SFTs have been reported at all sites [91, 93]. Usually, they present as a slow-growing mass. Local symptoms are related mainly to size and compression of surrounding structures [91, 94]. Hypoglycemia is seen in less than 5 % of the cases and is associated with a large tumor size and a high mitotic rate [94].

Macroscopy The range of tumor size is broad. SFTs have a (multi)nodular configuration with a coarse white-grayish cut surface [40].

Microscopy In its classic form, SFT shows a patternless architecture with variably hypo- and hypercellular areas composed of spindled to ovoid fibroblastic cells with scant cytoplasm and indistinct cell borders [39, 95]. There is a branching "staghorn" pattern of vasculature with often perivascular hyalinization. Cellular forms show more rounded nuclei and include numerous thin-walled vessels with little intervening fibrosis (Fig. 12.29) [93, 95]. The giant cell variant (giant cell angiofibroma) is defined by numerous multinucleated giant cells; some of them are lining pseudovascular spaces [95, 96]. Rare variants are fat-forming lesions including mature adipocytes and dedifferentiated SFT with abrupt transition to high-grade sarcoma [92].

Immunohistochemistry and genetics CD34 and STAT6 are expressed in more than 90 % of the cases [39]. The nuclear expression of STAT6 is due to the consistent *NAB2-STAT6* gene fusion, which can diagnostically be detected by RT-PCR [36–38]. Another novel diagnostic marker of SFT is GRIA2 showing cytoplasmic expression in 80 % of the cases based on overexpression of the corresponding gene [38, 97]. One has to be aware of GRIA2 expression in other soft tissue tumors (e.g., dermatofibrosarcoma protuberans) [97].

Differential diagnosis A huge number of lesions have to be considered, synovial sarcoma, cellular fibrous histiocytoma, nasopharyngeal angiofibroma, sinonasal-type hemangiopericytoma, spindle cell lipoma, especially in cases with a branching vasculature, sarcomatoid carcinoma, schwannoma, melanoma, malignant peripheral nerve sheath tumor, dedifferentiated liposarcoma, and in the giant cell variant, giant cell fibroblastoma (variant of dermatofibrosarcoma protuberans mainly occurring in children) [91, 93, 95, 96]. The patternless pattern and the relative monomorphic fibroblastic cells with nuclear immunohistochemical expression of STAT6 help in distinguishing SFTs from these other tumor types. Moreover, demonstration of the specific genetic change is discriminative.

Treatment and prognosis Complete excision is the treatment of choice. A notable feature is the unpredictable clinical behavior that is unreliably correlated with histologic parameters; however, mitotic count >4/10 HPF seems to be the best indicator for poor outcome. Other indicators for possible malignant behavior are site (e.g., meninges), size (>10 cm), and positive margins [92, 93]. Obtaining free margins in the head and neck region is a unique professional challenge for surgery by virtue of the close relationship to adjoining vital structures [91].

12.4.2 Inflammatory Myofibroblastic Tumor (IMT)

Definition IMT is a distinct myofibroblastic/fibroblastic neoplasm with a tendency for local recurrence and very rare metastases [30].

Epidemiology IMT has a predilection for children, adolescents, and young adults, with a median age at diagnosis of 9 years, although it can occur throughout life [30].

Clinical aspects The head and neck region is rarely involved. Up to one-third of patients with IMT present with an inflammatory syndrome with fever, malaise, weight loss, and laboratory abnormalities, such as microcytic hypochromic anemia, thrombocytosis, polyclonal hyperglobulinemia, and anemia. This syndrome may be related to the release of cytokine mediators by tumor cells [30].

Macroscopy IMT is a (multi)nodular circumscribed mass with a firm, tan cut surface [30].

Microscopy Lesions are composed of elongated or plump polygonal myofibroblasts with eosinophilic or amphophilic cytoplasm, variable cellularity and growth patterns, and an intermixed inflammatory infiltrate of plasma cells, lymphocytes, and eosinophils. There are three main morphological

patterns. The myxoid vascular pattern consists of interlacing bundles of spindle cells or haphazardly arranged polygonal myofibroblasts embedded in a myxoid matrix with small dilated blood vessels, inflammation, and ganglion-like cells with prominent nucleoli. This pattern resembles granulation tissue or nodular fasciitis. The cellular variant shows compact spindle cells arranged in fascicles, variable proportions of ganglion-like myofibroblasts, and prominent lymphoplasmocytic inflammation with eosinophils (Fig. 12.30). The collagenized pattern resembles a scar or desmoid and is hypocellular, with a collagenized background and sparse spindle cells intermingled with the characteristic but also relatively sparse inflammatory infiltrate [30].

Immunohistochemistry Ca. 50 % of IMT cases reveal ALK expression. The absence of ALK is more common in older individuals [98].

Genetics Approximately 50 % of IMTs contain clonal rearrangements of chromosome 2 at band 2p23, involving the *ALK* gene. A variety of gene partners can be fused to *ALK*, including *TPM3*, *TPM4*, *CLTC*, *RANBP2*, *CARS*, *ATIC*, and *SEC31L1* [30, 99].

Differential diagnosis depends on the histological pattern: Other myofibroblastic lesions as nodular fasciitis, myofibroblastic sarcoma, desmoid-type fibromatosis, scar, and other reactive (inflammatory) processes have to be considered. Further differential diagnoses are desmoplastic melanoma, sarcomatoid carcinoma, malignant peripheral nerve sheath tumor, spindle cell rhabdomyosarcoma, inflammatory leiomyosarcoma, and dedifferentiated liposarcoma [30]. The inflammatory background, *ALK* rearrangement, and expression of ALK in a lesion of a young patient may help to make the diagnosis. For the other myofibroblastic tumor types, see the corresponding Sects. (12.2.2, 12.3.1, and 12.5.3). The differential diagnostic aspects and other relevant data for laryngeal IMT are discussed in Chap. 7.

Treatment and prognosis The current treatment of choice is surgical excision. Chemotherapy may be useful for unresectable tumors. Newer therapeutic options include antitumor necrosis factor antibody and targeted treatment with an ALK inhibitor. IMT has a tendency for local recurrence. Very rarely metastases occur [30].

12.4.3 Infantile Fibrosarcoma

Definition Infantile or congenital fibrosarcoma is an intermediate (rarely metastasizing) neoplasm of infancy [30].

Epidemiology It occurs in the first 2 years of life, and nearly half are diagnosed at birth or antenatally [30].

Clinical aspects The head and neck region is one of the most frequent sites [30].

Macroscopy The solid mass has poorly defined margins and a fleshy cut surface with areas of necrosis and hemorrhage [30].

Microscopy Infantile fibrosarcoma displays a wide morphological spectrum. The highly cellular neoplasm is composed of sheets, bundles, or fascicles of spindle cells or sheets of immature round or polygonal cells with a high nuclear/cytoplasmic ratio (Fig. 12.31). A prominent hemangiopericytoma-like vascular pattern is often present,

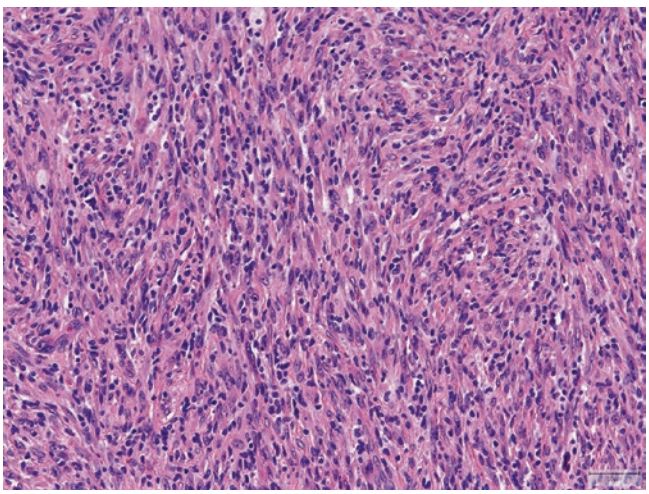

Fig. 12.30 This inflammatory myofibroblastic tumor is composed of plump polygonal myofibroblasts with eosinophilic or amphophilic cytoplasm and an intermixed inflammatory infiltrate

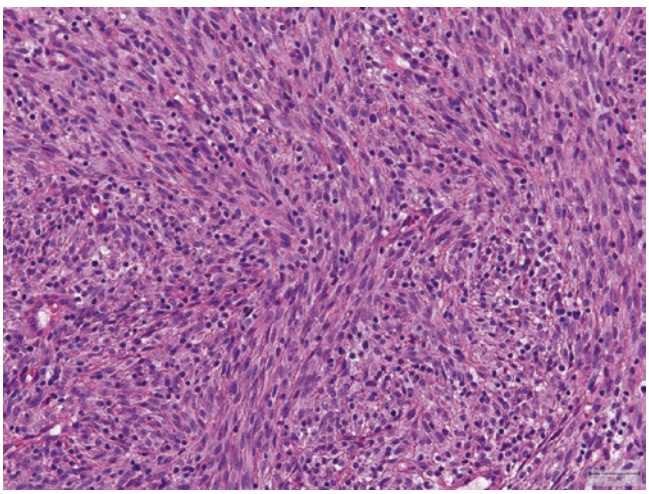

Fig. 12.31 Infantile fibrosarcoma is a cellular neoplasm composed of fascicles of plump monotonous spindle cells with enlarged nuclei

and a focal herringbone pattern of spindle cells may be seen. Less cellular areas resemble fibromatosis or myofibroma due to a collagenous background. Mitotic figures are frequent which has no prognostic impact. Necrosis and an inflammatory reaction may be present [30].

Immunohistochemistry The immunohistochemical profile is nonspecific with variable reactivity for SMA, MSA, and desmin in a subset of cases. Occasional individual cells may stain for cytokeratin and S100 [30].

Genetics *ETV-NTRK3* is the pathognomonic fusion gene [30].

Differential diagnosis Desmoid-type fibromatosis, myofibroma, and inflammatory myofibroblastic tumor are alternative possibilities, but they lack the specific genetic aberration mentioned above.

Treatment and prognosis In spite of rapid growth and the large size, the prognosis is favorable, with only rarely distant metastases [30].

12.5 Malignant

12.5.1 Leiomyosarcoma

Definition Sarcoma with smooth muscle differentiation. Any mitotic activity in a smooth muscle tumor should be regarded as indicative of potential malignancy.

Epidemiology Most cases arise in adults [67, 100]. Children are rarely affected [100, 101].

Clinical aspects Tumors present as a (painful) soft tissue mass. Symptoms depend on the site of involvement. The lesions also can affect the craniofacial bone, primarily or secondarily. Leiomyosarcomas metastasize to the lung, liver, and brain and to other soft tissue sites or bone. Lymph node metastases are rarely reported [67, 102]. Metastasized leiomyosarcoma from other sites should be excluded, e.g., uterus [67, 103].

Macroscopy Tumors are firm and either poorly defined or well circumscribed but unencapsulated. On sectioning, they are whorled, whitish, or tan grey with areas of hemorrhage, cystic degeneration, and necrosis [100, 101].

Microscopy The lesions show infiltrative growth or shapely demarcated borders. They are composed of spindle cells arranged in interlacing fascicles. A storiform architecture can be focally present. Tumor cell nuclei are oval to elongated and frequently blunt ended. There is variable atypia

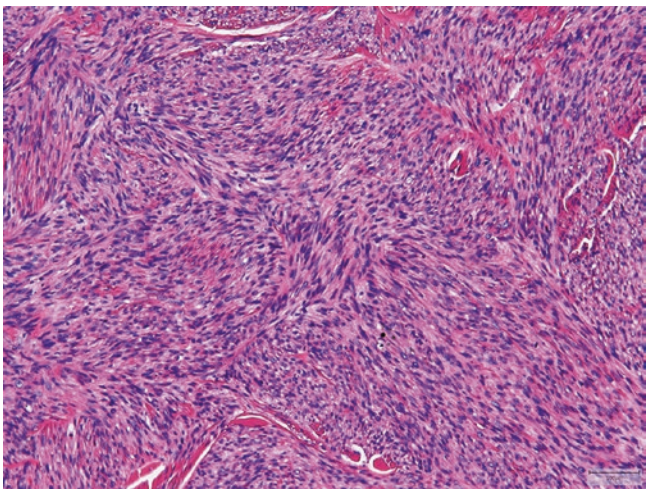

Fig. 12.32 This low-grade leiomyosarcoma displays slightly atypical leiomyocytes and few mitotic figures

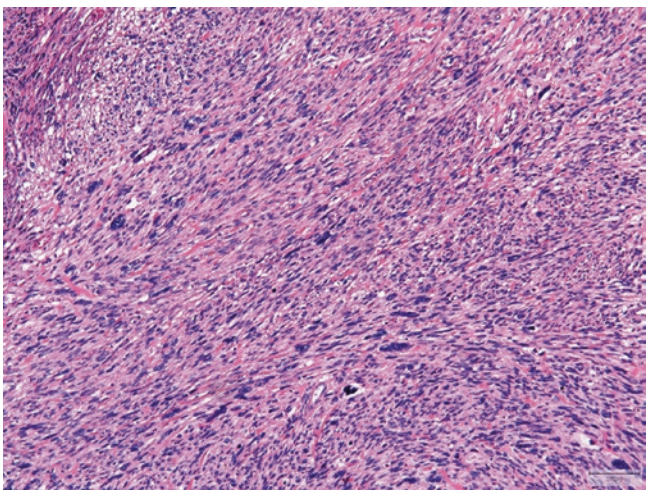

Fig. 12.33 This higher-grade leiomyosarcoma shows prominent pleomorphic features and numerous mitoses

with enlarged nuclei and hyperchromasia. Nucleoli are sometimes obvious. The eosinophil cytoplasm often shows small paranuclear vacuoles (Figs. 12.32 and 12.33). Epithelioid cytomorphology is rarely seen. Osteoclastic and pleomorphic giant cells may occur [67, 100]. A myxoid background should not lead to confusion with spindle cell myoepitheliomas. Scattered inflammatory cells are seen in some cases. Dystrophic or psammomatous calcification has been rarely reported [101]. Grading depends on mitotic activity, necrosis, and resemblance to normal tissue following the French criteria (FNCLCC) [104].

Immunohistochemistry Smooth muscle differentiation is demonstrated by diffuse staining for desmin, h-caldesmon, SMA, and MSA with at least positivity for two of these markers [105].

Genetics There are different genes involved in the pathobiology of leiomyosarcomas, *TP53*, *FANCA*, *ATM*, *RB1*, *CDK2NA*, *PTEN*, *MYOCD*, *ROR2*, and *MED12* [105, 106].

Differential diagnosis Spindle cell squamous carcinoma and spindle cell myoepithelioma have to be considered, especially in leiomyosarcoma cases with keratin expression. Loss of epithelial markers is not unusual in spindle cell carcinoma and SMA can be expressed. Therefore, careful examination of the superficial squamous epithelium is mandatory. Myoepithelioma is additionally positive for S100 and/or GFAP. Spindle cell melanoma can also show an aberrant immunophenotype with loss of lineage specific markers [107]. In children, myofibroma is the most important differential diagnosis, which may exhibit infiltrative growth and mitotic figures. For low-grade myofibroblastic sarcoma, see Sect. 12.5.3.

Treatment and prognosis Surgery is the treatment of choice. One-third of the patients die of their tumor, either as a result of distant metastases or of uncontrolled local recurrence involving vital head and neck structures. Complete surgical excision appears to be an important predictor of disease-free survival [67]. Morphologically high-grade sarcomas seem to be more aggressive [105].

12.5.2 Rhabdomyosarcoma

Rhabdomyosarcoma (RMS) is a heterogeneous group of mesenchymal tumors with skeletal muscle differentiation. They are divided into embryonal (including botryoid subtype), alveolar, spindle cell/sclerosing, and pleomorphic type with distinct clinical, pathologic, and molecular differences [108]. The recently described variant of epithelioid rhabdomyosarcoma is also reported herein. Whether this is a special type or a variant of a known type of rhabdomyosarcoma is still under debate.

Rhabdomyosarcoma is the most common soft tissue sarcoma in children but is distinctly unusual in adults [108–110]. In children, prognosis and response to chemotherapy correlate with the histologic type. In contrast, adult RMS is largely not chemosensitive and associated with a poor prognosis irrespective of the histologic type [110].

12.5.2.1 Embryonal Rhabdomyosarcoma (ERMS)

Definition ERMS is a sarcoma of infancy showing remarkable resemblance with developing skeletal muscle. It includes the botryoid and anaplastic subtype [108].

Epidemiology This sarcoma typically occurs in newborns and infants [108].

Clinical aspects In the head and neck, ERMS arise in the orbita (most commonly), nasal passages, paranasal sinuses, mouth, pharynx, parotid region, temporal region, pterygoid region, and cheek. Orbital lesions can cause proptosis and diplopia. Bone erosion and intracranial extension is sometimes present [111]. The botryoid type originates superficially from unusual sites as conjunctiva or ear [108].

Macroscopy These tumors present as bulging, infiltrative soft tissue masses that may be fungating when they present in external locations such as the conjunctiva. Botryoid lesions have a distinctive resemblance to a bunch of grapes with fleshy, nodular, polypoid outgrowths [108].

Microscopy ERMS is characterized by alternating foci of hypo- and hypercellularity. Like embryonic muscle, the dense zones typically contain areas of more overt myogenesis, whereas loose areas more closely resemble primitive mesenchyme set in a gelatinous matrix. The key cell is the strap-shaped or tadpole-shaped rhabdomyoblast with an eccentric round nucleus and brightly eosinophilic cytoplasm. However, the differentiation varies from poor to abundant with the latter resembling rhabdomyoma. The botryoid lesions produce a "cambium layer" as a key feature with subepithelial condensation of primitive cells (Figs. 12.34 and 12.35). They can be hypocellular and appearing innocuous and may be therefore initially misdiagnosed as inflammation or developmental anomaly [108]. In embryonal rhabdomyosarcoma with anaplasia, tumor cells with enlarged hyperchromatic pleomorphic nuclei containing prominent nucleoli are present [108].

Immunohistochemistry The combination of positive markers is desmin and myogenin. MyoD1 stains less specific. In

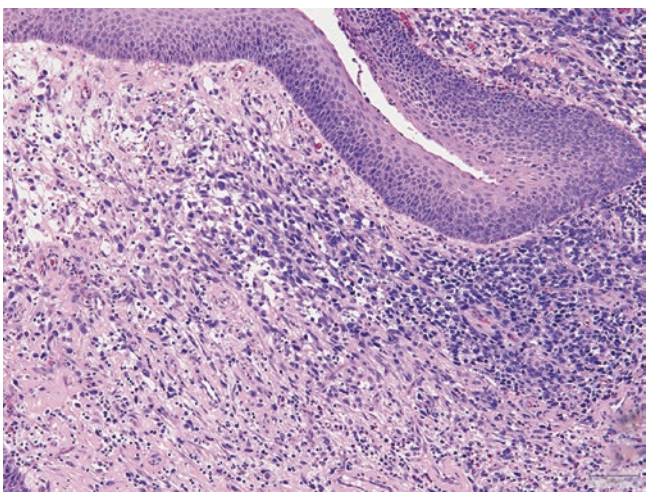

Fig. 12.34 Botryoid subtype of embryonal rhabdomyosarcoma with alternating foci of hypo- and hypercellularity

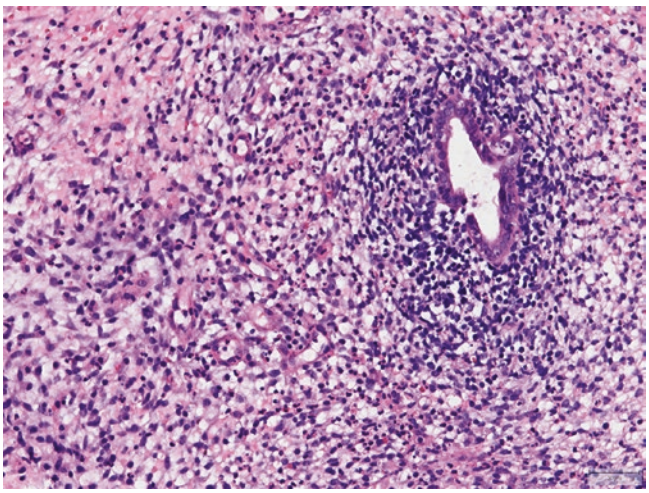

Fig. 12.35 Embryonal rhabdomyosarcoma. Note the subepithelial condensation of the primitive rhabdomyoblasts

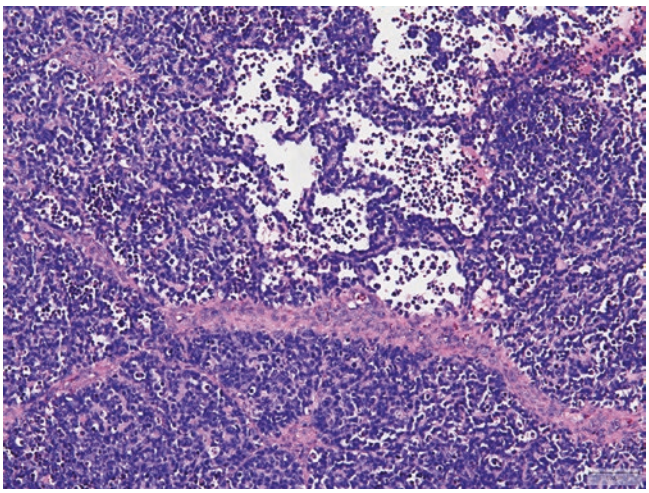

Fig. 12.36 Alveolar rhabdomyosarcomas are small blue round cell lesions commonly with an alveolar pattern

comparison to alveolar RMS (ARMS) with staining in almost 100 % of the nuclei, ERMS stain in a more heterogeneous fashion, which yields a clue to their subclassification [108].

Genetics In contrast to ARMS, ERMS possess no distinct molecular signature, although like other embryonal tumors they tend to exhibit loss of allelic heterozygosity and abnormalities in parental imprinting. Proto-oncogenes like *C-MET* are expressed. Also, there is hypermethylation of tumor suppressor genes. *IGFR I* amplification and *TP53* expression corresponds to anaplastic histology [108].

Differential diagnosis ERMS may be confused with reactive polyps in the botryoid subtype if one ignores the cellularity and atypia and with ARMS in cellular lesions in which case myogenin expression of almost all nuclei denotes ARMS (see below).

Treatment and prognosis All patients receive intensive multiagent chemotherapy, most of them radiotherapy, and some surgical resection [110]. The botryoid variant has a superior prognosis, whereas ERMS has an intermediate prognosis [108]. ERMS with prominent anaplastic (pleomorphic) features behave aggressively [108].

12.5.2.2 Alveolar Rhabdomyosarcoma (ARMS)

Definition Translocation-associated small blue round cell sarcoma with skeletal muscle differentiation.

Epidemiology These lesions affect all ages and are more common in adolescents and young adults [108, 112].

Clinical aspects See ERMS. These tumors also show a potential for leukemogenesis [108].

Macroscopy See ERMS.

Microscopy ARMS are highly cellular lesions typically forming nests of monotonous small blue round cells separated by fibrovascular septa imparting a mainly alveolar pattern due to floating cellular aggregates in the middle of empty spaces (Fig. 12.36). A solid pattern alternatively exists and applies the term solid variant. The latter may lack the typical septa. Scattered multinucleated (wreath-like) giant cells may be seen [108].

Immunohistochemistry The most reliable markers are desmin and myogenin. Myogenin decorates almost all nuclei helping to make the distinction from cellular ERMS [108].

Genetics *PAX3-FOXO1* or alternatively *PAX7-FOXO1* or rarely other fusion partners of *FOXO1* occur. Abnormalities of *ALK* are common [108, 110].

Differential diagnosis They encompass other small blue round cell tumors (Ewing sarcoma, olfactory neuroblastoma, small cell carcinoma, sinonasal undifferentiated carcinoma, midline carcinoma) [31]. Appropriate use of immunohistochemistry will rule out these other possibilities.

Treatment and prognosis For treatment, see ERMS. The prognosis is usually poor. Metastatic ARMS (mainly lung, bone, breast) with *PAX7* fusion appear to have a substantially better prognosis than those with *PAX3-FOXO1* [108].

12.5.2.3 Spindle Cell/Sclerosing Rhabdomyosarcoma

Definition Spindle cell rhabdomyosarcoma forms a morphological continuum with sclerosing rhabdomyosarcoma. It

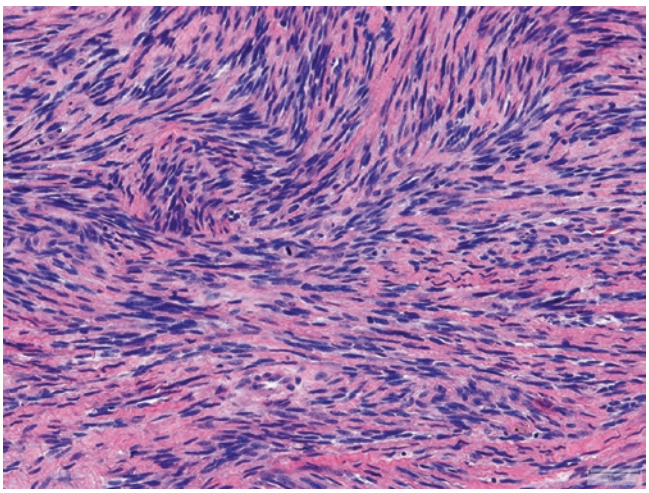

Fig. 12.37 Spindle cell rhabdomyosarcoma consists of bland spindle cells with scant, palely eosinophilic cytoplasm forming long intersecting fascicles

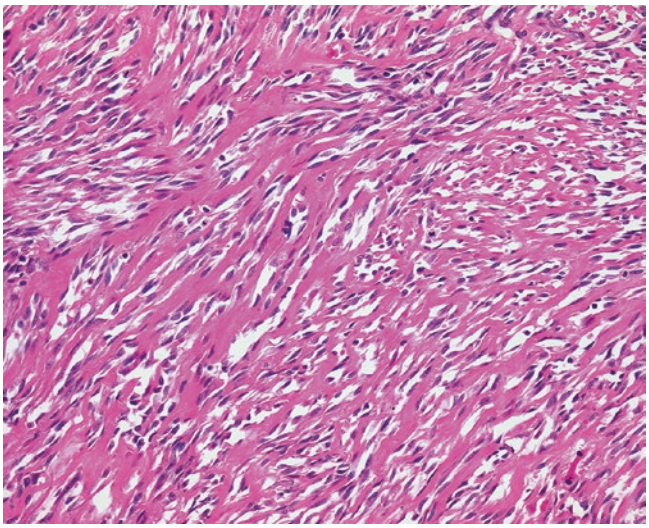

Fig. 12.38 Sclerosing rhabdomyosarcoma is composed of polygonal cells containing varying amounts of eosinophilic cytoplasm and atypical nuclei. Typically there is a hyaline matrix imparting a pseudovascular growth pattern (By courtesy of Prof. Dr. Thomas Mentzel)

is a rare subtype of rhabdomyosarcoma with a frequency of ca. 4 % of all rhabdomyosarcomas [113, 114].

Epidemiology This tumor is divided into two biological different groups, one occurring in children and the other in adults. Of the adult-type rhabdomyosarcomas, 8.7 % are spindle cell rhabdomyosarcomas [109]. In children and adolescents, ca. 4.4 % of rhabdomyosarcomas are of spindle cell-type [113].

Clinical aspects 50 % of the adult cases occur in the head and neck region and present as a mass. Symptoms depend on site and involvement of adjacent structures [109, 115]. In children, they arise more rarely in this region [108, 113].

Macroscopy Tumors are firm and relatively well demarcated from surrounding tissues but not encapsulated. On the cut surface, a nodular pattern with a gray-white and whorled appearance is reported [113].

Microcopy Spindle cell rhabdomyosarcoma consists of bland spindle cells with scant, pale eosinophilic cytoplasm forming long intersecting fascicles. The cells possess small, oval to elongated nuclei with vesicular chromatin and inconspicuous nucleoli (Fig. 12.37). Scattered around in variable amounts are more polygonal rhabdomyoblasts with eccentric hyperchromatic nuclei and bright eosinophilic cytoplasm. The latter population, if present, is a significant clue of this tumor type. Mitotic figures are often easily identifiable. There are variable amounts of intervening collagen [109, 116].

Sclerosing rhabdomyosarcomas are composed of spindle-shaped or round/polygonal tumor cells containing a varying amount of eosinophilic cytoplasm and enlarged, atypical nuclei, which are either hyperchromatic with irregular borders or vesicular with small nucleoli. The tumors contain an abundant, hyalinizing matrix that surrounds and entraps tumors cells imparting a pseudovascular growth pattern (Fig. 12.38) [117].

Hybrid tumors with features of spindle cell rhabdomyosarcoma and sclerosing rhabdomyosarcomas are reported [109, 117].

Immunohistochemistry Desmin and myogenin are the most reliable markers. MSA and SMA are possible positive markers. Rarely keratins and EMA are expressed focally. Caldesmon, S100, and GFAP are negative [108].

Genetics Consistent genetic data are available for the pediatric group with occurrence of *NCOA2* rearrangement in ca. 30 % of the cases [116].

Differential diagnosis Alternative diagnostic possibilities are age dependent, leiomyosarcoma, myofibroblastic sarcoma, infantile fibrosarcoma, MPNST with rhabdomyosarcomatous differentiation (malignant Triton tumor), sclerosing epithelioid fibrosarcoma, (desmoplastic) melanoma, and sarcomatoid squamous cell carcinoma [109, 113, 118, 119]. Immunohistochemistry with positivity for desmin and especially myogenin is specific for all RMS types.

Treatment and prognosis Standard protocols for rhabdomyosarcoma including surgery, chemotherapy, and radiation are in use. Owing to the complicated anatomy of the head and neck region and the local aggressiveness of the tumor, it is often difficult to obtain adequate tumor-free surgical

margins, which hampers local control of disease [119]. Children have a favorable prognosis with 95 % survival at 5 years. In contrast, the outcome in adults is significantly worse, with a rate of recurrence and metastasis of approximately 40–50 % [108, 113, 114, 119].

12.5.2.4 Pleomorphic (Adult-Type) Rhabdomyosarcoma

Definition Pleomorphic rhabdomyosarcoma is a high-grade sarcoma with skeletal muscle differentiation [120].

Epidemiology This sarcoma of adulthood accounts for 1 % of all adult soft tissue sarcomas. When these tumors arise in children, which however is distinctly uncommon, embryonal foci will be found [108]. It is the most common subtype of rhabdomyosarcoma in patients aged 40 years and older [112, 117].

Clinical aspects The head and neck region is rarely involved [120]. The tumors develop almost exclusively in deep soft tissue with involvement of striated muscle [112, 120].

Macroscopy Tumors are circumscribed and often lobulated. The cut surface is often grey reddish [120].

Microscopy Neoplasms are composed of large interlacing spindle, polymorphic, and epithelioid cells containing irregular, hyperchromatic nuclei, and numerous mitoses. Cells with copious, eosinophilic cytoplasm reminiscent of rhabdomyoblasts are often identifiable (Fig. 12.39). The mitotic range is broad. Necrosis may be seen [108, 120].

Immunohistochemistry By definition, desmin and myogenin are positive in a variable proportion of cells with a range for nuclear myogenin expression from 1 to 60 % [108, 120]. SMA, h-caldesmon, keratin, EMA, and CD34 expression may also occur in pleomorphic rhabdomyosarcoma. Rarely MDM2 and CDK4 are expressed without amplification of the corresponding genes [120].

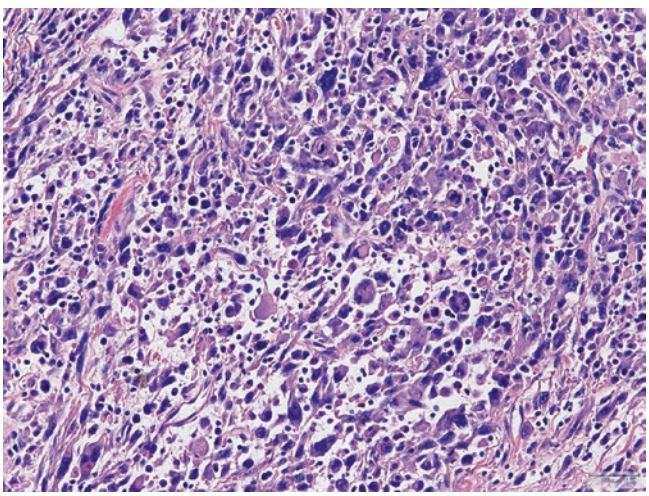

Fig. 12.39 Pleomorphic rhabdomyosarcomas are composed of polymorphic cells with hyperchromatic nuclei. Rhabdomyoblasts are often seen

Genetics Cytogenetic studies reveal a complex karyotype, with rearrangements and evidence of gene amplification offering no tools for distinction from other pleomorphic sarcomas [98, 109]. Gain of *MDM2* without amplification is described in few cases [120].

Differential diagnosis Expression of desmin together with myogenin rule out other pleomorphic sarcomas. Of note, dedifferentiated liposarcomas and rarely MPNST with heterologous rhabdomyoblastic differentiation are of differential diagnostic importance (see also Sects. 12.5.8.1 and 12.5.9) [108, 120].

Treatment and prognosis These tumors are very aggressive with a relative poor outcome [108, 120]. Surgery is the mainstay of management when it is feasible technically [112, 120]. In one study, the only predictive factor for metastasis was FNCLCC tumor grade [120].

12.5.2.5 Epithelioid Rhabdomyosarcoma

Definition Epithelioid rhabdomyosarcoma is a morphologically distinct variant or subtype of rhabdomyosarcoma, which has been described recently [121, 122].

Epidemiology It originates in adults and children [121, 122].

Clinical aspects Lesions may occur anywhere in the head and neck region [121, 122]. The neoplasms are mostly located in the deep soft tissue. Lymph node metastases and spread to the lung, liver, and bone are reported [121].

Macroscopy These are poorly circumscribed lesions having nodular fleshy cut surfaces [121].

Microscopy The tumors show infiltrative growth and arrangement in sheets or nests of relatively uniform large epithelioid cells with large vesicular nuclei, prominent nucleoli, and abundant amphophilic-to-eosinophilic cytoplasm (Fig. 12.40). Rhabdoid cytoplasmic inclusions are sometimes present. High mitotic counts and tumor necrosis are often observed [121, 122].

Immunohistochemistry There is a strong and diffuse desmin immunoreactivity. Myogenin shows a diffuse-to-multifocal staining pattern. Keratin, EMA, and PLAP expression is rare [121, 122].

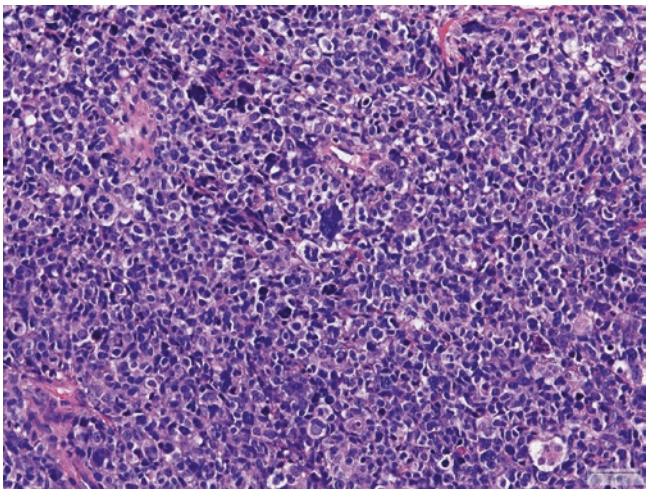

Fig. 12.40 Epithelioid rhabdomyosarcoma shows sheets of large epithelioid cells resembling carcinoma or melanoma

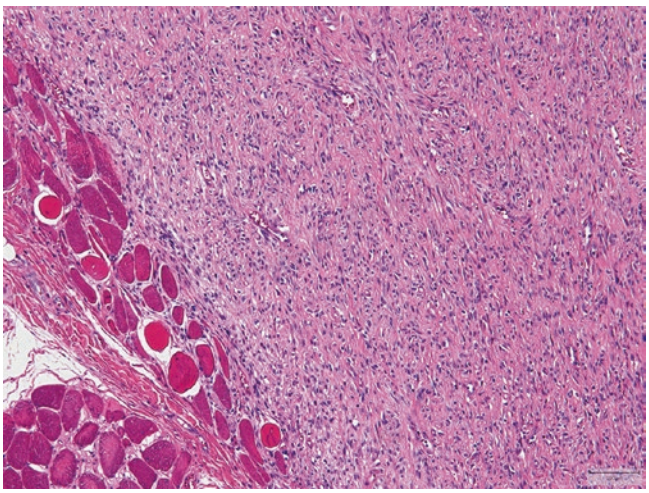

Fig. 12.41 Low-grade myofibroblastic sarcoma with a fibromatosis-like appearance and checkerboard infiltration of the voluntary muscle. Note the slight nuclear atypia

Genetics Currently, no genetic data are detected other than the finding that the tumors lack the typical transcripts of ARMS [121, 122].

Differential diagnosis The tumors morphologically closely resemble carcinoma or melanoma [121]. Appropriate immunohistochemistry will rule out these alternative possibilities.

Treatment and prognosis In adults, epithelioid RMS show aggressive behavior, regardless of treatment modalities and surgical margin status. A favorable clinical course is documented in children after neoadjuvant chemotherapy and resection [122].

12.5.3 Low-Grade Myofibroblastic Sarcoma

Definition Low-grade myofibroblastic sarcoma is an infiltrative tumor of the deep soft tissue with a predilection for the head and neck. It displays a range of microscopic appearances from fasciitis- or fibromatosis-like to fibrosarcoma-like [123, 124].

Epidemiology They occur at any age with a mean of 40 years [123].

Clinical aspects The oral cavity including the tongue is a preferred site. Origin in the bone, notably the maxilla and mandibular bone, is possible. Low-grade myofibroblastic sarcoma can arise subcutaneously or in a submucosal localization, but is, in the majority of cases, deep seated [123, 125].

Macroscopy The lesions are firm with fibrous cut surfaces and usually ill-defined margins [124].

Microscopy The usual pattern is of a rather cellular fibromatosis-like or fibrosarcoma-like lesion composed of fascicles or broad sheets of cells, with or without focal herringbone or storiform arrangement. Checkerboard-like infiltration of the adjacent voluntary muscle is a key diagnostic feature. Furthermore, the tapered myofibroblastic nuclei are atypical and show hyperchromasia. Mitotic figures are variably present. There are scant or moderate amounts of cytoplasm. The background can be collagenous or myxoid (Fig. 12.41). Transformation into high-grade sarcomas has been reported [123–125].

Immunohistochemistry Variable expression of SMA, desmin, calponin, and CD34 is typical for a myofibroblastic phenotype [124]. Expression of β-catenin does not rule out this tumor type [126].

Differential diagnosis Differential diagnoses encompass nodular fasciitis, desmoid-type fibromatosis, and leiomyosarcoma. Nodular fasciitis shows a cell culture-like appearance and no atypical nuclei of the more plump myofibroblasts. In desmoid-type fibromatosis, the nuclei are monomorphous without atypia. Leiomyosarcomas possess cigar-shaped nuclei and express more prominent smooth muscle markers, especially desmin and caldesmon.

Treatment and prognosis Excision is the treatment of choice. Local recurrences are common but metastatic spread occurs rarely [124].

12.5.4 Epithelioid Hemangioendothelioma (EHE)

Definition EHE is a translocation-associated malignant vascular neoplasm with indolent behavior in the majority of cases. Although a progressive clinical course with tumor-related fatality has been documented in a small proportion of cases, this tumor does not behave as aggressively as a conventional angiosarcoma [127–129].

Epidemiology There is a wide age distribution with children being rarely affected [127, 130].

Clinical aspects EHE may arise at any site including the head and neck region. Bone may be involved, primarily and secondarily. There is a propensity for lymph node metastases. Very rarely, a lymph node can be a primary site [131].

Macroscopy The (multi)nodular mass typically shows a pale solid cut surface, sometimes with some hemorrhage [130].

Microscopy Classically, the epithelioid and histiocytoid-appearing endothelial cells are arranged in short cords and strands in a myxohyaline stroma. They typically show subtle intracytoplasmic lumina and an abundant hyaline cytoplasm (Fig. 12.42). Striking nuclear atypia is seen in approximately 30% of the cases. Mitotic activity is usually low. Multicellular vascular channels are present in individual cases [127–129, 131].

Immunohistochemistry Vascular markers are expressed with CD31, ERG, and FLI1 being the most sensitive. Keratin expression is seen in ca. 30% of the cases, which can be confused with carcinomas or myoepithelial tumors [128, 131].

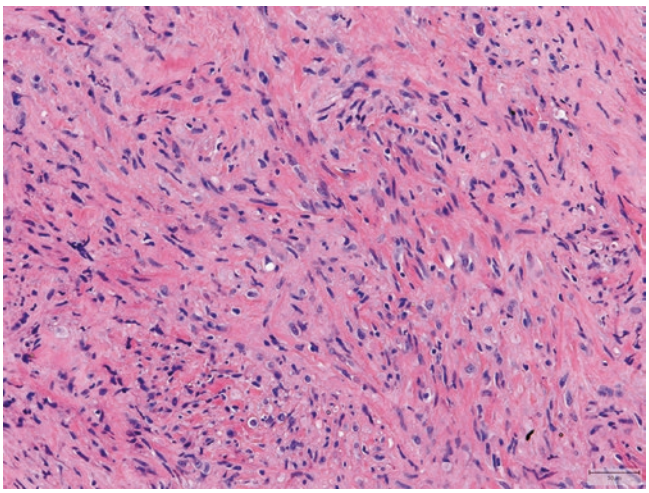

Fig. 12.42 The epithelioid- and histiocytoid-appearing cells of epithelioid hemangioendothelioma are arranged in short cords and strands in a myxohyaline stroma. There are subtle intracytoplasmic lumina. Note the hyaline cytoplasm

Genetics The fusion gene *WWTR1-CAMTA1* has been detected in the majority of cases, whereas a small subset harbors a *YAP1-TFE3* fusion [131–133].

Differential diagnosis Differential diagnosis includes epithelioid hemangioma, pseudomyogenic (epithelioid sarcoma-like) hemangioendothelioma, carcinoma, and myoepithelial tumors [131]. The specific fusion genes may help to distinguish EHE from other vascular tumors. Carcinoma and myoepithelial tumors do not express vascular markers.

Treatment and prognosis Wide excision is the treatment of choice. The majority of cases behave indolent. However, aggressive behavior has been documented in a small proportion of cases. In 2008, a proposal for risk stratification was made showing that higher mitotic activity (>3/50 HPF) and tumor size exceeding 3 cm are associated with higher mortality, irrespective of anatomic site and presence of cytologic atypia, tumor cell spindling, or necrosis [129].

12.5.5 Low-Grade Fibromyxoid Sarcoma (LGFMS)

Definition LGFMS is a translocation-associated fibroblastic neoplasm characterized by deceptively bland spindle cells arranged in an alternating myxoid and fibrous stroma.

Epidemiology Young adults are mainly affected but the age range is wide including children [54, 134].

Clinical aspects Tumors typically arise in the deep soft tissue of the proximal extremities or trunk and less often in the head and neck region [134, 135]. Superficial LGFMS comprise ca. up to 20% of the cases [54].

Macroscopy Neoplasms are typically well circumscribed with a fibrous to myxoid cut surface [54, 134, 135].

Microscopy These tumors are characterized by contrasting fibrous and myxoid areas of varying size, a swirling, whorled growth pattern, low to moderate cellularity, and bland, deceptively benign-appearing fibroblastic spindle cells with no or slight nuclear pleomorphism and few mitotic figures (Figs. 12.43 and 12.44). Typically, there are arcades of small blood vessels in the myxoid areas [134, 135]. In some cases, hyaline rosettes surrounded by a palisade of round to oval tumor cells are present. Such cases were formerly called hyalinizing spindle cell tumor with giant rosettes [136]. Cellular examples often contain cells with epithelioid/polygonal morphology showing overlap with sclerosing epithelioid fibrosarcoma (SEF) (Fig. 12.45) [137]. A shift of the prevailing morphologic pattern from LGFMS to SEF in local recur-

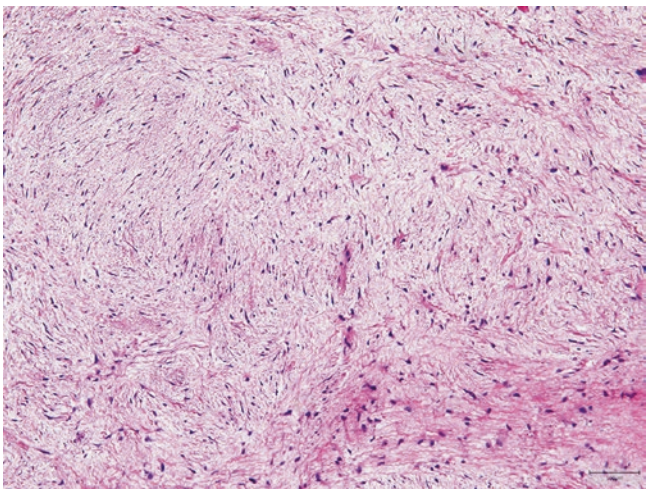

Fig. 12.43 Myxoid area of a low-grade fibromyxoid sarcoma. Note the deceptive bland cytomorphology

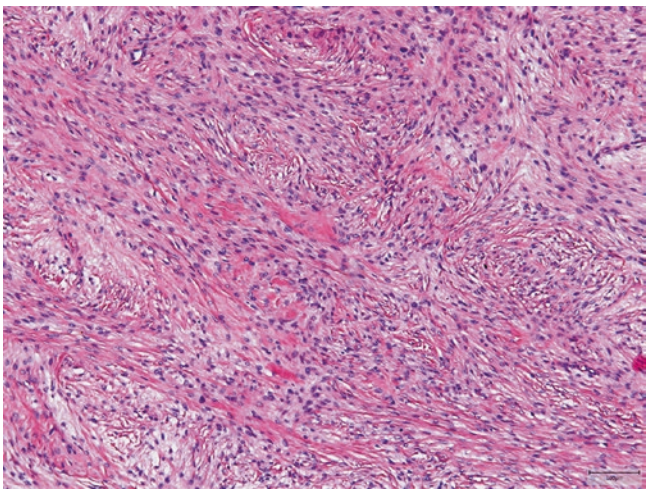

Fig. 12.44 Fibrous more cellular area of a low-grade fibromyxoid sarcoma

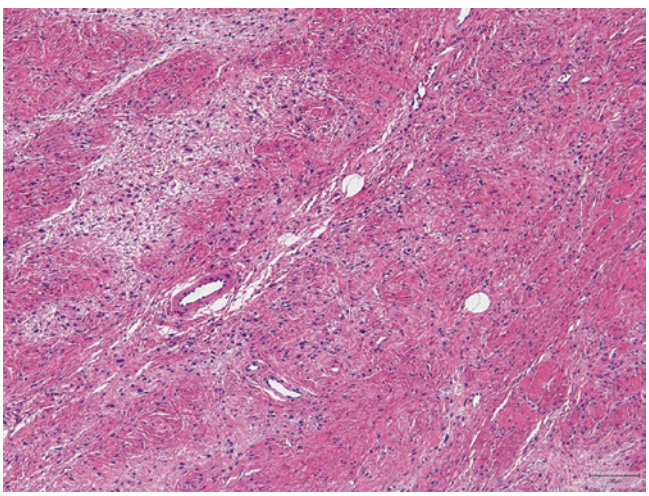

Fig. 12.45 More cellular area of a low-grade fibromyxoid sarcoma with epithelioid morphology reminiscent of sclerosing epithelioid fibrosarcoma

rences/metastases has been described in the literature [134, 137, 138]. Other growth pattern variations included storiform, fascicular-herringbone, and patternless [134].

Immunohistochemistry MUC4, a very specific marker for LGFMS, is negative in exceptional cases [139]. EMA is focally expressed in up to 90 % and claudin-1 in up to 100 % of the cases [63, 137]; individual cases show SMA and CD34 positivity [54, 137].

Genetics A recurrent t(7;16)(q32-34;p11) translocation results in *FUS-CREB3L2* fusion in the majority of cases. Rare cases disclose a *FUS-CREB3L1* fusion [140]. *FUS* can exchange with *EWSR1* in individual cases [141].

Differential diagnosis The tumor has to be distinguished from desmoid-type fibromatosis, ossifying fibromyxoid tumor, neurofibroma, fibroma nuchae, cellular myxoma, and myxoid dermatofibrosarcoma protuberans (DFSP) (see also Chap. 15 that describes skin lesions) [79, 135, 142]. In addition to differences in histomorphology, none of these entities shows positivity for MUC4 [139].

Treatment and prognosis Wide excision is the treatment of choice. LGFMS is an indolent tumor showing a prolonged clinical course with often late recurrences (ca. 60 % of the cases) and late metastases (ca. 50 % of the cases). (Multiple) Recurrences are often associated with positive margins [134].

12.5.6 Sclerosing Epithelioid Fibrosarcoma (SEF)

Definition SEF is an uncommon, often aggressive sarcoma of fibroblastic type with a subset of cases being related to LGFMS, so-called hybrid tumors [137, 143, 144].

Epidemiology SEF occurs in an older age group (middle-aged adults) compared with LGFMS [134].

Clinical aspects SEF rarely develop in the head and neck [142–144]. These infiltrative lesions can invade the bone/cranium and intracranial contents [143]. Reported sites of metastases are the lung, bone and soft tissue, breast, pericardium, brain, and lymph nodes [142, 143].

Macroscopy Tumors are (multi)nodular with a gray-white whorled cut surface and a firm to elastic consistency [142, 143].

Microscopy Epithelioid/polygonal tumor cells are arranged in single files, cords, nests, or sheets, situated within a densely sclerotic collagenous background. An alveolar or pseudovascular appearance is sometimes seen. Nuclei are round to oval.

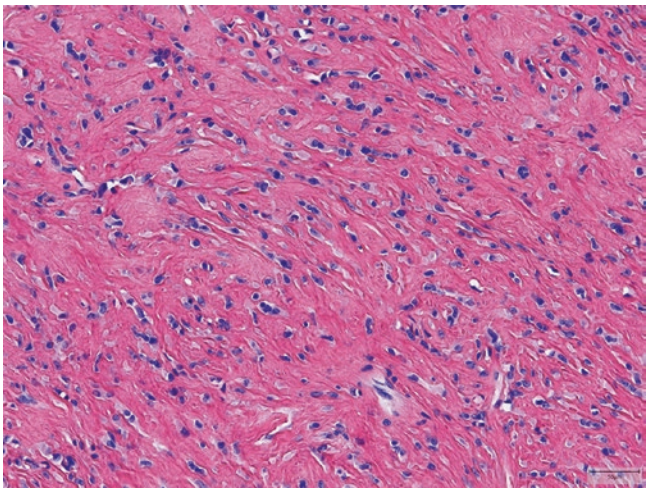

Fig. 12.46 Epithelioid tumor cells of sclerosing epithelioid fibrosarcoma are arranged in single files and cords set in a densely sclerotic collagenous background

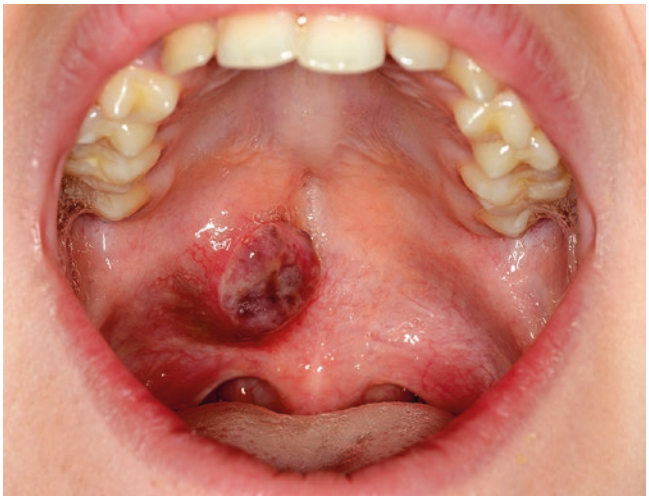

Fig. 12.47 Ulcerating swelling at the soft palate due to synovial sarcoma

The mitotic count is variable. There is an eosinophilic to clear cytoplasm. The latter is probably due to cellular shrinkage leaving a space between cellular contents and the encasing collagen (Fig. 12.46). Fibroma-like or LGFMS-like areas may be present (hybrid tumors) [137, 143].

Immunohistochemistry MUC4 is positive in a subset of cases (up to 70%). MUC4 negative cases harbor usually no FUS rearrangement [138, 145]. EMA and S100 expression may be seen [142, 143]. Keratin positivity is exceptionally rare [142].

Genetics In contrast to the marked predominance of *FUS-CREB3L2* in LGFMS and hybrid LGFMS/SEF, the genetics of SEF seems more heterogeneous [138]. A subset of MUC4-positive tumors is *FUS* related [139]. However, it seems that more than a half of the pure SEF harbor a *EWSR1-CREB3L1* gene fusion [138].

Differential diagnosis Differential diagnoses are ossifying fibromyxoid tumor, carcinoma (lobular or signet ring), or, less commonly, a sclerosing lymphoma. Other alternatives are synovial sarcoma, extraskeletal osteogenic sarcoma, or clear cell sarcoma [79, 142, 143].

Treatment and prognosis Surgery is the treatment of choice. In contrast to LGFMS, patients with SEF have a higher rate of recurrences and metastases and show a much shorter survival [134, 143].

12.5.7 Synovial Sarcoma

Definition Translocation-associated sarcoma with either monophasic spindle cell or biphasic appearance (spindle cell areas with alternating epithelioid structures).

Epidemiology Synovial sarcoma is most prevalent in adolescents and young adults 15–35 years of age [146]. However, there is a wide age range from infancy to the elderly [147].

Clinical aspects The head and neck region is outnumbered by far by extremities and the trunk [147]. Most tumors are located in the deep soft tissue. Organ-related tumors are reported in the tonsil, thyroid gland, salivary gland, and others (e.g., palate; Fig. 12.47) [148–150]. Superficial tumors may arise in the dermis [147]. Tumors can present as a painless mass or can cause site-specific symptoms [147].

Macroscopy The nodular or lobulated mass has often infiltrative borders. The cut surface is firm and pink or grey (Fig. 12.48) and may be focally mucoid, necrotic, or hemorrhagic, with occasional cyst formation. Calcification may be extensive. The size varies from <1 cm to very large. Lymph nodes should be evaluated, if present in the specimen, for metastases [147]. However, spread to lymph nodes accounts for <1 % of patients [151].

Microscopy At low power, hypercellular and hypocellular zones may be seen. Monophasic tumors show a fascicular growth pattern of monotonous spindle cells with oval to elongated nuclei and scant amphophilic cytoplasm. The mitotic rate varies. The background can be collagenous/keloidal (Fig. 12.49), sometimes with calcification and/or metaplastic ossification. Hemangiopericytoma-like vessels are sometimes obvious. A variable mast cell infiltrate is present. In case of a myxoid stroma, spindle cells display a reticular, lacy, or fascicular arrangement [147]. Biphasic tumors comprise spindle cell and epithelioid cell areas. The latter consist of glands or clusters of rounded, cuboidal, or columnar cells (Fig. 12.50). Papillary structures, whorls, or anastomosing strands are also present [147]. Poorly differentiated

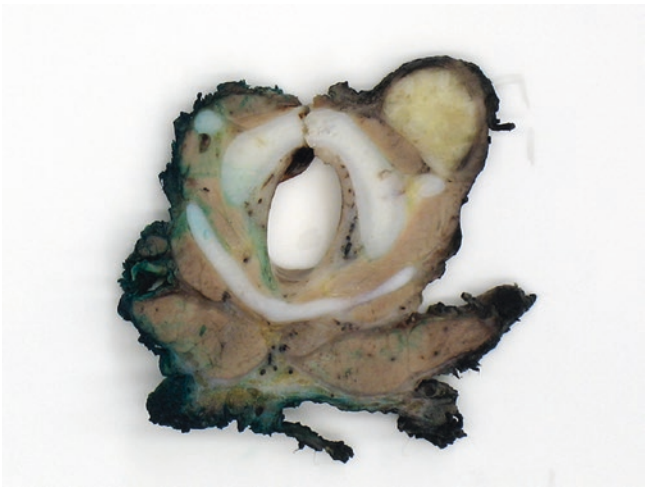

Fig. 12.48 Paralaryngeal localized synovial sarcoma appearing grossly as a circumscribed nodular white to yellowish nodus

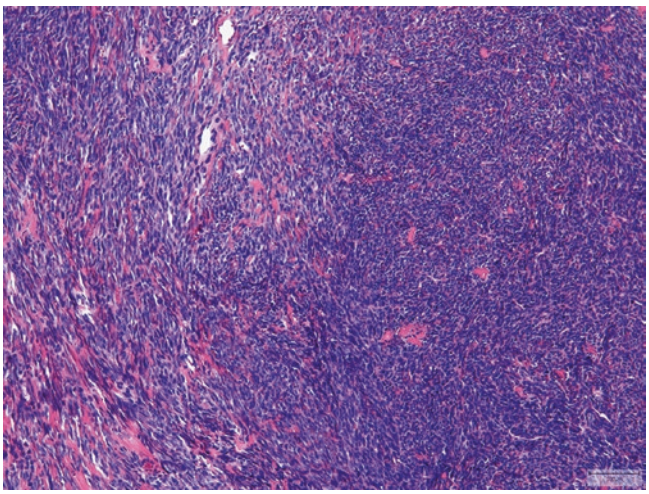

Fig. 12.49 Monophasic synovial sarcomas show a fascicular growth pattern of monotonous spindle cells

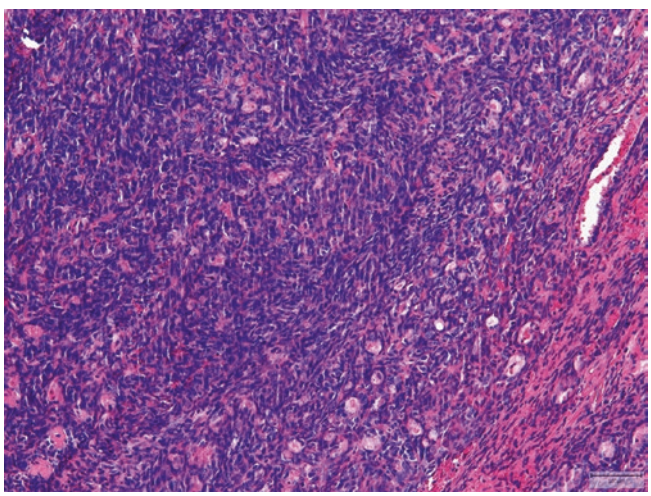

Fig. 12.50 Biphasic synovial sarcomas comprise spindle cells and epithelioid areas. The latter consist of glands or clusters of rounded cells

tumors encompass an Ewing-like round cell proliferation, a highly cellular spindle cell proliferation, or plump tumor cells with nuclear atypia [147].

Immunohistochemistry Cytokeratins, EMA, and bcl-2 are commonly positive. In addition to the broad-spectrum cytokeratins AE1/3 and CAM5.2, keratins 7, 13, and 19 are relatively specific. TLE1 is a sensitive marker without high specificity and can especially be positive in the most important differential diagnosis of MPNST. CD99, CD57, CD56, S100, neurofilament, and other markers are potential diagnostic pitfalls because they can be found in MPNST and Ewing sarcoma too. Positivity for calponin, SMA, and MSA is exceptionally rare, and desmin and caldesmon are negative [147].

Genetics The balanced reciprocal translocation t(X;18)(p11.2;q11.2) results in a fusion of *SYT-SSX1* or *SYT-SSX2*. Other exceptional fusions are *SYT-SSX4* or *SS18L1-SSX1* [147].

Differential diagnosis Monophasic tumors can imitate MPNST, solitary fibrous tumors, sinonasal hemangiopericytoma, and other spindle cell sarcomas. In myxoid tumors perineurioma is an important differential diagnosis. Biphasic lesions can resemble myoepithelial tumors and MPNST with glandular differentiation or adenocarcinomas. Molecular genetic tests discriminate synovial sarcoma.

Treatment and prognosis This aggressive tumor is characterized by a high rate of local and metastatic recurrences occurring in 40–50 % of patients usually within 2 years after the initial diagnosis. Wide surgical excision completed by radiotherapy in cases of large tumors or invaded margins is the standard for the locoregional treatment [146]. When the FNCLCC grading systems are applied, patients with grade 2 do significantly better than those with grade 3 tumors [152].

12.5.8 Liposarcoma (LS)

Liposarcoma is one of the most common malignant soft tissue tumors in adult patients, accounting for up to 20 % of adult sarcomas [153–155]. In contrast, liposarcomas are extremely unusual in children [154]. There are three biologically distinct categories: well-differentiated/dedifferentiated LS, myxoid LS, and pleomorphic LS [155]. In adult patients, atypical lipomatous tumors (well-differentiated liposarcomas)/dedifferentiated liposarcomas account for approximately 60 % of cases and occur at a median of 60 years of age. Myxoid/round cell liposarcomas comprise approximately 35 % of cases and occur at a median age of 45 years, and pleomorphic liposarcoma, the most rare subtype, constitutes only 5 % of cases, with a median of 60 years of age [154, 156]. Head and neck liposarcoma is markedly less common than in other body sites, comprising only 2–9 %

of sarcomas found in this region [1, 157]. Most of them occur within the subcutaneous connective tissues of the face, neck, and scalp (80 %), with the oral cavity (4 %), skin (4 %), salivary glands (4 %), and a collection of sites throughout the upper aerodigestive tract (5 %) comprising the remaining cases [157].

12.5.8.1 Atypical Lipomatous Tumor/Well-Differentiated Liposarcoma and Dedifferentiated Liposarcoma

Definition Atypical lipomatous tumors/well-differentiated and dedifferentiated liposarcomas form a continuum. Dedifferentiation occurs in up to 10 % of well-differentiated LS and is defined as the transition to a nonlipogenic sarcoma [155, 158].

Epidemiology They develop rarely in the head and neck region [155, 158].

Etiology and pathogenesis *MDM2* and *CDK4* amplification plays an essential role (see genetics) [155, 159].

Clinical aspects Reported localizations are the head (scalp and face), pharynx, mouth, larynx, and neck [1]. The tongue is a common location for intraoral cases [3]. A commonly reported symptom is that of a slowly growing, painless mass, sometimes present already for a period of time [158].

Macroscopy Tumors can be large; they are mostly well-circumscribed fatty nodules. Multinodularity and gelatinous consistency can be present [158].

Microscopy Lipoma-like subtype shows variation in adipocyte size with hyperchromatic, enlarged nuclei. In the often irregular fibrous septa and in the vessel walls, there are atypical stromal cells (Fig. 12.51). Lipoblasts are not a requirement for this tumor type [158]. Dedifferentiated nonlipogenic areas may show a wide variety of growth patterns and cytomorphology (e.g., spindle cell, pleomorphic, giant cell, round cell, or meningothelial-like) (Fig. 12.52). The transition to dedifferentiated areas usually occurs abruptly, although in some cases this can be more gradual and, exceptionally, well-differentiated and dedifferentiated areas are comingled [155]. Heterologous elements are found in up to 10 % of cases (cartilage, bone, rhabdomyoblasts, angio(sarco)matous elements) [155].

Immunohistochemistry MDM2 and CDK4 are useful immunohistochemical markers with positivity in more than 90 % of the cases [155, 160]. Even though MDM2 immunostaining is more sensitive, CDK4 is more specific. It is therefore recommended to perform both stainings for a given tumor [160]. One study recommends the diagnostic utility of the panel p16, CDK4, and MDM2 to distinguish well-differentiated/dedifferentiated LS from other adipocytic tumors [13].

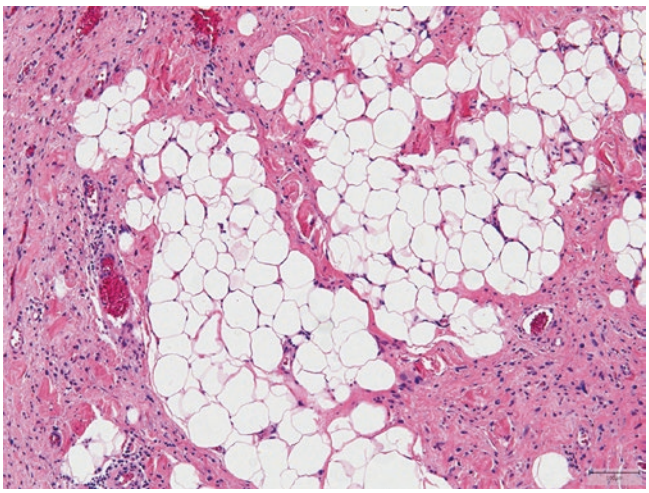

Fig. 12.51 Lipoma-like/well-differentiated liposarcoma shows variation in adipocytic size with hyperchromatic, enlarged nuclei. There are atypical stromal cells in the fibrous septa

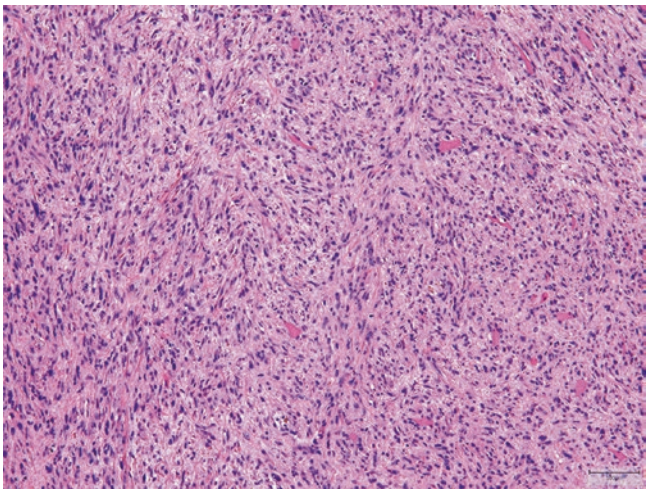

Fig. 12.52 Dedifferentiated liposarcoma showing short bundles of atypical spindle cells

Genetics Well-differentiated/dedifferentiated LS show by definition 12q13-15 amplification including constant amplification of *MDM2* and frequent amplification of *CDK4* [160].

Differential diagnosis Lipoma with reactive changes may imitate atypia. Of note, rare cases show MDM2 and/or CDK4 expression; however, there is no amplification of the corresponding gene [12, 13, 160]. Dedifferentiated liposarcoma should be separated from other poorly differentiated sarcomas by the presence of well-differentiated areas and/or immunohistochemistry and molecular analysis as described above [155, 160].

Treatment and prognosis Surgery is the treatment of choice. Multiple recurrences in lipoma-like/well-differentiated lesions may occur with possible dedifferentiation. Tumor site appears to have some influence on prognosis. Laryngeal and head liposarcomas have the best outcome, possibly due to early recognition. Oral liposarcoma is associated with a poor outcome despite the high proportion of low-grade tumors [1].

12.5.8.2 Myxoid/Round Cell Liposarcoma (MLS)

Definition This translocation-associated liposarcoma is the most common subtype in children, adolescents, and young adults [154].

Epidemiology MLS comprise up to 35 % of liposarcomas. Patients are younger (mean 45 years of age) than for other liposarcoma subtypes [154].

Clinical aspects Tumors present commonly as a painless mass in the soft tissue of the head and neck. The mouth, pharynx, and larynx are also reported sites [154, 157]. One-third of the patients develop distant metastases often arising in soft tissue, bone, and lung. Metastases can even be synchronous [161].

Macroscopy Lesions are mostly well circumscribed and (multi)nodular with a gelatinous to firm cut surface [7].

Microscopy Myxoid liposarcoma are infiltrating lesions showing a multinodular appearance and a prominent myxoid matrix with pools of mucin. They consist of small uniform primitive cells and lipoblasts in different stages of maturation. There is a peripheral condensation of cells within the nodules. A delicate chickenwire-like capillary network is very typical (Fig. 12.53). High-grade tumors are characterized by sheets of primitive round cells without intervening myxoid stroma [7, 154, 161].

Immunohistochemistry S100 is variably positive in these neoplasms [161].

Genetics *FUS-DDIT3* and alternatively *EWSR1-DDIT3* are the consistent fusion genes [154, 161, 162].

Differential diagnosis The main alternative diagnoses are (spindle cell) lipoma with myxoid changes or well-differentiated/dedifferentiated liposarcoma with a prominent myxoid background. Classical features in these tumors will help to distinguish them from MLS (see Sect. 12.2.1). In contrast to lipoblastoma, lobulation is incomplete, and age is a helpful finding because most of the lipoblastomas develop in the first 3 years of life [7]. Round cell liposarcomas resemble other small blue round cell tumors. The lipoblasts, if present, the delicate capillary network, and the fusion genes are discriminating.

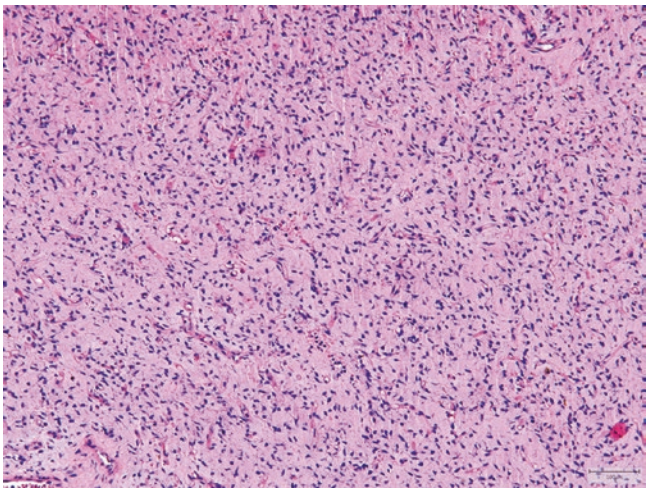

Fig. 12.53 Myxoid liposarcoma with small uniform primitive cells in a prominent myxoid matrix. As here, lipoblasts can be absent

Treatment and prognosis Combined radiotherapy and surgery is the treatment of choice, at least in difficult anatomic situations in which adequate surgical excision is not feasible [163]. In children, adolescents, and young adults, myxoid liposarcoma shows an excellent prognosis [154]. Low-grade lesions are associated with a metastatic risk of <10 %. High-grade lesions (>5 % round cell component) and necrosis are signs of unfavorable outcome [161].

12.5.8.3 Pleomorphic Liposarcoma

Definition Pleomorphic liposarcoma, the rarest and most aggressive liposarcoma subtype, is infrequently seen in the head and neck region. It is defined as a high-grade pleomorphic sarcoma with varying numbers of multivacuolated lipoblasts [153, 155, 156, 164].

Epidemiology Patients age ranges from 18 to 95 years with a mean age of approximately 60 years [153, 154, 156, 164].

Clinical aspects Most patients present with a rapid growing painless mass [153, 156].

Macroscopy Tumors are often (multi)nodular and well demarcated, but a number of cases show infiltrative margins. On sectioning, most tumors are whitish to brown yellow. Myxoid changes as well as foci of necrosis are occasionally observed [153, 164].

Microscopy The diagnosis of pleomorphic liposarcoma relies on the identification of lipoblasts (often pleomorphic) within a given high-grade sarcoma, which may be very scanty.

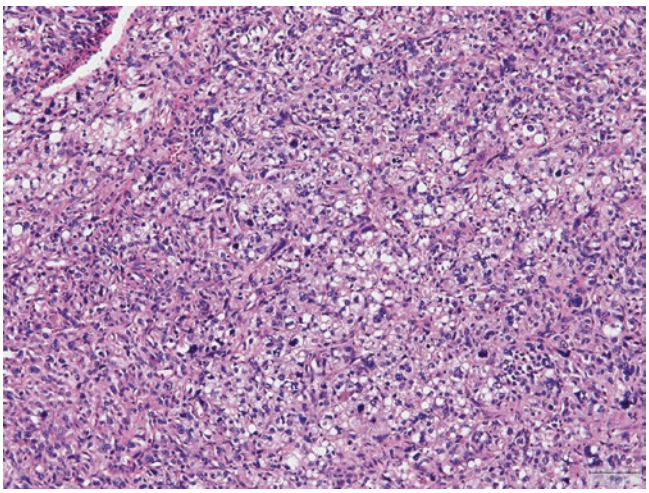

Fig. 12.54 The identification of lipoblasts within a given high-grade sarcoma is essential for the diagnosis of pleomorphic liposarcoma

Prominent adipocytic differentiation is reported in the minority of neoplasms (Fig. 12.54). There is a great morphological diversity, especially in the nonlipogenic component, including storiform-pleomorphic/myxofibrosarcoma-like, epithelioid/carcinoma-like, round cell liposarcoma-like (without a plexiform capillary network typical for myxoid/round cell liposarcoma), and spindle cell pattern often with fascicular arrangement (occasionally with floret-like cells). It is not rare to observe within the same lesion more than one morphological pattern. A frequent but aspecific finding is the presence of extracellular or intracellular hyaline droplets. A hemangiopericytoma-like vasculature can be prominent. Rarely, heterologous elements as osteoid, cartilage, or striated muscle differentiation are present [153, 156].

Immunohistochemistry There is an unspecific immunophenotype with variable expression of S100, SMA, desmin, CD34, and EMA. MDM2 and CDK4 are negative. Lipoblasts may be positive for S100, and epithelioid cells may express keratins and/or EMA, pondering the diagnosis of a carcinoma [153, 155, 156, 164].

Genetics At the cytogenetic level, pleomorphic LS is characterized by a complex karyotype with multiple chromosomal gains and losses that do not include the 12q13-15 region. Therefore, it differs from well-differentiated/dedifferentiated LS. The resulting gene expression profiles place pleomorphic LS closer to other high-grade sarcomas [155].

Differential diagnosis Dedifferentiated liposarcoma with pleomorphic liposarcoma-like morphology has to be differentiated from pleomorphic liposarcoma by the presence of MDM2/CDK4 expression or amplification of the corresponding genes. In case of epithelioid cytomorphology and expression of keratins, a carcinoma should be excluded by extensive sampling to find lipoblasts and by considering the clinical context. A melanoma is another diagnostic possibility when lipoblasts are not obvious [153, 156, 164].

Treatment and prognosis Oncological resection is the treatment of choice. Neoadjuvant and adjuvant chemotherapy and/or radiation therapy is occasionally administered [153]. Pleomorphic liposarcoma is a relatively aggressive tumor with a reported 5-year survival ranging from 30 to 60 % [156]. It exhibits consistently a more aggressive clinical behavior than dedifferentiated LS [155].

12.5.9 Malignant Peripheral Nerve Sheath Tumor (MPNST)

Definition MPNSTs are soft tissue neoplasms that usually arise from peripheral nerves and show variable differentiation toward one of the cellular components of the nerve sheath (Schwann cells, fibroblasts, and perineurial cells) [57].

Epidemiology They occur mainly in adults with a wide age range. NF1 patients tend to be younger. More rarely, MPNST develop during childhood [57].

Clinical aspects Tumors arise either de novo, commonly in a major nerve trunk, or from a preexisting neurofibroma or, rarely, schwannoma. Patients may present with a painful and/or rapidly enlarging mass with associated neurologic deficits [57].

Macroscopy Tumors may be within or attached to a nerve trunk or neurofibroma. They often have a fusiform appearance and can extend within a nerve. Lesions tend to be white, solid, and fleshy, sometimes with myxoid change and frequent necrosis and hemorrhage [57].

Microscopy MPNSTs are usually highly infiltrative lesions that display a varied range of cell morphologies (including spindle, epithelioid, pleomorphic, or small round cell). Frequent growth patterns include a “marbled” effect with alternating cellular and myxoid areas, perivascular cuffs, poorly defined nuclear palisading, and neuroid whorls. Rosette-like appearance is less common. Tumors may harbor a variety of patterns within the same lesion, including pleomorphic or small cell areas. Spindle cell MPNSTs are often arranged in long fascicles or a herringbone fashion. The cells have elongated, tapered, buckled, or wavy nuclei and scanty amphophilic cytoplasm. The nuclei may be hyperchromatic or vesicular, the latter with coarse chromatin. Mitoses, hemorrhage, and necrosis are frequent (Figs. 12.55 and 12.56). Heterologous elements are rarely seen (osteoid, cartilage, striated muscle and glandular structures, or angiosarcoma) [57].

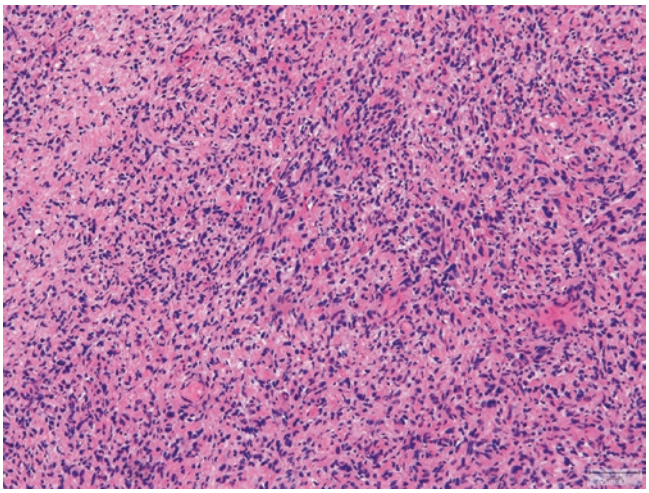

Fig. 12.55 Malignant peripheral nerve sheath tumor with short fascicles of pleomorphic tumor cells

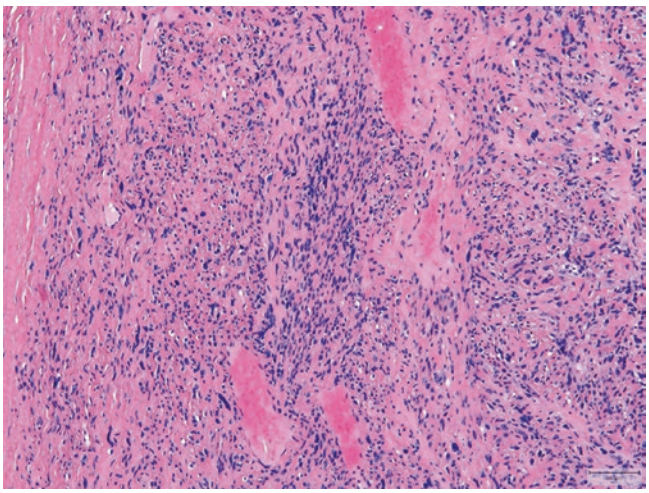

Fig. 12.56 Atypical neuroid spindle cells arranged in fascicles in a malignant peripheral nerve sheath tumor

Immunohistochemistry There is no diagnostic immunoprofile. Epithelioid MPNSTs show strong S100 expression in comparison to spindle cell MPNST being positive in scattered cells in ca. 60 % of the lesions. SOX10 is found in up to 50 % of MPNST, while melanomas are almost always positive. Nestin shows strong cytoplasmic staining and is useful in combination with the other markers. Cytokeratins, EMA, and CD34 may be positive [57].

Genetics The most frequent gene alterations include loss of *NF1* on17q11 and of *p53* on 17q13, and these can include inactivation of the *NF1* tumor suppressor gene, both in sporadic cases and NF1 patients [57].

Differential diagnosis Both spindle cell melanoma and cellular schwannoma show strong S100 reactivity [31]. Other differential diagnoses are cellular neurofibroma (does not show mitotic figures), low-grade fibromyxoid sarcoma, low-grade myofibroblastic sarcoma, and synovial sarcoma (see Sects. 12.5.3, 12.5.5, and 12.5.7) [57].

Treatment and prognosis MPNSTs are aggressive tumors. Radical surgery continues to be the mainstay of current management, as these tumors have limited sensitivity to chemotherapy and radiation. Poorer prognosis is associated with large tumors (>5 cm) and those associated with NF1 as well as those of higher grade and with truncal location. Other unfavorable features include a mitotic index of greater than 6/10 high-power fields and incomplete resection. The recurrence rate is up to 40 %, and approximately two-thirds of the cases metastasize, usually hematogenously to the lungs and bone. Five-year survival has varied in series from 26 to 60 %, and 10-year survival is approximately 45 %. There are currently no effective targeted therapies [57].

12.5.10 Alveolar Soft Part Sarcoma (ASPS)

Definition ASPS is a very rare, morphologically distinct translocation-associated sarcoma [165].

Epidemiology Young adults are mainly affected [165]. Head and neck tumors, especially of the orbit and the tongue, arise often in children [166–168].

Clinical aspects The head and neck region may be involved, primarily or as metastatic site [169]. Tumors are described as erythematous mass or raised red papule. Several cases are clinically misinterpreted as hemangioma [167]. Metastases can occur very early (synchronous) and also very late. Metastatic sites include the lymph nodes, lung, bone, liver, and brain [169].

Macroscopy ASPS is multinodular circumscribed tumors with a fleshy gray-red appearance [167].

Microscopy ASPS is characterized by uniform nests of polygonal tumor cells separated by fibrovascular septa with delicate capillary-sized vascular channels. In these nests there is prominent cellular dyscohesion, leading to a distinctive pseudoalveolar pattern (Fig. 12.57). A solid (non-alveolar) appearance occurs in individual cases. Intravascular tumor extension is often seen [165, 167]. The lesional cells have distinct borders and abundant eosinophilic to clear, somewhat granular cytoplasm, resulting in an epithelioid appearance. PAS-positive, diastase-resistant crystals are a typical finding which are also detectable with Alcian Blue or trichrome stains. The nuclei are round and typically eccentrically placed. They possess vesicular chromatin and

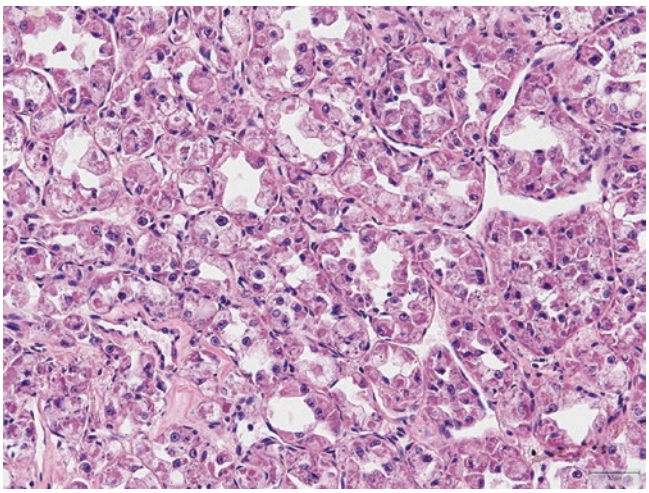

Fig. 12.57 Typical example of an alveolar soft part sarcoma. Note the distinctive pseudoalveolar pattern of large polygonal cells

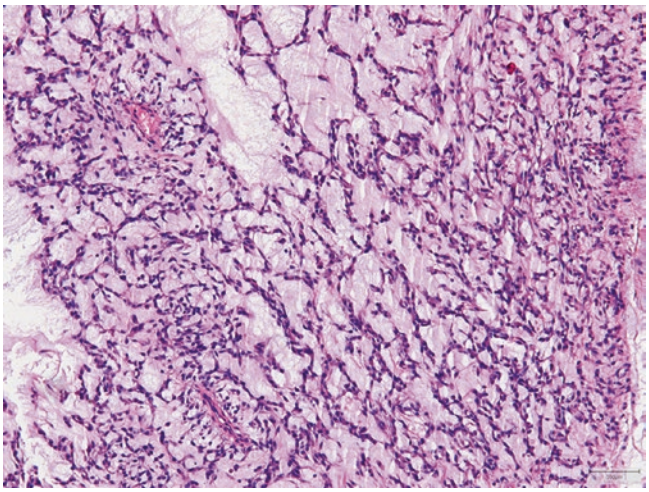

Fig. 12.58 Extraskeletal myxoid chondrosarcoma with bland-looking small round to spindled cells set in a myxoid matrix resulting in a lace-like or reticular appearance

a prominent nucleolus. Multinucleation is a relative frequent finding. In some cases, marked variation in nuclear size or cell spindling has been reported. Myxoid or pseudocystic changes, hemorrhage, and a prominent inflammatory infiltrate are present in the minority of cases [165].

Immunohistochemistry TFE3 shows nuclear expression using an antibody that binds to the C-terminal portion [170]. SMA is frequently expressed, and desmin positivity has been reported in around 50 % of ASPS, although they tend to be present in a small number of the neoplastic cells. Staining for S100 can be positive, but in contrast to PEComa, HMB is negative [171].

Genetics *ASPL-TFE3* is a robust diagnostic marker [171].

Differential diagnosis Paraganglioma, adult-type rhabdomyoma, granular cell tumor, acinus cell adenocarcinoma, metastatic renal cell carcinoma, PEComa, and melanoma can be distinguished with appropriate immunohistochemical markers and/or the detection of the mentioned fusion gene.

Treatment and prognosis Surgery still offers the best chance to control the disease [166]. Overall survival is around 70 % [168]. A poorer prognosis is seen in patients with metastatic disease [165]. Response to sunitinib malate has been reported recently in advanced disease [172].

12.5.11 Extraskeletal Myxoid Chondrosarcoma (EMC)

Definition EMC is a translocation-associated rare soft tissue sarcoma, which infrequently arises in the neck [173–175].

Epidemiology Adults are mainly affected [173–176].

Clinical aspects This is a mostly deep located soft tissue tumor. Rare cases have been reported in the neck and intracranial cavity [173–176]. They present often as a painless slowly growing mass [173].

Macroscopy Tumors show a (multi)nodular configuration with relatively well-defined margins and an incomplete fibrous pseudocapsule. The cut surface is gray to tan brown and shows a gelatinous appearance, often accompanied by intralesional hemorrhage [176].

Microscopy EMC is commonly a hypocellular lesion characterized by a multinodular growth pattern. The tumor nodules show peripheral accentuation of cellularity and are composed of bland-looking small round to spindled cells with scanty eosinophilic cytoplasm set in a myxoid matrix resulting in a lacelike or reticular appearance (Fig. 12.58) [173, 176]. The monotonous nuclei are round to oval with hyperchromasia or vesicular chromatin. Mitotic activity is usually low. Cellular areas (devoid of a myxoid matrix) may be present in primary and recurrent lesions sometimes showing pleomorphic cells displaying an epithelioid, rhabdoid, or spindle cell phenotype [176].

Immunohistochemistry EMCs are, often focally, positive for S100. GFAP, EMA, ASMA, keratins, and p63 are expressed in the minority of cases, mostly with a focal staining pattern [176, 177].

Genetics EMCs are defined by specific reciprocal translocations, involving obligatory *NR4A3*. The described fusion partners in decreasing frequency are *EWSR1*, *TAF15*, *TCF12*, and *TGF* [176, 177].

Differential diagnosis The most important differential diagnosis are myoepithelial tumors of soft tissue [177]. They show morphological, immunophenotypical, and genetic overlap with rearrangement of *EWSR1* in a subset of cases [177].

Treatment and prognosis Surgery is the treatment of choice. EMC shows a protracted clinical course with a high metastatic potential. In one study from two referral centers, the overall survival at 5, 10, and 15 years were 82 %, 65 %, and 58 %, respectively [178].

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Benign and Malignant Lymphoid Lesions of the Head and Neck

13

Daniel Martinez, Lluis Colomo, Davide Soldini, and Elias Campo

Contents

13.1 Introduction

The reactive and malignant lesions of the lymphoid system are a broad and heterogeneous group of diseases that usually require a multidisciplinary approach for their diagnosis integrating clinical information together with morphology, phenotypic and molecular studies. The relevance of these studies in the final diagnosis may vary in different lesions. In most cases morphology will orient the diagnosis that will be confirmed by ancillary studies. The precise diagnosis of infectious disease will require the identification of the causal agent by serologic, tissue microbiological or molecular studies. In some immunological disorders such as Kikuchi's lymphadenopathy, the morphological appearance may be undistinguishable of the systemic lupus erythematosus lymphadenopathy, and only clinical and biological criteria will establish the differential diagnosis. In most neoplastic diseases, the cell lineage and immunophenotypic profile will define the diagnosis, but in certain cases, specific findings are essential for the diagnosis. For instance, in the extranodal NK/T lymphoma, nasal type, Epstein-Barr virus needs to be demonstrated.

The diversity of these lesions is due to several factors that include the complexity of the cell composition and regulatory networks of the normal immune system, the different etiological factors triggering these processes, the influence of different topographic microenvironments and the individual genetic background of the patients that may modulate many of these interactions. Most of the lymphoid lesions involving the head and neck region are similar to those occurring in other topographic sites and may represent the local involvement of a systemic process. However, there are a number of benign and malignant disorders that preferentially develop in the lymph nodes or extranodal lymphoid tissues of these regions. For instance, toxoplasmic lymphadenitis, Kikuchi's disease and Rosai-Dorfman lymphadenopathy, although may be detected in any topographic site, are frequently diagnosed in cervical lymph nodes. Similarly, extranodal NK/T lymphoma, nasal type or plasmablastic

D. Martinez, MD (✉) • E. Campo, MD, PhD
Hematopathology Unit, Pathology Department, Hospital Clinic, University of Barcelona, Villarroel 170, Barcelona 08036, Spain
e-mail: dmartin1@clinic.ub.es; ecampo@clinic.ub.es

L. Colomo, MD, PhD
Hematopathology Unit, Pathology Department, Hospital del Mar, University Pompeu Fabra, Passeig Marítim 25-29, Barcelona 08003, Spain
e-mail: lcolomo@parcdesalutmar.cat

D. Soldini, MD, PhD
Institute of Surgical Pathology, University Hospital Zurich, Schmelzbergstrasse 12, Zurich 8091, Switzerland
e-mail: Davide.Soldini@usz.ch

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lymphoma are more frequently diagnosed in mucosae of the upper aerodigestive tract than in other regions. In this chapter, we review the benign and malignant lymphoid proliferative disease that frequently originate or secondarily involve the head and neck lymph nodes and extranodal sites. For further review of malignant lymphomas, see the World Health Organization (WHO) classification system for tumors of haematopoietic and lymphoid tissues [1, 2].

13.2 Cervical Lymph Nodes and Waldeyer Ring

13.2.1 Atypical Marginal Zone Hyperplasia

Definition Atypical marginal zone hyperplasia is an uncommon reactive condition described in tonsil and appendix of children.

Clinical aspects It usually presents in the head and neck area as unilateral or bilateral tonsil enlargement and has a benign course without need of therapy.

Microscopy These lesions show a follicular hyperplasia with an expansion of the marginal zone by centrocyte-like cells with many transformed blasts. Some of the follicles had changes of progressive transformation of the germinal centres.

Immunohistochemistry Stains show that these cells are CD20, BCL2, IgD and IgM positive and have an aberrant co-expression of CD43 and a lambda light chain restriction, and are negative for CD10 and IRF4/MUM1.

Genetics Molecular studies of DNA samples demonstrate a polyclonal pattern in all cases.

Differential diagnosis The main differential diagnosis is with paediatric marginal zone lymphoma that usually has *IGH* clonal rearrangements and present chromosomal aberrations. An integrated diagnosis of histology, immunohistochemistry and molecular findings is essential for the distinction between atypical marginal hyperplasias and marginal zone lymphoma.

Treatment and prognosis These lesions have an indolent behaviour and should be managed conservatively, with a watch-and-wait approach [3, 4].

13.2.2 Viral Infections

13.2.2.1 EBV Lymphadenitis

Definition Infectious mononucleosis is a disease caused by Epstein-Barr virus (EBV) infection.

Epidemiology It commonly produces lymphadenopathy and enlargement of tonsils in adolescent and young adults, although cases in young children and elderly adults have been reported.

Clinical aspects Clinical features include fever, pharyngitis, cervical lymphadenopathy, rash, hepatosplenomegaly and reactive peripheral blood lymphocytosis. Although these manifestations usually lead to a diagnosis without a biopsy, the excision of lymph nodes or tonsils may be performed due to airway obstruction or to exclude a lymphoma [5].

Microscopy The lymph node or tonsil architecture may be distorted but not effaced, by a polymorphous paracortical infiltrate with a 'moth-eaten' appearance composed of large immunoblasts, medium-sized and small lymphocytes, tingible bodies and plasma cells associated with variable degrees of follicular hyperplasia and distended sinuses. The immunoblasts may be atypical and occasionally binucleated, resembling Reed-Sternberg cells [6].

Immunohistochemistry Immunophenotype shows that most immunoblasts are B cells and often express CD30, including Reed-Sternberg-like cells, although they are typically EMA and CD15 negative. The small- and intermediate-sized cells in the paracortex are predominantly CD8+ T cells. In situ hybridization for EBER shows numerous paracortical positive immunoblasts (Fig. 13.1). LMP-1 or EBNA-2 are also expressed in a variable proportion of cells [7, 8].

Differential diagnosis The differential diagnosis includes high-grade non-Hodgkin lymphoma and classical Hodgkin lymphoma (cHL) [9]. When paracortical immunoblasts are numerous, large cell lymphoma must be considered. Lack of architectural effacement; presence of areas recognizable as reactive hyperplasia; a polymorphous background of medium-sized lymphocytes, plasma cells and immunoblasts; and the presence of high endothelial venules among the large cells favour the diagnosis of infectious mononucleosis. The presence of classic Reed-Sternberg-like cells may suggest cHL, but these cells lack CD15 expression.

Absolute and atypical lymphocytosis and a positive heterophile antibody test support a diagnosis of EBV-associated infectious mononucleosis. In cases in which the diagnosis is unclear, EBV-specific serologic testing may be used to definitively diagnose primary EBV infection.

Treatment and prognosis Treatment should be supportive, with steroids given only in cases of airway compromise. Treatment with antiviral agents has yet to be shown to be of benefit [10, 11].

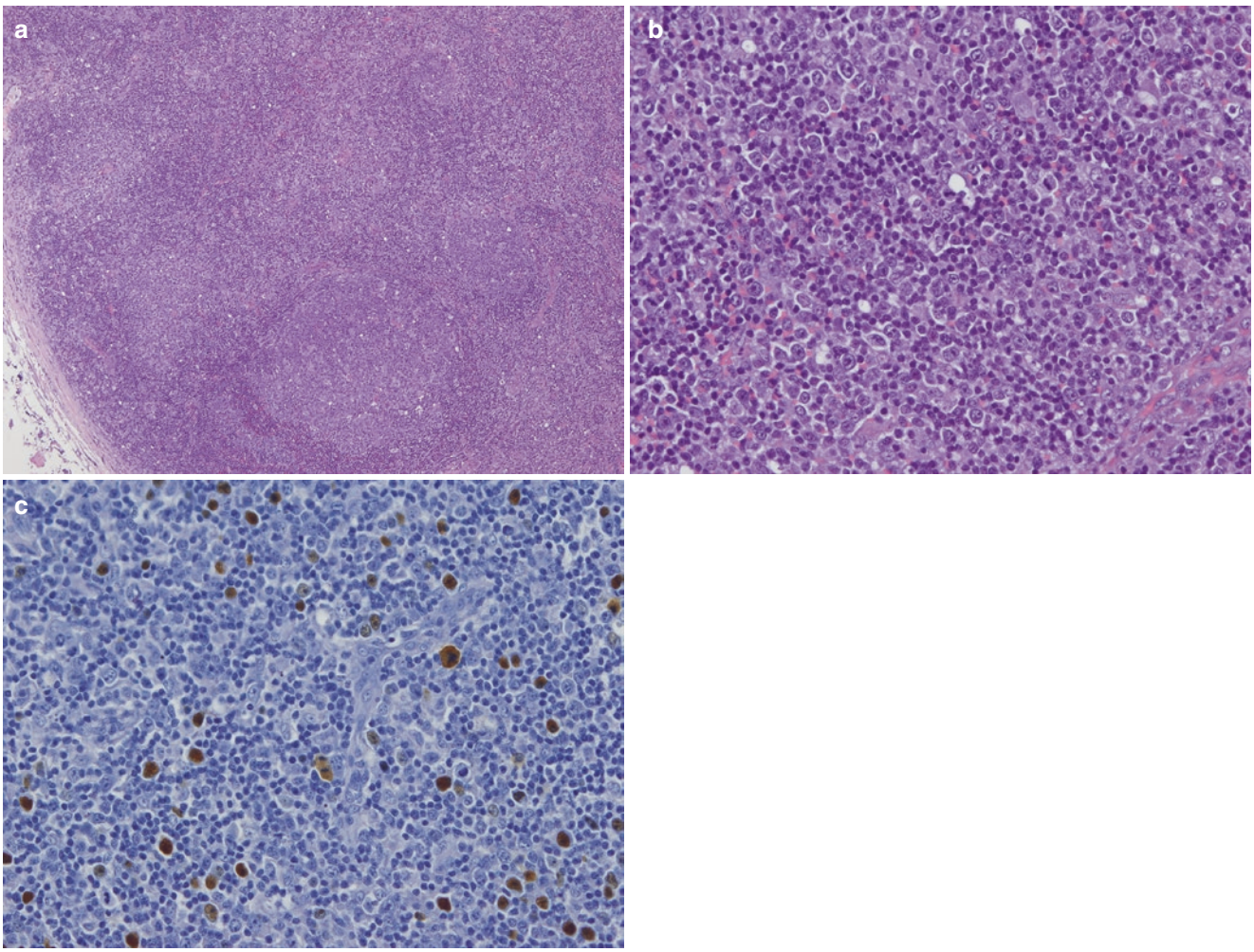

Fig. 13.1 (**a**) EBV lymphadenitis. Conserved lymphoid architecture with polymorphous paracortical infiltrate. (**b**) Presence of large immunoblasts. (**c**) EBER is positive in large cells

13.2.2.2 Herpes Simplex and Cytomegalovirus Lymphadenitis

Infection by other members of the herpes family can produce a localized or generalized lymphadenopathy or may show a clinical and haematologic picture indistinguishable from infectious mononucleosis. Infection by herpes simplex virus (type I or II) shows a lymph node with follicular and prominent paracortical hyperplasia with foci of necrosis that contain neutrophils, karyorrhectic debris and a variable number of large cells with prominent nuclear inclusions. Histiocytes often surround necrotic foci, but granulomas are absent. Immunohistochemical studies and serologies can confirm easily this diagnosis [12, 13]. Cytomegalovirus (CMV) lymphadenitis shows a follicular or paracortical hyperplasia with immunoblastic and perivascular monocytoid B-cell proliferation. Infected cells contain large eosinophilic intranuclear ('owl's eye') and cytoplasmic viral inclusions and may be found among monocytoid B cells, within epithelioid histiocytes or vascular endothelial cells [14]. Immunophenotype shows paracortical T cells and CD30-positive immunoblasts. Infected cells may express CD15, with a cytoplasmic pattern of staining. Immunostaining with CMV-specific antibodies is useful to make the diagnosis of CMV lymphadenitis [15].

13.2.2.3 HIV-Associated Lymphadenopathy

Epidemiology HIV lymphadenopathy is more common in young children as they are exposed to new infections/antigens.

Clinical aspects Human immunodeficiency virus (HIV) may cause a variety of reactive lesions. Primary infection may be associated with an acute mononucleosis-like syndrome, with fever, pharyngitis and cervical lymphadenopathy. Persistent generalized lymphadenopathy is common in HIV-infected patients and is defined as of at least 3-month duration involving two or more noncontiguous sites. It is often accompanied by constitutional symptoms. Biopsies are most often performed to exclude treatable infections or malignant neoplasms [16].

Microscopy Lymph node changes in benign HIV-infected lymph nodes suffer a range of changes as the disease evolves. In the early stage, there is a florid follicular hyperplasia with expanded follicles and effaced mantle zones. Germinal centres are commonly very large, irregular and with a high mitotic rate. There is often follicular lysis (small lymphocytes infiltrate the germinal centres, often associated with haemorrhage). Paracortical hyperplasia is also seen, with a mixture of immunoblasts, plasma cells, lymphocytes and histiocytes. Commonly, there is also a marked monocytoid B-cell reaction in the sinuses. As the disease progresses, follicles decrease in number and become smaller, and in the late stages, there is lymphoid depletion, absence of follicles and a prominent vascular network with immunoblasts and plasma cells in the interfollicular region. Although these features are nonspecific for HIV infection, the set of clinical and histological findings is highly characteristic of HIV-related lymphadenopathy [17, 18].

Immunohistochemistry HIV-associated antigens can be detected in follicular dendritic cells with immunohistochemistry or in situ hybridization. Antibodies useful to localize HIV include p24, p17, gp41 and gp120 (Fig. 13.2).

Differential diagnosis The differential diagnosis includes nonspecific reactive hyperplasia and lymphomas. In cases of explosive follicular hyperplasia with ill-defined follicular mantles, the apparent architecture distortion may lead to confusion with non-Hodgkin's lymphomas.

Treatment and prognosis The use of highly active antiretroviral therapy produces in many cases a recovery of the lymphoid tissue architecture with reappearance of follicular structures [19].

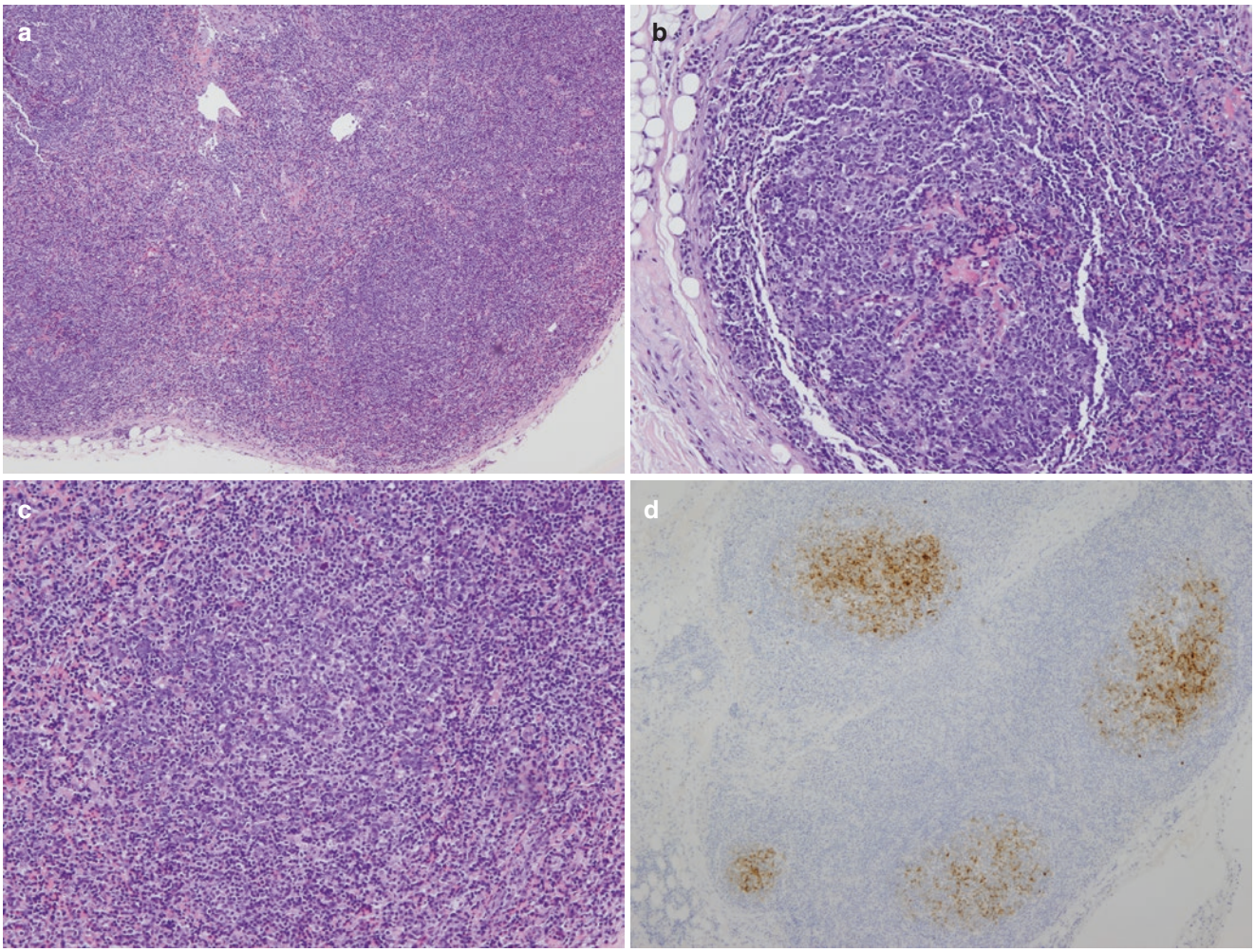

Fig. 13.2 (**a**) HIV-associated lymphadenopathy. Lymph node show expanded follicles with effaced mantle zones and a paracortical hyperplasia. (**b**) Germinal centres are large and irregular. (**c**) Some germinal centres show follicular lysis. (**d**) Staining for p24 shows immunoreactivity in the follicular dendritic cells

13.2.3 Bacterial and Protozoal Infections

13.2.3.1 Pyogenic Bacteria and Cat-Scratch Disease

The bacterial agents that most commonly cause cervical lymphadenitis are group A *β-haemolytic streptococci*, *Staphylococcus aureus* and various oral anaerobes [20]. Histology shows a lymph node with paracortical hyperplasia with neutrophilic infiltration of the lymph node capsule and abscess formation. Organisms can be identified using Gram stains [21]. Another disease caused by bacterial infection is cat-scratch disease. It is a self-limited benign condition characterized by regional lymphadenopathy affecting most frequently the head and neck region in children and young adults and often accompanied by fever. It is caused most commonly by *Bartonella henselae* and *B. quintana*, as a result of a cat scratch or bite [22, 23]. The histopathologic appearance of the lymph nodes depends on the duration of the disease. The first changes show follicular hyperplasia with proliferation of monocytoid B cells. Paracortical areas are initially filled with immunoblasts and histiocytes with focal areas of necrosis and few neutrophils. In later stages, necrotic foci enlarge and coalesce, with palisading epithelioid macrophages, forming the stellate microabscess or granuloma [24]. The Warthin-Starry stain demonstrates the small and curved bacteria in macrophages, in sinus histiocytes, in blood vessels or in the extracellular space.

Differential diagnosis should include other entities that course with necrotizing granulomatous lymphadenitis, like lymphogranuloma venereum or mycobacterial infection.

13.2.3.2 Mycobacteria

Definition Tuberculosis is a chronic worldwide disease caused by *Mycobacterium tuberculosis*.

Epidemiology In Africa, tuberculosis is one of the most common causes of lymphadenopathy, [25, 26] but it has re-emerged as a public health concern in developed countries in recent years [27]. Increasing tuberculosis rates in children born abroad or with immigrant parents have also been described, and cervical lymphadenitis is a frequent clinical presentation [28].

Clinical aspects Cervical lymphadenitis, referred also as scrofula, is the most common manifestation of mycobacterial infections encountered in the otolaryngologic practice. It may be manifestation of a systemic tuberculous disease or a unique clinical entity localized to the neck and can result from direct extension or haematogenous spread of the infection [29]. It may present as a unilateral single or multiple painless mass, mostly located in the posterior cervical or supraclavicular region [30].

Microscopy Histopathologic examination is one of the most important means for diagnosing mycobacterial cervical lymphadenitis. Lymph nodes show multiple granulomas, composed of epithelioid macrophages and Langerhans multinucleated giant cells. Central caseation necrosis is present to a variable extent of granulomas. Ziehl-Neelsen stain is useful to identify *Mycobacteriae* bacilli and to establish a definitive diagnosis.

Differential diagnosis It includes sarcoidosis, fungal infections as histoplasmosis and cat-scratch disease. Lymph nodes in sarcoidosis show well-formed granulomas with no central necrosis.

Treatment and prognosis Tuberculosis of the cervical lymph nodes responds well to antituberculous drugs and the surgical role is limited to guidance in fine-needle aspiration, incision and drainage and incisional and limited excisional biopsy [31].

13.2.3.3 Toxoplasmic Lymphadenitis

Definition Toxoplasmic lymphadenitis is caused by *Toxoplasma gondii*.

Clinical aspects Infection commonly presents as localized posterior cervical lymphadenopathy associated with mild constitutional symptoms, as malaise, sore throat and fever. The disease is self-limited, but occasionally serious extranodal disease occurs, including myocarditis, pneumonitis, encephalitis, chorioretinitis and maternal transmission of the infection to the foetus [32].

Microscopy Histologically, the lymph nodes show a triad of florid follicular hyperplasia, parasinusoidal and paracortical monocytoid B-cell hyperplasia and small clusters of epithelioid histiocytes within follicles (Piringer-Kuchinka lymphadenitis). The germinal centres contain numerous tingible body macrophages (Fig. 13.3). The microorganisms are seen only rarely, although antibodies for immunohistochemistry are available. Serologic studies or polymerase chain reaction method from frozen tissues can confirm the diagnosis [33, 34].

Differential diagnosis It includes leishmania lymphadenitis, cat-scratch disease, sarcoidosis or mycobacterium infection.

Treatment and prognosis Patients with toxoplasmic lymphadenitis can be treated with co-trimoxazole.

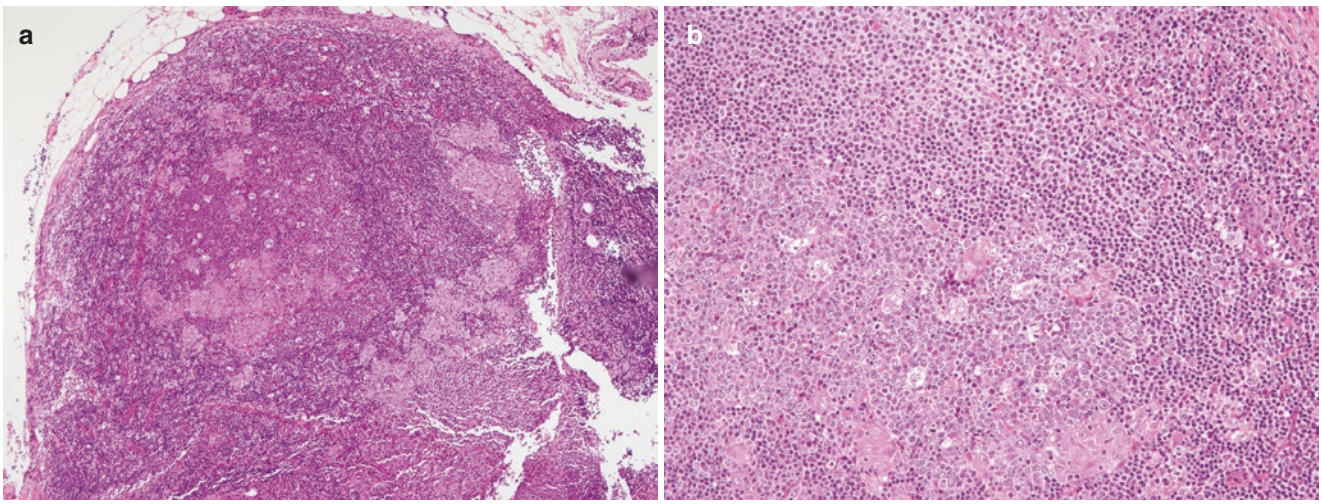

Fig. 13.3 (**a**) Toxoplasmic lymphadenitis. Lymph node presents the classical triad of florid follicular hyperplasia, paracortical monocytoid B-cell hyperplasia and large clusters of epithelioid histiocytes within follicles. (**b**) Germinal centre contains numerous tingible body macrophages and clusters of epithelioid histiocytes. Monocytoid hyperplasia is seen at the periphery of the follicle

13.2.3.4 *Haemophilus influenzae* Lymphadenitis

Definition *Haemophilus influenzae* lymphadenitis is a recently described type of polyclonal cervical paediatric lymphadenopathy associated with invasive *H. influenzae* infection [35].

Epidemiology It affects children and adolescents without sex predilection.

Clinical aspects This disorder is characterized by prominent, unilateral or bilateral, cervical lymphadenopathy in children who are otherwise healthy and without history of immunodeficiency or frequent upper airway infections. The lymph nodes may be large, but the lymphadenopathy is indolent.

Microscopy Lymph nodes show partial destruction of the architecture with nodular pattern and presence of fibrotic bands. The follicles have different morphologies, some are strongly expanded, mimicking progressively transformed germinal centres, and others are hyperplasic secondary follicles. Variable monocytoid B-cell expansion with marginal zone hyperplasia is present. The germinal centres and marginal zones contain highly activated medium-sized lymphoid cells, with some degree of apoptosis (Fig. 13.4).

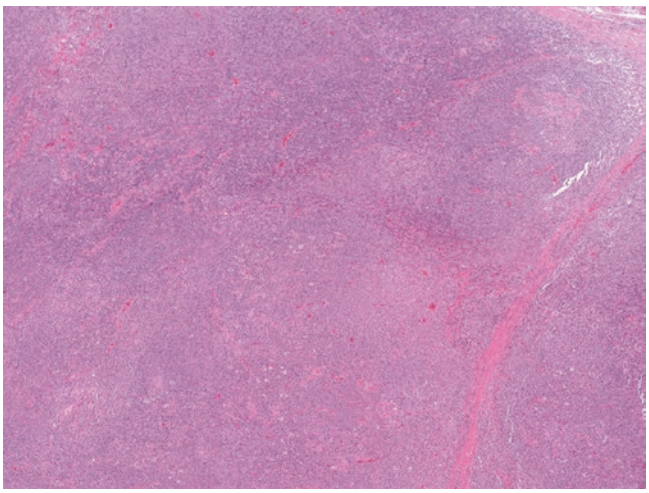

Fig. 13.4 *Haemophilus influenzae* lymphadenitis. This biopsy shows prominent nodular structures mimicking progressively transformed germinal centres and germinal centres with a marginal zone hyperplasia

Immunohistochemistry Stain for IgD is strong on small lymphocytes corresponding to the residual follicle mantles and weak on the blasts in the marginal zones and germinal centres. BCL2 staining is absent or weak in nodules and marginal zones. CD21 staining show disrupted meshworks of follicular dendritic cells. IRTA1/CD307d staining is variable in nodules and marginal zones. Many cases have light chain-restricted plasma cells.

Molecular studies Cytogenetic studies did not show abnormalities, and molecular studies of DNA samples demonstrate *IGH* polyclonal rearrangement.

Differential diagnosis The main differential diagnosis should be established with paediatric marginal zone lymphoma that tends to affect older children and presents with masses other than cervical. Although both entities share some features, a useful differential feature is that the

marginal zones of paediatric marginal zone lymphoma are BCL2 positive. *IGH* clonality studies can be also a very helpful tool in the differential diagnosis between these two entities.

Treatment and prognosis It is a self-limited disease with an indolent behaviour that resolves spontaneously, although local recurrences may occur.

13.2.4 Specific Clinical Entities

13.2.4.1 Systemic Lupus Erythematosus

Definition Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown etiology.

Epidemiology It affects adolescents and young adults, with a female predominance.

Clinical aspects Localized or generalized lymphadenopathy may frequently be associated with systemic symptoms, such as fever. Cervical lymph nodes are the most common site involvement [36]. Patients with lymph node enlargement had more constitutional symptoms of fatigue, fever and weight loss, more cutaneous symptoms and signs, a higher rate of hepatomegaly and splenomegaly, increased anti-dsDNA antibodies and decreased complement levels [37]. Histological features of lupus lymphadenitis are extremely variable, reflecting the variability of clinical manifestations and the disease course in individual patients.

Microscopy On morphological grounds, the lymph nodes include nonspecific findings such as reactive follicular hyperplasia with giant follicles, atypical paracortical hyperplasia with lymphocytes and immunoblasts and foci of coagulative necrosis surrounded by a moderate number of foamy macrophages and small lymphocytes, scattered neutrophils, plasma cells and B-immunoblasts. Haematoxylin bodies, extracellular amorphous haematoxyphilic structures, although uncommon, are highly specific of SLE and are found in areas of necrosis [38, 39].

Differential diagnosis It includes infectious processes and Kikuchi's lymphadenitis, because of the extensive necrosis. The presence of polymorphous neutrophils and plasma cells is a useful finding for distinguishing lupus lymph node necrosis from that in Kikuchi's disease. Cases with abundant large cells may be mistaken for non-Hodgkin's lymphoma, but preservation of architecture, necrosis, histiocytes and haematoxylin bodies favours de diagnosis of lupus.

13.2.4.2 Hyaline Vascular Castleman's Disease

Definition Castleman's disease (CD) is a rare lymphoproliferative disorder with two primary subtypes that vary in presentation and course.

Clinical aspects Localized Castleman's disease (hyaline-vascular type (HV) and plasma cell type (PC)) presents as a solitary mass, most commonly in the mediastinum, and rarely in the head and neck. Systemic or multicentric Castleman's disease has distinctive clinical presentation with peripheral lymphadenopathy and numerous systemic symptoms. Localized CD in head and neck most often affects neck level II and III and usually presents as an otherwise asymptomatic or slowly enlarging mass [40, 41].

Microscopy The histological findings show a preserved architecture but distorted. Lymphoid follicles are small, but increased in number, with depleted germinal centres. Mantle zones are expanded and are composed of concentric rings in an 'onion skin' pattern. In some cases, there may be atypical follicular dendritic cells with enlarged irregular nuclei. Interfollicular region shows a prominent vascular proliferation, and blood vessels may penetrate into the germinal centre to form a 'lollipop' follicle (Fig. 13.5).

Differential diagnosis The morphologic features of HV-CD are relatively specific, although may rise the differential diagnosis with late-stage of HIV-associated lymphadenopathy, early stages of angioimmunoblastic T-cell lymphoma, follicular lymphoma, mantle cell lymphoma and nonspecific reactive lymphadenopathy.

Treatment and prognosis Regional excision is the first choice for the treatment of localized HV-CD, with excellent prognosis [42].

13.2.4.3 Kikuchi's Disease

Definition Kikuchi's disease, Kikuchi-Fujimoto lymphadenitis (KFL) or histiocytic necrotizing lymphadenitis was first reported by Kikuchi and Fujimoto simultaneously in 1972 [43, 44].

Epidemiology It affects predominantly young adults, especially in females of Asian origin.

Clinical aspects It classically presents as tender cervical lymphadenopathy associated with fever and systemic symptoms. The disease is self-limited and most patients recover without therapy. Less common findings include generalized lymphadenopathy, hepatomegaly, splenomegaly, cutaneous

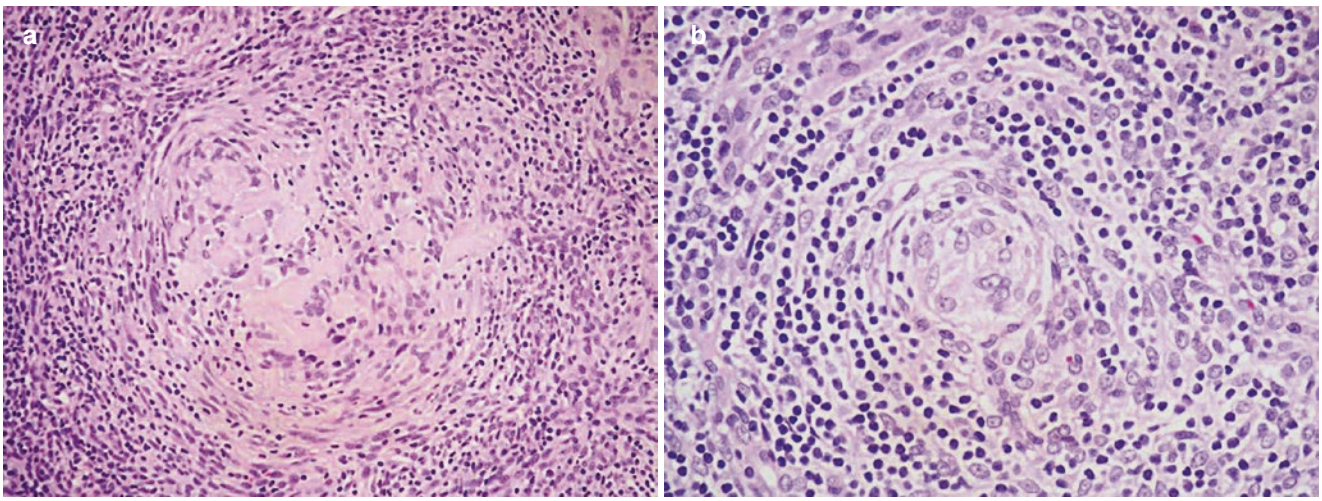

Fig. 13.5 (**a**) Castleman's disease. Lymphoid follicle is small, with depleted germinal centre. Mantle zones present concentric rings in an 'onion skin' pattern. (**b**) Some vascular vessels penetrate into a depleted germinal centre

eruption, fatigue, joint pain, nausea, vomiting and sore throat [45–49].

Microscopy The lymph nodes characteristically show partially effaced architecture by paracortical expansion composed of numerous immunoblasts with prominent nucleoli, admixed with histiocytes of different types and aggregates of plasmacytoid dendritic cells and foci of apoptotic necrosis with abundant karyorrhectic debris. The so-called crescentic histiocytes are typically seen in these necrotic foci. Neutrophils and eosinophils are characteristically absent (Fig. 13.6).

Kikuchi's disease can be classified into three evolving histological phases: proliferative, necrotizing and xanthomatous. In the proliferative phase, the lymph node shows expanded paracortex with the mixture of cells described above, with apoptosis but without necrosis. In the necrotizing phase, necrosis of any degree is observed in addition to the changes of the proliferative phase. The xanthomatous phase is characterized by the predominance of foamy histiocytes in the lesions, despite the presence or absence of necrosis [50].

Immunohistochemistry The lymphoid cells express pan-T-cell markers and most of them are CD8+. Histiocytes are CD68+ and may express myeloperoxidase. The plasmacytoid dendritic cells are CD68+, CD4+ and CD123+.

Differential diagnosis The histologic differential diagnosis of KFL includes mainly reactive lesions such as lymphadenitis associated with lupus, herpes lymphadenitis, tuberculosis, non-Hodgkin lymphoma, blastic plasmacytoid dendritic cell neoplasm, Kawasaki disease, nodal colonization by acute myeloid leukaemia and even metastatic adenocarcinoma. Morphological features favouring this diagnosis are the patchy distribution, abundance of karyorrhectic debris and the presence of admixed plasmacytoid dendritic cells. Therefore, KFL should be taken into consideration in the diagnostic process in all patients with persistent lymphadenopathy and prolonged fever because misdiagnosis may lead to unnecessary surgery and/or chemotherapy [51–54].

Treatment and prognosis KFD is a self-limited disease that resolves spontaneously in few weeks although local recurrences may occur.

13.2.4.4 Sinus Histiocytosis with Massive Lymphadenopathy

Definition Sinus histiocytosis with massive lymphadenopathy (SHML) or Rosai-Dorfman disease is a disease of unknown etiology that was first reported by Rosai and Dorfman in 1969 [55].

Epidemiology It mainly affects children and young adults.

Clinical aspects Typically presents as bilateral painless cervical lymphadenopathy, accompanied by low-grade fever, weight loss, leukocytosis, polyclonal gammopathy and elevated erythrocyte sedimentation rate. In about 40 % of cases, extranodal sites are involved. These sites include the sinonasal region, salivary gland, skin, orbit, bone, upper respiratory tract, central nervous system and kidney. The clinical course is self-limited and usually regresses spontaneously, although lymphadenopathy may persist or recur [56, 57].

Microscopy The lymph nodes show a marked sinusoidal dilatation by large histiocytes with round vesicular nuclei,

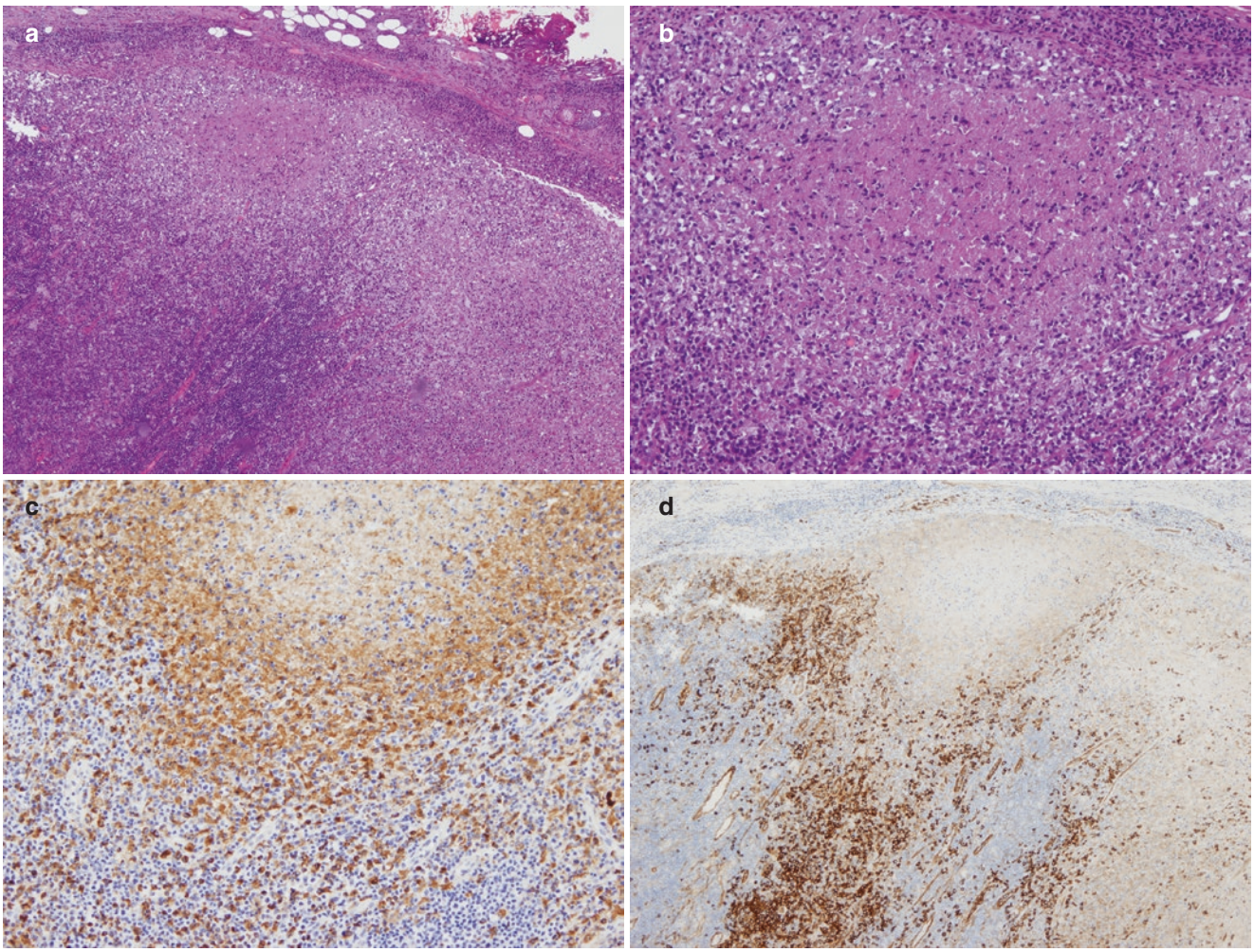

Fig. 13.6 (**a**) Kikuchi's disease. Focus of apoptotic necrosis with abundant karyorrhectic debris at the periphery of the lymph node. (**b**) Necrosis is surrounded by abundant histiocytes and immunoblasts. (**c**) CD68 highlights the histiocytes. (**d**) CD123 shows the presence of aggregates of plasmacytoid dendritic cells

prominent nucleoli and foamy cytoplasm. Emperipolesis is the most frequent finding, with viable lymphocytes, plasma cells, neutrophils or erythrocytes within the cytoplasm of macrophages. Medullary cords contain lymphocytes and a prominent population of polytypic plasma cells. The lymph node capsule is often fibrotic. In extranodal sites, the histiocytes form aggregates that resemble dilated sinuses and emperipolesis may be less conspicuous.

Immunohistochemistry The histiocytes are positive for S100 protein and other macrophage-associated markers such as CD4, CD11c, CD14, CD33 and CD68 and negative for CD1a (Fig. 13.7).

Differential diagnosis The main differential diagnosis is Langerhans cell histiocytosis. Cells have elongated grooved nuclei, inconspicuous nucleoli and pale cytoplasm. Emperipolesis does not occur. Eosinophils are more frequent and plasma cells are absent. Another important differential diagnosis is IgG4-related sclerosing disease. Both entities share the histologic feature of intense chronic inflammatory cell infiltrates. A varying degree of stromal fibrosis sometimes found in extranodal SHML cases further difficult the distinction. A link between SHML and IgG4-related sclerosing disease has been proposed [58, 59].

Treatment and prognosis SHML regresses spontaneously in most patients and the prognosis is excellent.

13.2.4.5 Kimura's Disease

Definition Kimura's disease is a rare and benign chronic inflammatory condition of unknown origin.

Epidemiology It affects most often young adult males of Asian origin.

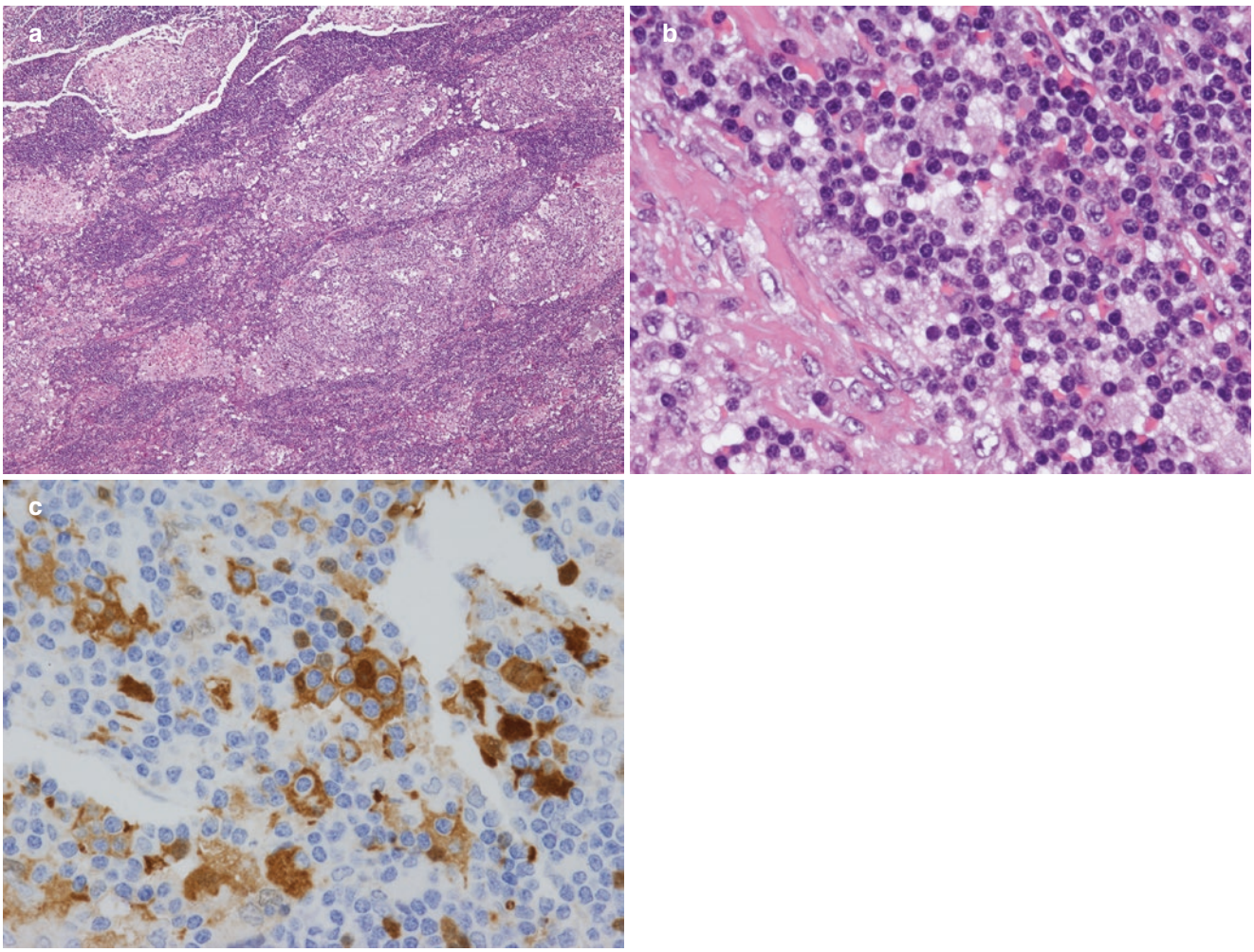

Fig. 13.7 (**a**) Sinus histiocytosis with massive lymphadenopathy. The lymph node shows marked dilated sinusoids by aggregates of histiocytes. (**b**) Emperipolesis with viable lymphocytes and plasma cells within the cytoplasm of macrophages are seen. (**c**) Histiocytes are positive for S100. Note the presence of lymphocytes within the cytoplasm

Clinical aspects Subcutaneous soft tissue masses occur predominantly in the head and neck region and often involve the parotid, submandibular or minor salivary glands. Peripheral blood examination shows eosinophilia and increased serum IgE levels [60].

Microscopy Microscopic findings include florid follicular hyperplasia, prominent eosinophilia and vascularization of the germinal centres. The interfollicular areas show prominent high endothelial venules with a mixture of lymphocytes, plasma cells, eosinophils and mast cells. Eosinophilic abscesses and polykaryotic cells may be seen within germinal centres as well as in the paracortex. In many cases, there is a deposition of homogeneous eosinophilic material in germinal centres.

Differential diagnosis The main differential diagnosis is with angiolymphoid hyperplasia with eosinophilia, which is a vascular neoplasm characterized by the proliferation of blood vessels lined by prominent endothelial cells with abundant eosinophils. Unlike Kimura's disease, it has a female predominance and occurs in all races. The lesions are superficial and usually present as isolated or grouped papules, plaques or nodules in the skin of the head and neck. Regional lymphadenopathy, serum eosinophilia and elevated IgE levels are rare [61, 62].

Treatment and prognosis The disease is self-limited, although recurrences can occur.

13.2.5 Haematopoietic Neoplasms

13.2.5.1 Classical Hodgkin Lymphoma

Definition Classical Hodgkin lymphoma (cHL) is a monoclonal lymphoid neoplasm of B-cell origin, composed of

Reed-Sternberg (RS) cells and mononuclear Hodgkin cells in the context of an abundant and variable infiltrate of non-neoplastic inflammatory and accessory cells.

Epidemiology It represents approximately 4 % of all lymphomas of the head and neck. Most of these neoplasms involve lymph nodes. Extranodal involvement by HL is very rare, and the Waldeyer ring is the most frequently involved area in approximately 1–2 % of cases. It affects mostly young people, with a median age at diagnosis between 30 and 40 years, and occurs mostly in males.

Clinical aspects Patients usually present with peripheral lymphadenopathy at stage I or II [63]. B symptoms are present in up to 40 % of patients.

Microscopy Nodular sclerosis (NS) and mixed cellularity (MC) are the most frequent variants of cHL. The former variant is the most frequent subtype in people younger than 30, whereas MC is most frequent in older people. Characteristically there is an infiltrate composed of variable numbers of RS cells admixed with a rich inflammatory background of lymphocytes, eosinophils and histiocytes. RS cells usually have at least two nucleoli in two separate nuclear lobes and often present a retraction of the cytoplasm. In NS, RS cells usually form aggregates and nodules that are surrounded by broad fibrous bands (Fig. 13.8), whereas in MC there are no fibrotic bands. The other histological types of cHL, lymphocyte-rich and lymphocyte-depleted, are very rarely found in head and neck region.

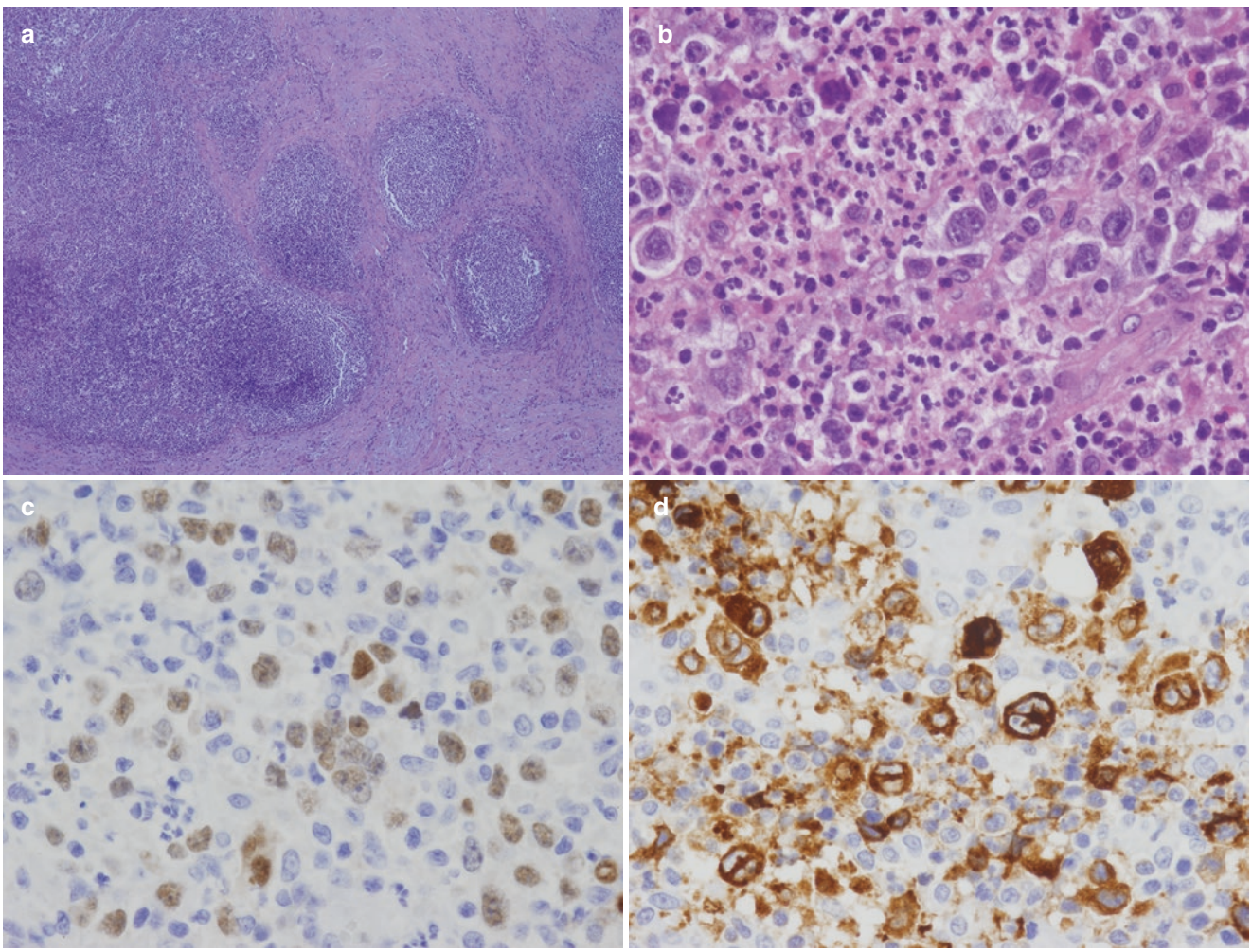

Fig. 13.8 (**a**) Classical Hodgkin lymphoma, nodular sclerosis. Cervical mass with nodules that contain neoplastic RS cells, surrounded by broad fibrous bands. (**b**) Aggregates of RS cells admixed with an inflammatory background of neutrophils, eosinophils and histiocytes. (**c**) PAX5 is weakly positive in neoplastic cells. (**d**) RS cells are also positive for CD30

Immunohistochemistry RS cells are positive for CD30 and CD15 and negative for CD45. CD20 and CD79a are frequently negative and PAX5 is usually weakly positive. The Epstein-Barr virus positivity is frequently observed in MC and usually negative in NS [1, 2].

Treatment and prognosis The treatment of cHL with chemotherapy and consolidative radiation therapy appears to offer excellent long-term local and systemic control in patients who present with HL, with an overall survival of up 85 % at 10 years [63].

13.2.5.2 Nodular Lymphocyte Predominant Hodgkin Lymphoma

Definition Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is a monoclonal B-cell neoplasm characterized by a nodular proliferation of scattered large neoplastic cells, known as lymphocyte predominant (LP) cells, in the context of expanded lymphoid follicles with abundant follicular dendritic cells and non-neoplastic lymphocytes and histiocytes.

Epidemiology It represents around 5 % of all Hodgkin lymphomas and has a male predominance.

Clinical aspects The disease affects mainly cervical and axillary lymph nodes and usually presents as localized lymphadenopathy (stage I or II) [64].

Microscopy NLPHL is characterized by a nodular or a nodular and diffuse infiltrate, consisting of small lymphocytes, histiocytes and scattered intermingled LP cells. These cells are large and usually have one large vesicular and polylobulated nucleus, with multiple peripheral nucleoli, smaller than those seen in classical RS cells. LP cells proliferate in the context of expanded nodular lymphoid follicles with meshworks of follicular dendritic cells associated with mature lymphocytes and variable number of histiocytes.

Immunohistochemistry The immunophenotype of NLPHL is significantly different from that of cHL. LP cells express B-cell antigens as CD19, CD20, CD79a and PAX5, express BCL6 and CD45, and, with some exceptions, lack CD30 and CD15 expression. The follicular T helper cells (CD4+, CD57+, PD1+) often rosette the LP cells in the nodules (Fig. 13.9). Epstein-Barr virus infection is absent in the neoplastic LP cells in virtually all cases, although may be present in small lymphocytes [2, 65].

Differential diagnosis The main differential diagnoses are progressive transformation of germinal centres (PTGC) and T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL). PTGC is a reactive condition characterized by lymph nodes with large, well-defined follicles containing abundant small B cells infiltrating the germinal centres. THRLBCL is a diffuse large B-cell lymphoma characterized by the presence of few scattered large atypical B cells surrounded by a background of abundant T cells and histiocytes. Lack of residual follicular dendritic cell meshwork in THRLBCL usually helps in the distinction between these two entities [66, 67].

Treatment and prognosis The treatment options vary widely. Surgical excision alone or radiotherapy are usually effective therapies in stages I and II, with a median overall survival of 90 % at 10 years. Patients in stages III and IV usually receive a combination of chemotherapy and radiotherapy. Some patients may relapse and may transform into a diffuse large B-cell lymphoma [64, 68].

13.2.5.3 Follicular Lymphoma

Definition Follicular lymphoma (FL) is a B-cell neoplasm derived from germinal centre cells that usually growth in a follicular pattern.

Epidemiology It is the second most common lymphoma arising in cervical lymph nodes (14 %), although most patients with FL have widespread nodal disease at the time of diagnosis. It affects predominantly adults with a mean age of 60 years, and there is no gender preference [69].

Microscopy Histologically, FL is composed of germinal centre (GC) B cells (centrocytes and centroblasts) with a follicular pattern of growth. Centrocytes are small, with irregular or angulated nuclei, pale chromatin and one or more small nucleoli. Centroblasts are larger, with round or oval nuclei, vesicular chromatin and one to three nucleoli apposed to the nuclear membrane. In contrast to reactive follicular hyperplasia, mitotic activity is low in most cases, and the 'starry sky' pattern with histiocytes is absent [2]. Grading of FL is important for predicting outcome and is made by counting the number of centroblasts per 40× microscopic high power field (HPF). FL with up to 5 centroblasts/hpf is grade 1; 6–15 centroblasts is grade 2; and greater than 15 centroblasts is grade 3 [2]. The WHO classification recommends subdividing grade 3 into grade 3A (more than 15 centroblasts/hpf, but centrocytes still present) and grade 3B (solid sheets of centroblasts), since FL 3A is more closely related to low-grade FL, whereas grade 3B is more closely related to diffuse large B-cell lymphoma (DLBCL) [70, 71].

Lymph nodes are enlarged with architecture effaced by neoplastic follicles that are typically closely packed, poorly defined and lack mantle zone or polarization. Diffuse areas comprised of predominantly centrocytes may be present. However, if diffuse areas comprised predominantly of

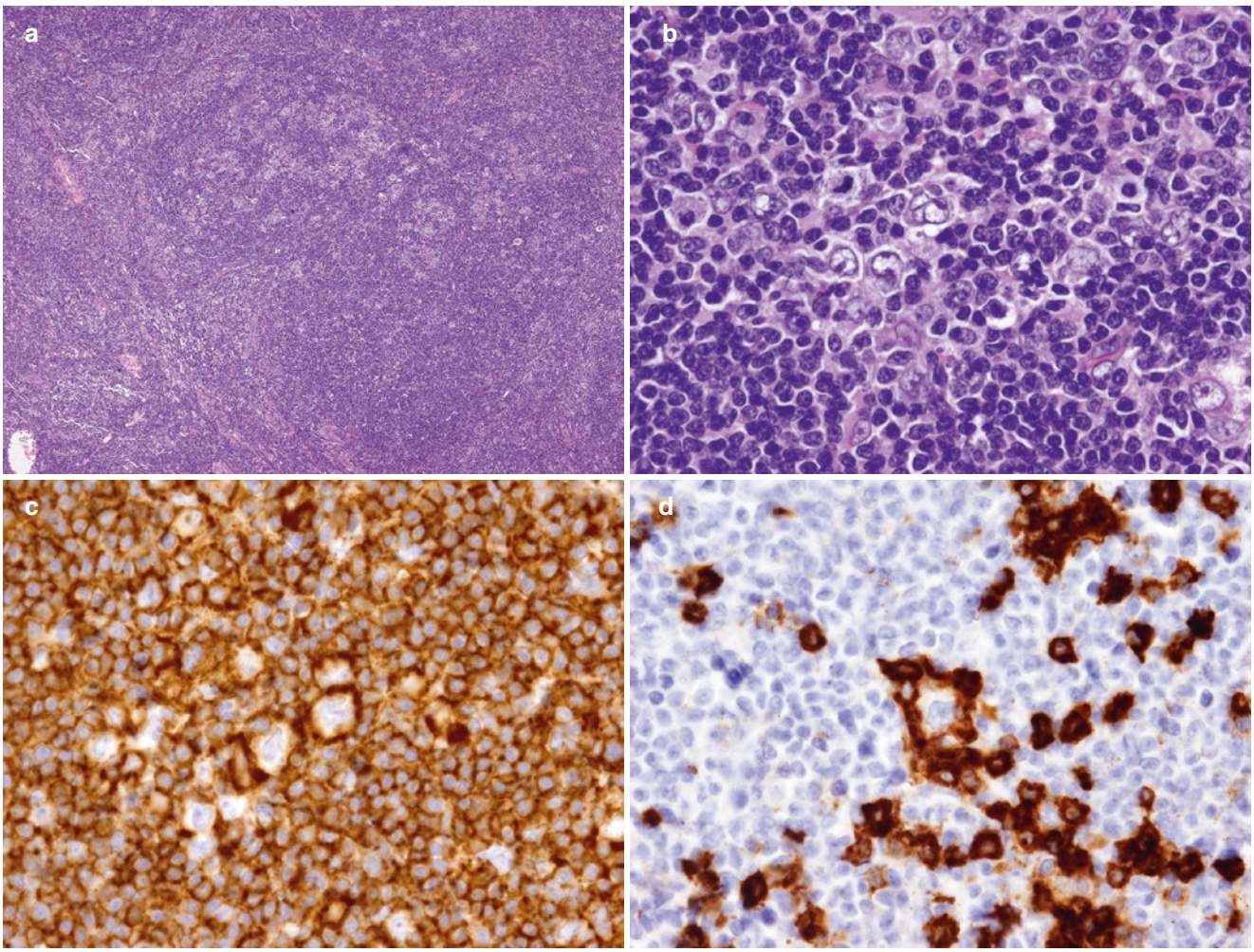

Fig. 13.9 (**a**) Nodular lymphocyte predominant Hodgkin lymphoma. Nodular infiltrate consisting of small lymphocytes, histiocytes and scattered intermingled LP cells. (**b**) LP cells are large and have one vesicular and polylobulated nucleus. (**c**) LP cells express CD20. (**d**) CD57 staining show rosettes of T helper cells around the neoplastic cells

centroblasts are seen, diagnosis of DLBCL should be established [2].

For diagnostic purpose, excision of cervical lymph node is preferable to fine-needle aspiration because it allows to asses grade and pattern [72, 73].

Immunohistochemistry Tumor cells express B-cell-associated antigens (CD19, CD20, CD79a) and express GC-associated markers such as BCL6 and CD10, but both may be downregulated in interfollicular neoplastic cells. BCL2 is usually overexpressed in the neoplastic cells located in the expanded germinal centres, which contain nodular aggregates of follicular dendritic cells CD21 or CD23 positive. FL is usually negative for CD5 and CD43 (Fig. 13.10).

Genetics FL is characterized by the translocation between genes 14 and 18, t(14;18), that is identified in up to 90 % of cases, which places the *BCL2* gene on chromosome 18 under the influence of the *IGH* promoter on chromosome 14 [74, 75]. This alteration is an initial genetic event in the pathogenesis of FL. Secondary chromosomal alterations that are typical of FL include gains in 1q, 2p, 7, 8,12q,18q and X as well as losses in 1p, 6q, 10q, 13q and 17p, combinations of which are present in almost all t(14;18)-positive FL [76, 77].

Paediatric follicular lymphoma is a variant of FL that presents as localized lymphadenopathy in children involving cervical lymph nodes or Waldeyer ring. Unlike conventional adult FL, paediatric FL have early-stage disease and typically does not recur or progress, with a highly indolent behaviour. Morphologically, paediatric FL tends to have large expansive follicles with serpiginous shapes. Germinal centre cells are monotonous with blastoid cytologic features. This variant typically is grade 3 with high proliferative index and a 'starry sky' pattern. When involving the tonsils,

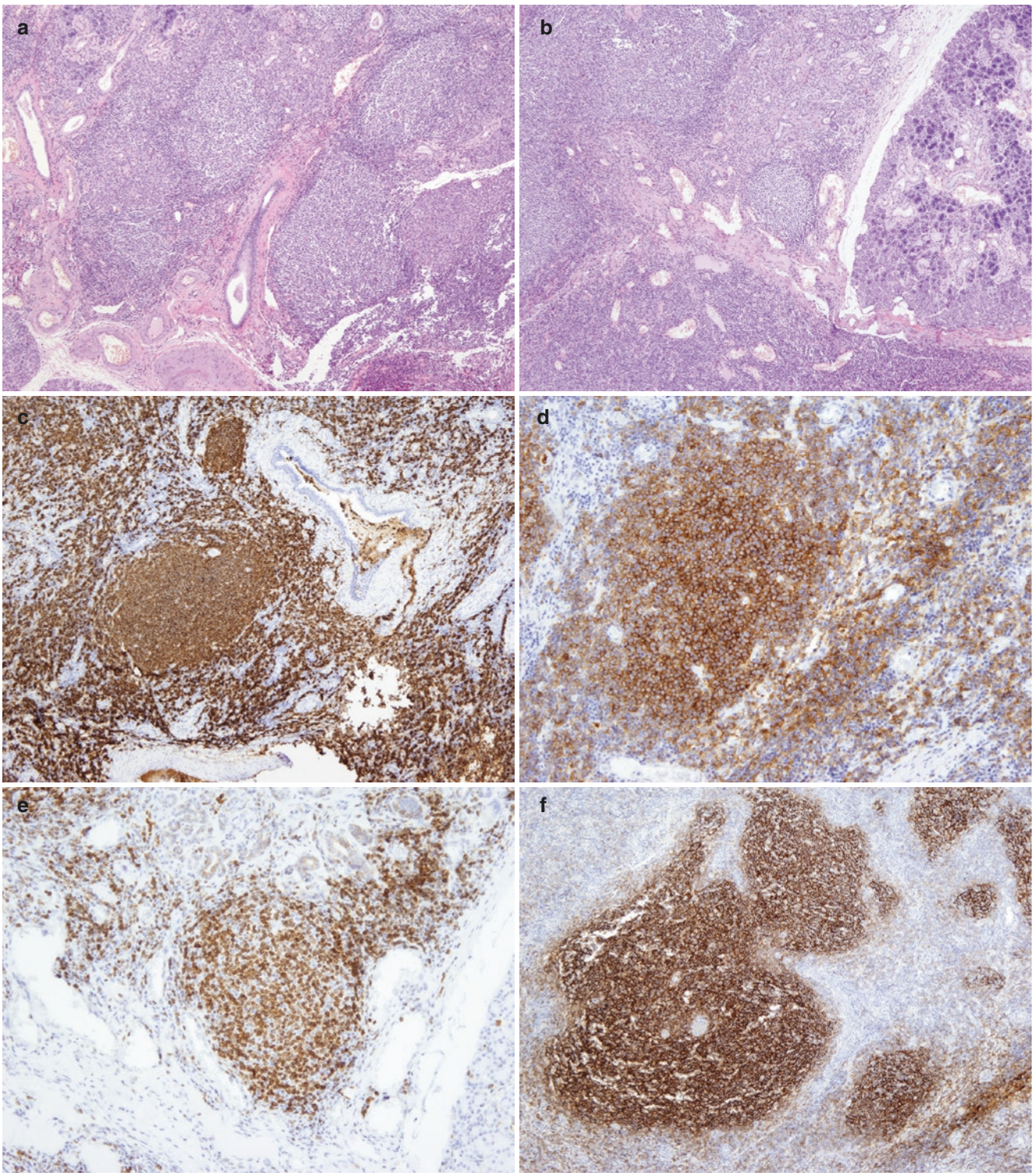

Fig. 13.10 (**a, b**) Follicular lymphoma in parotid gland. Nodular infiltration in the parotid gland composed by small lymphocytes. (**c**) Aggregates are positive for CD20. (**d**) Aggregates are positive for CD10. (**e**) Aggregates are positive for BCL2. (**f**) Aggregates are supported by a meshwork of follicular dendritic cells, highlighted with CD21 stain

neoplastic cells usually express BCL2 and MUM1, whereas when it involves the cervical lymph nodes, it tend to be more frequently BCL2 and MUM1 negative. Unlike conventional FL, this variant usually lacks t(14;18) [78].

Differential diagnosis Reactive follicular hyperplasia is the major differential diagnosis in FL. In the majority of cases, architectural and cytological features are enough to diagnose FL. In difficult cases, overexpression of BCL2 in the atypical cells may distinguish FL and follicular hyperplasia. However, BCL2 is negative in 10–15 % of cases, and this may be due to *BCL2* gene mutations interfering with the antibody recognition, but the tumor still carries the translocation (pseudo-negative) or a real absence of the translocation. In these cases, the FL has *BCL6* rearrangements or other genetic alterations. Therefore, the absence of BCL2 does not exclude lymphoma [79]. Other lymphomas with a nodular pattern may resemble FL and include mantle cell lymphoma, marginal zone lymphoma, small lymphocytic lymphoma and Hodgkin's lymphoma.

Treatment and prognosis Treatment options for patients with naïve or recurrent FL range from a 'watch-and-wait' policy to haematopoietic stem cell transplantation, according to aggressiveness of the disease. Rituximab with chemotherapy is considered the standard treatment of patients in need of treatment [80].

13.2.5.4 Mantle Cell Lymphoma

Definition Mantle cell lymphoma (MCL) is a mature B-cell neoplasm characterized by the t(11;14) and cyclin D1 overexpression that commonly involves the lymph nodes.

Epidemiology Waldeyer ring is involved at presentation in 20 % of patients, generally with stage I or II.

Clinical aspects Many patients present clinical features of stage III or IV with lymphadenopathy and peripheral blood involvement [81, 82].

Microscopy MCL is characterized by a nodular or diffuse proliferation of small to intermediately sized lymphoid cells with irregular nuclei, with dispersed chromatin and inconspicuous nucleoli, and scarce cytoplasm. Scattered single epithelioid histiocytes and hyalinized small vessels are frequently seen. Blastoid and pleomorphic variants of MCL are more aggressive forms, with higher proliferative activity that include a spectrum of intermediate to large cells with round or irregular nuclei and finely dispersed chromatin [2, 83].

Immunohistochemistry The tumor cells have a B-cell phenotype and co-expression of CD5. Most of cases are positive for cyclin D1 and SOX11 and negative for CD10, CD23 and BCL6 (Fig. 13.11). The overexpression of cyclin D1 is very helpful in differential diagnosis, especially in small biopsy specimens.

Genetics Cyclin D1 overexpression is the result of the chromosomal t(11;14), that is present in almost all cases and considered the primary genetic event of the disease. This alteration is usually followed by additional chromosomal aberrations that target genes regulating DNA damage response, cell cycle, and cell survival pathways. Infrequent cases that are negative for t(11;14) and cyclin D1 are still SOX11 positive, and 55 % harbour a translocation involving the gene *CCND2*. Some MCL cases display an indolent behaviour that might not necessitate treatment at diagnosis. These cases may be recognized because frequently present with leukemic non-nodal disease and splenomegaly and lack expression of SOX11 [84, 85].

Treatment and prognosis Immunochemotherapy is the main treatment modality in patients with advanced MCL. For localized disease, combination with radiotherapy may obtain long-term remissions. The median overall survival of patients with advanced MCL is under 5 years [86].

13.2.5.5 Diffuse Large B-Cell Lymphoma

Definition and epidemiology Diffuse large B-cell lymphoma (DLBCL) is a neoplasm of large B-cell lymphocytes, which has a diffuse growth pattern.

Epidemiology It is the most common type of non-Hodgkin lymphoma and accounts approximately for 30 % of cases. It is also the most frequent B-cell lymphoma in the head and neck region [87]. Although DLBCL is classified as a single category, it represents a heterogeneous group of lymphomas and many clinicopathologic variants, distinct subtypes and disease entities. DLBCLs that are not classified into the different subtypes or groups are considered as DLBCL not otherwise classified (NOS).

Clinical aspects Patients with DLBCL involving Waldeyer ring (compared with nodal DLBCL) present more frequently with early-stage disease, absence of B symptoms or bone marrow infiltration, normal serum lactate dehydrogenase and low- to low/intermediate-risk international prognostic index [88, 89].

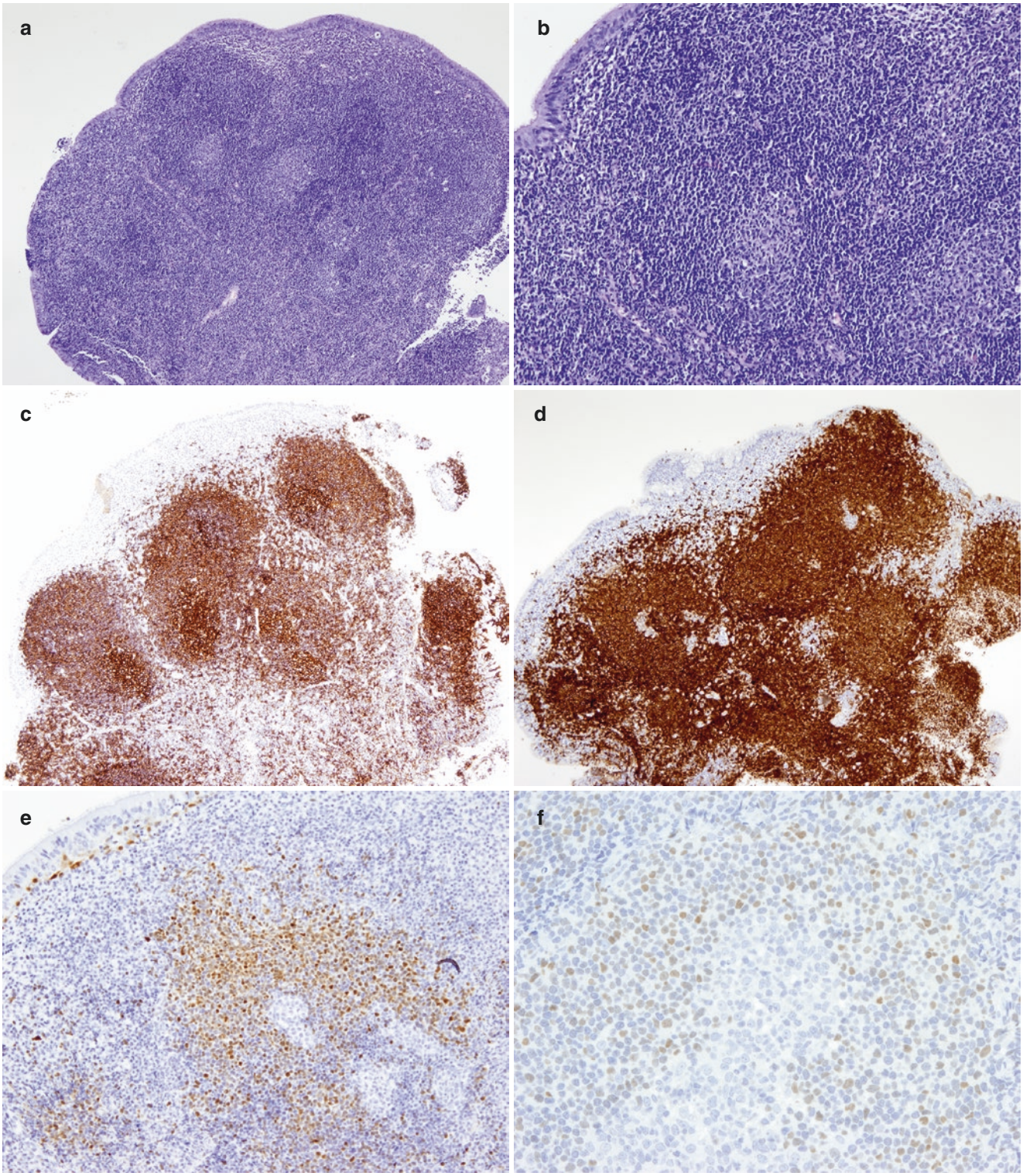

Fig. 13.11 (**a, b**) Mantle cell lymphoma in cavum. The tonsil with germinal centres that present an expansion of the mantle cell zone. (**c**) Cells are positive for CD20. (**d**) Cells co-express CD5. (**e**) Cells are positive for cyclin D1. (**f**) Cells are positive for SOX 11

Microscopy An effacement of the architecture by a diffuse atypical population of large lymphoid cells is identified in the involved tissues. Coexisting low-grade lymphoma should be ruled out for the diagnosis of DLBCL, NOS. The cytological features are also variable and different morphologic variants can be identified. Among them, centroblastic and immunoblastic are the most common. Variable numbers of reactive cells in the background, such as small lymphocytes (mostly T cells), plasma cells, histiocytes and neutrophils, can be seen.

Immunohistochemistry DLBCLs usually express pan-B markers such as PAX-5, CD19, CD20, CD22 and CD79a, with no expression of T-cell markers. Approximately 50 % of cases express BCL2 protein, whereas CD10 expression occurs in 20–40 % of cases and BCL6 in 50–70 %, and IRF4/MUM1 may be expressed in 30–50 % of cases depending on the cut-off used in different studies [89–91]. CD10 and BCL6 are frequently expressed in cases involving the Waldeyer ring in adults. A particular subtype is the 'large B-cell lymphoma IRF4/MUM1 positive' described in children and young adults that affects preferentially the Waldeyer ring and head and neck lymph nodes. Most of these cases have an *IRF4-IGH* translocation [88, 89, 92]. Ki-67 staining usually shows a high proliferation index in all DLBCL (Fig. 13.12).

Genetics DLBCL carry recurrent translocations involving *BCL2*, *BCL6* and *MYC* genes in approximately 20–30 %, 30 % and 10 % of cases, respectively. However, in the Waldeyer ring, the frequency of *BCL2* translocations may be lower (7 %) [88]. Recently, translocations activating *IRF4* have been described in FL/DLBCL of the Waldeyer ring in young patients (Fig. 13.13) [92].

DLBCL can be molecularly divided into germinal centre B-cell-like (GCB) and activated B-cell-like (ABC) subtypes by gene expression profiling (GEP) [93, 94]. GCB-DLBCL associate with the t(14;18) translocation and *C-REL* amplification, which occur with a frequency of approximately 25 % and 15 %, respectively, whereas ABC-DLBCL is frequently associated with constitutive activation of the NF-κB pathway, resulting in enhanced cell proliferation and decreased apoptosis [94–96].

Treatment and prognosis DLBCLs are treated currently with chemotherapy protocols that include anti-CD20 drugs. Many prognostic factors have been suggested for DLBCL. The International Prognostic Index remains the best available index in patients with DLBCL treated with immunochemotherapy. In addition, GEP signatures associate with significantly different survival rates, showing that the patients with ABC-like DLBCL had a poorer outcome [91, 97]. Although this classification can be mimicked by different immunostaining algorithms, their reliability is object of controversy [91].

13.2.5.6 Burkitt Lymphoma

Definition Burkitt lymphoma (BL) is an aggressive B-cell lymphoma of germinal centre derivation composed by highly proliferating mature B cells that carry *MYC* rearrangements with *IG* genes.

Epidemiology BL frequently occurs in extranodal sites in children and young adults, and other sites than the maxilla and mandible are uncommon in the head and neck region. The World Health Organization Classification describes three variants of BL: endemic, sporadic and immunodeficiency-associated types [2]. The endemic BL occurs in African children and involves the bones of the jaw and other facial bones in approximately 50 % of the cases. Other extranodal sites include the kidneys, gastrointestinal tract, ovaries and breast. Thyroid and salivary glands may be involved but at a lower frequency. Sporadic BL occurs worldwide, and the abdominal area is the most common site of involvement. Lymph node involvement is more common among adults than among children. Immunodeficiency-associated BL occurs mainly in HIV-infected patients, and the disease often involves lymph nodes, bone marrow, and extranodal sites, most often in the abdomen.

Microscopy The microscopic features of BL are similar in all the epidemiological forms described. The tumor cells are medium sized and show a monotonous and diffuse pattern of growth. The nuclei are round and may show several small nucleoli. The proliferation rate is very high, and many mitotic figures are identifiable. In addition, many apoptotic figures may be also identified giving a 'starry sky' pattern, since *MYC* activation in BL induces both proliferation and apoptosis. The tumors associated with immunodeficiency states may show plasma cell differentiation.

Immunohistochemistry Burkitt lymphoma is a germinal centre-derived lymphoma, and therefore it is composed of cells with germinal centre phenotype. Thus, in addition to common B-cell markers (CD20, CD79a), the tumor cells express CD10 and BCL6. BCL2 is negative or weakly expressed in some cases and more that 95 % of the cells are positive for Ki67. EBV is positive in nearly all cases of the endemic form, common but not uniformly positive in immunodeficiency-associated BL, and it is present in 15–30 % of sporadic cases.

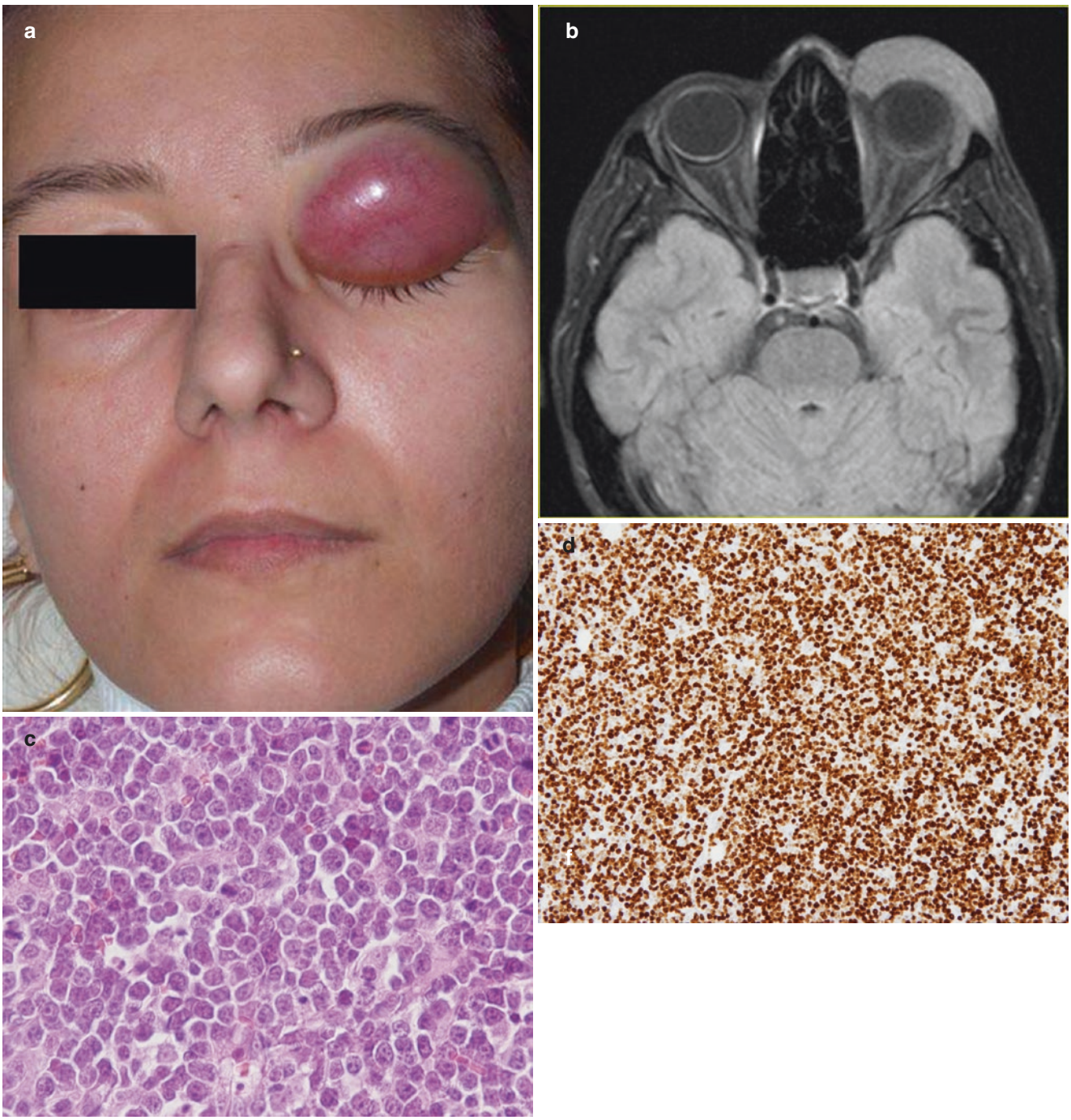

Fig. 13.12 (**a**) Diffuse large B-cell lymphoma. Young woman with a DLBCL in the left eyelid (Courtesy of Dr. P. Claros, Barcelona, Spain). (**b**) CT scan in the same young woman displaying a mass in the left eyelid (Courtesy of Dr. P. Claros, Barcelona, Spain). (**c**) The cells are large with centroblastic appearance. (**d**) Ki67 proliferation rate is very high, nearly 100 %

Genetics The genetic hallmark of BL is the *MYC* translocation, usually associated with few additional alterations [2, 98].

Treatment and prognosis The treatment of BL is similar to acute lymphoblastic leukaemia, and short, intensive chemotherapeutic regimens have obtained excellent response rates, and prognosis has changed significantly with the introduction of these regimens.

13.2.5.7 T-Cell Lymphoma

Definition Peripheral (or mature) T-cell lymphomas (TCL) represent a heterogeneous group of relatively uncommon diseases with mostly aggressive behaviour and poor outcome.

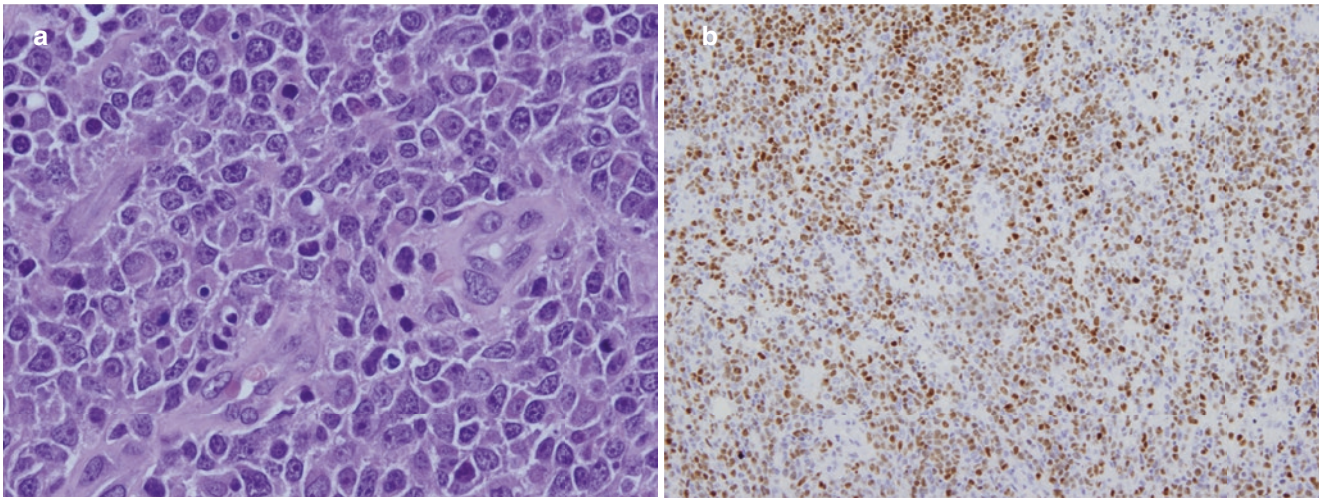

Fig. 13.13 (**a**) Diffuse large B-cell lymphoma. DLBCL arising in a tonsil in a 5-year-old girl. (**b**) Large neoplastic cells are positive for IRF4

Epidemiology They account for 10–15 % of all non-Hodgkin lymphomas worldwide; however, their incidence shows geographic variation, being more common in Asia (up to 20 % of all lymphomas classified as mature TCL) than in Europe or North America [2]. In order of frequency, the most common subtypes of mature TCL are peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS, 26 %), angioimmunoblastic T-cell lymphoma (AITL, 19 %), extranodal NK-/T-cell lymphoma (10 %), adult T-cell leukaemia/lymphoma (ATLL, 10 %), anaplastic large cell lymphoma (ALCL), anaplastic lymphoma kinase (ALK)-positive (ALCL-ALK+, 7 %), anaplastic large cell lymphoma, ALK-negative (ALCL-ALK-, 6 %) and enteropathy-associated T-cell lymphoma (5 %). The incidence of the remaining subtypes is less than 2 % of the total [2, 99].

Clinical aspects In peripheral TCL, the clinical presentation is of major importance for the classification of the disease. Peripheral TCL with predominantly nodal manifestation includes PTCL-NOS, AITL, ALCL-ALK+ and ALCL-ALK-. For these subtypes, involvement of cervical lymph nodes represents a frequent initial manifestation of the disease. However, this occurs in the context of generalized lymphadenopathy rather than as a specific feature for these different entities. ATLL usually presents as disseminated/leukemic disease with widespread lymphadenopathy, whereas involvement of the cervical lymph nodes in extranodal NK-/T-cell lymphoma (discussed in Sect. 13.3.2) and enteropathy-associated T-cell lymphoma, when present, must be considered a late manifestation.

Microscopy The morphological spectrum is very broad. Most commonly, the nodal architecture is diffusely effaced. In PTCL, NOS and AITL, the neoplastic population usually shows variable cytology, with a mixture of medium- to large-sized cells characterized by irregular nuclei and prominent nucleoli. Proliferation of high endothelial venules and inflammatory background with reactive cells (lymphocytes, eosinophils, plasma cells) are frequently observed. Epithelioid histiocytes can be numerous. In ALCL-ALK+ and in ALCL-ALK-, large cells with eccentric, kidney-shaped nuclei and perinuclear eosinophilic region are invariably present (so-called 'hallmark cells'). Finally, in ATLL, the neoplastic population can show a broad cytologic spectrum, ranging from small to large transformed cells with pleomorphic nuclear features.

Immunohistochemistry Neoplastic cells express T-cell markers, such as CD2, CD3, CD5 and CD7, but aberrant loss of any of these pan T-cell markers can be observed. Most cases are TCRαβ+ and CD4+/CD8-. Cytotoxic markers (such as TIA-1) are expressed in ALCL (both ALK+ and ALK-) in addition to CD30. CD10 and follicular helper T-cell markers (BCL6, CXCL13, PD1, ICOS) are usually identified in AITL, in addition to a marked proliferation of follicular dendritic cells [100]. The ALK protein represents the most important marker in ALCL-ALK+ and the immunostaining can be nuclear and cytoplasmic, cytoplasmic only, or rarely in the cell membrane, depending on the ALK translocation partner. In situ hybridization for EBV-encoded RNA (EBER) is variable and is usually positive in the B-cell population present in AITL, whereas it is consistently negative in ALCL-ALK+ and ALCL-ALK- [100].

Genetics TCR rearrangement can be found in most cases. Up to 30 % of the cases of AITL show additional monoclonal immunoglobulin gene rearrangement, particularly those with expansion of EBV-infected B lymphocytes [101]. PTCL-NOS usually show complex karyotypes, and various recurrent

chromosome gains/losses have been described, some with putative prognostic value. ALCL-ALK+ carries a reciprocal translocation involving the gene *ALK* (2p23). In the majority of cases, the translocation partner is nucleophosmin (*NPM*, 5q35), but other genes can be involved (e.g. *TPM3*, 1q25). All translocations up-regulate the *ALK* gene [102].

Treatment and prognosis Peripheral TCL are usually characterized by a poor outcome, with 5-year overall survival rates <40 % with different treatments (usually anthracycline-based combinations, like CHOP) [99].

13.2.5.8 Dendritic Cell Tumors

Definition Follicular dendritic cell (FDC) sarcoma is a neoplastic proliferation with immunophenotypic features similar to those of normal follicular dendritic cells, whereas interdigitating dendritic cell (IDC) sarcoma expresses the phenotype of these cells, which reside in the paracortical areas of the lymph nodes.

Epidemiology Both are very uncommon neoplasms that may involve the head and nek lymph nodes. No age, gender or racial predilection have been appreciated. Most studies in both sarcomas consist of single case reports or small series [103].

Clinical aspects FDC sarcoma rarely has been reported in children. The most common site involved is the cervical lymph nodes, but supraclavicular, axillary, mediastinal, mesenteric, retroperitoneal and lymph nodes may be involved. Extranodal sites, particularly the nasopharyngeal region, may be involved, as well as the liver, spleen and digestive tract. In these patients, simultaneous extranodal and nodal disease may occur. In 10–20 % of patients, there is an association with Castleman's disease, most frequently the hyaline-vascular type. IDC sarcomas (IDCS) do not have topographic nodal predilection and can also be found at extranodal sites, especially in the head and neck [104, 105].

Microscopy Microscopically FDC sarcoma shows a storiform or whorled proliferative pattern. Cytologically, the cells are oval shaped or spindled and contain a clear nuclei with small nucleoli. Binucleated cells are common. Cytologic atypia is variable and most cases are considered as low-grade sarcomas, but it may increase in recurrent or disseminated tumors. Typically, there is an accompanying population of small lymphocytes admixed with the tumor cells.

Immunohistochemistry FDC sarcoma retains one or more follicular dendritic markers such as CD21, CD23, CD35, CNA.42 or KiMp4. Both normal and neoplastic follicular dendritic cells are also positive for clusterin, vimentin, fascin, epidermal growth factor receptor and HLA-DR.

IDCS involve the paracortical areas of the lymph nodes but may infiltrate diffusely. The cells are oval or spindled and atypia is variable. As in FDC sarcomas, associated small lymphocytes are common. Immunohistochemically, the neoplastic cells are characterized by positive staining for S-100, vimentin, HLA-DR or CD68 and negative for CD-1α, CD21, CD35, CD3 or CD20.

Genetics The V600E *BRAF* mutation has been described in 19 % of FDC sarcomas.

Treatment and prognosis Common treatments for FDC and IDC sarcomas consist of surgical excision, systemic chemotherapy or radiotherapy. Surgical resection is the mainstay of treatment in patients with localized disease, but the optimal approach is not well established. The biologic behaviour of FDC sarcoma is similar to low-grade soft tissue sarcomas. Local recurrences occur in 40–50 % of cases and distant metastases in 25 % of patients. IDC sarcoma has more aggressive clinical course, and half the patients die of their disease, generally within 1 year of diagnosis.

13.3 Sinonasal Region and Oral Cavity

13.3.1 EBV Mucocutaneous Ulcer

Definition and epidemiology Epstein-Barr virus-positive mucocutaneous ulcer (EBVMCU) has been recently described as a localized mucosal or cutaneous ulcerative EBV-positive lymphoproliferative disease characterized by a polymorphous proliferation with Hodgkin/Reed-Sternberg (HRS) cell-like morphology and immunophenotype.

Epidemiology This lesion is usually seen in patients with immunosuppression. It affects predominantly adults with a median age of approximately 75 years and a slight female preference.

Clinical aspects EBVMCU presents as an isolated, slowly developing ulcer involving in most cases the oropharyngeal mucosa, lip and facial skin or, less frequently, the gastrointestinal tract and skin of other locations. Local lymphadenopathy can occur, however, with no evidence of systemic lymphadenopathy, hepatoesplenomegaly or bone marrow involvement.

Common to all patients is the immunosuppressive state. This can be due to HIV infection, age-related immunological senescence or administration of immunosuppressors (such as methotrexate, azathioprine and cyclosporine, with or without steroids) for the treatment of autoimmune conditions or following allogeneic stem cell bone marrow transplantation. Patients

with age-related disease tend to be older than those with iatrogenic EBVMCU (median age 80 versus 69) [106–108].

Microscopy The shallow and sharply circumscribed ulcer of EBVMCU presents an underlying polymorphous infiltrate of lymphocytes and immunoblasts, accompanied by plasma cells, histiocytes and eosinophils. Scattered large pleomorphic blasts reminiscent of HRS cells and apoptotic cells with plasmacytoid appearance are present in all cases. A rim of small lymphocytes sharply limits the base of the lesions. In some cases, large cells infiltrate arteries and both thrombosis and necrosis can be observed. Adjacent squamous epithelium can show pseudoepitheliomatous hyperplasia [107, 109].

Immunohistochemistry Large B-cell blasts and HRS-like cells express CD45, PAX5, CD79a, Oct-2 and CD20, although the intensity of the latter may be low. Further, these cells express strongly CD30, with co-expression of CD15 in half of the cases. Large numbers of small B lymphocytes, as well as CD4+ or CD8+ T lymphocytes, are present in the lesion, some of which may be large. In situ hybridization for EBV-encoded RNA (EBER) highlights a large number of small to large pleomorphic cells, which co-express B-cell markers and LMP1. Importantly, T lymphocytes and the adjacent epithelium are negative for EBER [107, 109].

Genetics Monoclonal immunoglobulin gene rearrangement can be found in approximately one third of the cases and this can be accompanied by a restricted T-cell receptor (TCR) pattern. Another third of the cases shows merely monoclonal TCR gene rearrangement. The evidence of clonal T cells might be related to the reduction in the T-cell repertoire diversity [110].

Differential diagnosis This includes other immunodeficiency-associated lymphoproliferative disorders, EBV-positive diffuse large B-cell lymphoma, primary cutaneous CD30 positive T-cell lymphoproliferative disorders and classical Hodgkin lymphoma. Finally, lymphomatoid granulomatosis must be excluded when vasculitic changes are observed.

Treatment and prognosis EBVMCU is characterized by an indolent and self-limited course. In patients with iatrogenic immunosuppression, reduction or discontinuation of the medication leads to complete remission. Radiotherapy and/or chemotherapy have been applied to some patients with achievement of complete remission. However, most cases of age-related immunosuppression regress spontaneously, and only a minority of cases shows relapsing and remitting course. No disease-related deaths have been reported so far [109, 111].

13.3.2 Lymphoid Neoplasms

Mature B-cell lymphomas comprise the vast majority of all upper aerodigestive lymphomas in western countries, while T-cell lymphomas are more frequently in the East. The most common mature B-cell lymphoma neoplasm is DLBCL. A list of the most frequent lymphomas in the head and neck region is summarized in Table 13.1.

13.3.2.1 Plasmablastic Lymphoma

Definition Plasmablastic lymphoma (PBL) is an aggressive B-cell tumor composed by large cells with predominantly immunoblastic morphology and plasma cell immunophenotype.

Clinical aspects PBL occurs predominantly in patients with immunodeficiency, most commonly HIV infection, but also in patients with iatrogenic immunosuppression (transplant and autoimmune diseases) and ageing [90, 112]. PBL is commonly an extranodal tumor that involves the head and neck region, in particular the oral cavity, but the nasal cavity and respiratory sinuses may be also involved. Other sites that may be involved are the skin, digestive tract, soft tissues and lymph nodes.

Table 13.1 Common lymphoid neoplasm of the upper aerodigestive tract (UADT)

Waldeyer ring (approximately 70 % of UADT lymphomas)
Diffuse large B-cell lymphoma
Mantle cell lymphoma
Follicular lymphoma
Extranodal marginal zone lymphoma
Burkitt lymphoma
T-cell lymphoma
Sinonasal region (approximately 13 % of UADT lymphomas)
Diffuse large B-cell lymphoma
NK-/T-cell lymphoma, nasal type
Follicular lymphoma
Small lymphocytic lymphoma
Lymphoblastic lymphoma/leukaemia
Oral cavity (approximately 13 % of UADT lymphomas)
Diffuse large B-cell lymphoma
Plasmablastic lymphoma
Follicular lymphoma
Mantle cell lymphoma
Extranodal marginal cell lymphoma
Plasmacytoma
Lymphoblastic lymphoma
Burkitt lymphoma
Larynx (approximately 4 % of UADT lymphomas)
Diffuse large B-cell lymphoma
Plasmacytoma

[a]Adapted from Mills et al. [87, 150–162]

Microscopy On histological grounds, PBL is composed of a monomorphic and cohesive proliferation of immunoblasts with no or minimal plasmacytic differentiation, but cases with a certain degree of plasmacytic differentiation and additional mature plasma cells may be seen [90]. A 'starry sky' pattern is common in monomorphic cases, and mitotic activity is high and apoptotic bodies are frequent.

Immunohistochemistry The neoplastic cells have an immunophenotype consistent with plasma cell differentiation. Thus, IRF4/MUM1, CD38, CD138, EMA and VS38c are positive, whereas CD79a is variably expressed and PAX5 and CD20 are negative. Immunoglobulins are also variably expressed. EBV is positive in 75% of cases, and HHV8 is constantly negative.

Genetics *MYC* translocations have been identified in approximately 50% of the PBL, more in monomorphic tumors than in cases with plasmacytic differentiation. The rearrangement usually occurs with IG genes in the context of complex karyotypes [113].

Differential diagnosis The differential diagnosis includes plasmablastic plasmacytomas/myeloma, especially in cases of PBL with plasmacytic differentiation. Other DLBCLs with plasmablastic features such as primary effusion lymphoma, HHV8-positive lymphoma, ALK+ large B-cell lymphomas and undifferentiated carcinoma have to be also considered.

Treatment and prognosis Prognosis is generally poor, but some recent reports have observed patients with longer survivals related to the new antiretroviral treatments, better immunologic status of the patients and improvements in the supportive care and delivery of chemotherapy [114].

13.3.2.2 Extranodal NK-/T-Cell Lymphoma, Nasal Type

Definition and epidemiology Extranodal NK-/T-cell lymphoma is a predominantly extranodal lymphoma composed of neoplastic cells expressing cytotoxic markers characterized by vascular damage, prominent necrosis and a strong association with Epstein-Barr virus.

Epidemiology It is an uncommon neoplasm, which affects mostly male adults and is more prevalent among Far-East Asians and patients of Native American ethnicity [99]. It is the second most frequent lymphoma in sinonasal region after diffuse large B-cell lymphoma.

Clinical aspects Extranodal NK-/T-cell lymphoma shows ulceration and destruction of the affected organ or presents with a mass lesion. Symptoms reflect the major site of involvement. Commonly, it involves the upper aerodigestive tract, in particular the nasal cavity and nasopharynx, and presents with early-stage disease. Eventually, it disseminates to adjacent tissues, but bone marrow involvement is rare [115]. Less frequently, extranodal NK-/T-cell lymphoma arises outside the upper aerodigestive tract. These 'extranasal' cases preferably involve the skin, gastrointestinal tract, soft tissue, lung and testis and are associated with systemic symptoms and more advanced disease [116].

Microscopy Biopsies show a dense lymphoid infiltrate, frequently with angiocentric growth pattern, which is accompanied by fibrinoid necrosis of the vessels and by geographic necrosis and/or ulceration of the affected mucosa. The cytological morphology of the neoplastic cells is variable, ranging from small- to large-sized and anaplastic cellular elements. Most frequently, medium-sized neoplastic cells with folded nuclei and pale to clear cytoplasm are recognized. The mitotic rate is high and apoptotic bodies are commonly detected. Cytoplasmic azurophilic granules can be detected in most cases with a Giemsa staining. The accompanying inflammatory infiltrate may be prominent and is usually composed of small lymphocytes, plasma cells and neutrophils.

Immunohistochemistry The neoplastic cells are CD2+, surface CD3− and cytoplasmic CD3ε+. Other T-cell markers (CD4, CD5, CD8) and TCRαβ or TCRγδ are usually not expressed. Most cases are positive for CD56, in contrast with the other NK markers CD57 and CD16, which are absent. Cytotoxic molecules (TIA-1, granzyme B and perforin) are commonly expressed, and CD30 can be detected, though only focally. The proliferation index (Ki-67 staining) is high. In situ hybridization for EBV-encoded RNA (EBER) is nearly always positive and labels all lymphoma cells (Fig. 13.14). Immunohistochemistry for LMP1 is not reliable. In the absence of CD56 expression and EBV infection (in EBER), cases should be classified as peripheral T-cell lymphomas, not otherwise specified [99].

Genetics In most cases, the T-cell receptor and immunoglobulin genes are in germline configuration, and those few cases with clonal rearrangement of the T-cell receptors are presumed to derive from cytotoxic T cells. No specific cytogenetic anomaly has been identified [117].

Differential diagnosis The differential diagnosis entails other EBV-associated T-cell/NK-cell lymphoproliferative disorders. In case of bone marrow infiltration, aggressive

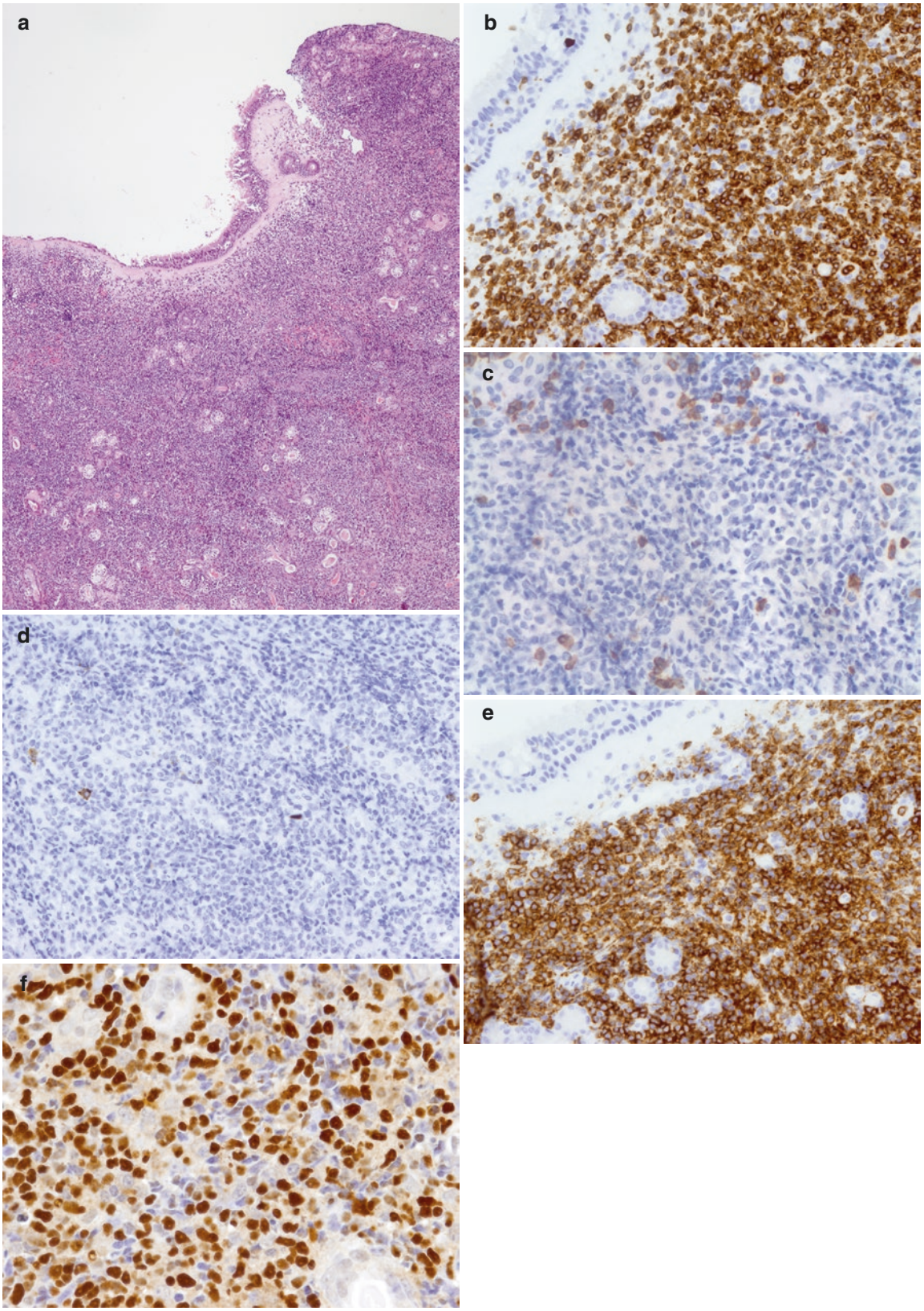

Fig. 13.14 (**a**) Extranodal NK-/T-cell lymphoma. Nasopharynx infiltrate of atypical lymphoid cells. (**b**) Neoplastic cells are positive for CD3. (**c**) Neoplastic cells are negative for CD5. (**d**) Neoplastic cells are negative for CD20. (**e**) Neoplastic cells co-express CD56. (**f**) Neoplastic cells are positive for EBER

NK-cell leukaemia must be considered. Lymphomatoid granulomatosis and subcutaneous panniculitis-like T-cell lymphoma represent other differential diagnoses. Cases composed of small or mixed cellular elements with prominent accompanying inflammatory infiltrate must be distinguished from an inflammatory process. Clinically, primary nasal DLBCL, which has worse outcome than other DLBCL in the head and neck area, has to be considered in the differential diagnosis [118].

Treatment and prognosis Extranodal NK-/T-cell lymphoma involving the upper aerodigestive tract presents initially with localized disease, but frequently progresses and disseminates, despite treatment, with a 5-year survival rate of approximately 50 %. Extranodal NK-/T-cell lymphoma arising outside the upper aerodigestive tract presents with advanced disease, is highly aggressive and shows poor treatment response and short survival [119]. For localized disease, treatment consists of radiotherapy, with or without chemotherapy, whereas chemotherapy is applied to patients with stage III and IV disease [120, 121]. Different protocols have been applied, and recently some L-asparaginase-based regimens have shown promising results [122, 123].

13.4 Salivary Glands, Eye and Ocular Adnexa and the Thyroid

13.4.1 IgG4-Related Diseases

Definition IgG4-related disease is an uncommon sclerosing and inflammatory mass-forming disease that may affect a single organ or be systemic.

Epidemiology The head and neck region is the second most common site to be affected, after the pancreatobiliary system. It usually affects middle-aged and older adults, and the sign and symptoms depend on the affected organs.

Clinical aspects Salivary glands, lacrimal glands and other tissues in the head and neck region can be involved. Salivary gland involvement has been called chronic sclerosing sialadenitis or Kuttner tumor and usually presents as unilateral mass lesion. Chronic sclerosing dacryoadenitis of the lacrimal glands presents with swollen lacrimal glands, swollen eyelids, extraocular muscle swelling, pain, diplopia or proptosis [124, 125]. Involvement of thyroid or IgG4 thyroiditis is more frequently seen in male and presents as a rapid clinical progression with a subclinical hypothyroidism with high level of circulating autoantibodies [126, 127]. All these IgG4-related diseases have an increase of serum IgG4 levels, which are several to more than tenfold that of normal concentrations.

Microscopy The histological pattern of the lesions is similar in all locations, and it is characterized by an acinar atrophy of the glandular tissue with preservation of lobular architecture, a dense chronic inflammation with lymphoplasmacytic infiltrates, absence of prominent lymphoepithelial lesions, obliterative phlebitis and presence of prominent cellular interlobular fibrosis, composed of activated fibroblasts, lymphocytes and plasma cells. Lymphoid follicles with large germinal centres and occasional eosinophils may be seen. In the thyroid, a moderate-to-severe giant cell/histiocyte infiltration may also be present located in the fibrous stroma, where they are mixed with destroyed thyroid follicles, lymphocytes and plasma cells (Fig. 13.15) [124].

Immunohistochemistry Immunohistochemical stains for IgG4 reveal an elevated number of IgG4-positive plasma cells, with a range from more than 60 cells per high power field (HPF) to a few hundred cells/HPF, although a wide range exists. IgG4+/IgG plasma cell ratio is usually high, over 40 %. The lymphoid infiltrate is polyclonal [124].

Treatment and prognosis In cases with localized disease, it may be not necessary treatment, and a watchful waiting is a correct approach. When several or vital organs are affected, glucocorticoids are usually the first-line therapy that can be associated or not with other immunosuppressors. Patients with refractory or recurrent disease can be treated with rituximab, which decreases IgG4 levels sharply [128].

13.4.2 Extranodal Marginal Zone Lymphoma

Definition and epidemiology Extranodal marginal zone lymphoma or mucosa-associated lymphoid tissue (MALT) lymphoma is an extranodal lymphoma composed of heterogeneous small B cells, including marginal zone cells, monocytoid cells and small lymphocytes. Preferentially arises in patients with autoimmune disorders, being Sjögren syndrome the most frequently involved [129].

Clinical aspects It occurs most frequently in the stomach (60–70 %) but has also been described in the head and neck area. Salivary glands, lacrimal glands, conjunctiva, orbit and thyroid are frequent sites of involvement, especially parotid gland and ocular adnexa. MALT lymphoma is characterized by an indolent clinical course. Involvement of the parotid gland usually presents as unilateral or bilateral swelling. MALT lymphoma of the ocular adnexal structures presents with slow onset of erythema, pain, conjunctival chemosis, or distorted vision and has been associated with *Chlamydia*

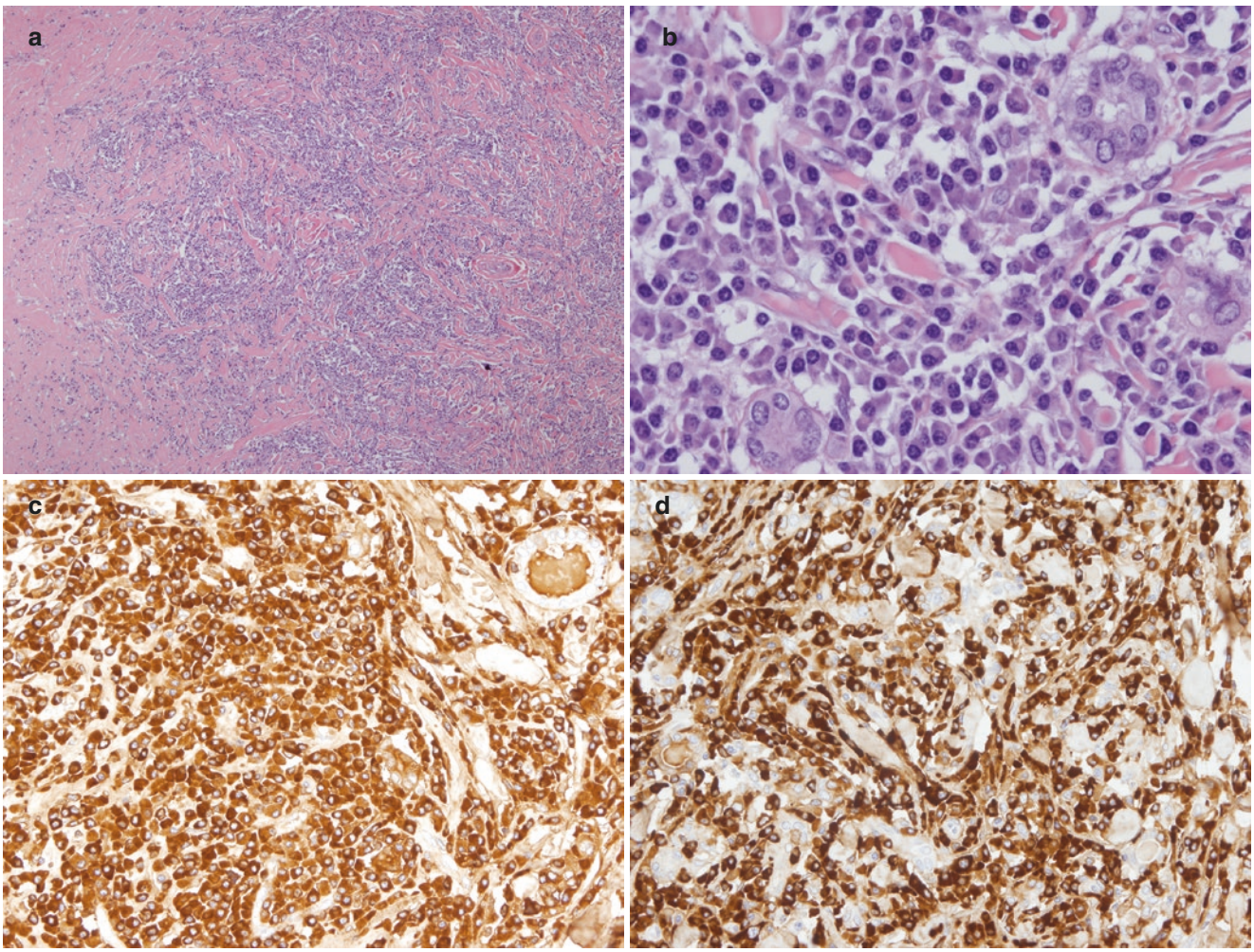

Fig. 13.15 (**a, b**) IgG4 thyroiditis. Atrophic thyroid parenchyma with fibrosis, residual follicles and dense infiltrates of plasma cells. (**c**) These plasma cells express IgG. (**d**) Almost all plasma cells express IgG4

psittaci infection [130–132]. In thyroid, the most common presenting feature is a swelling or a mass in the neck [133, 134].

Microscopy Histologically, MALT lymphoma shows a destructive lymphoid infiltrate with no preserved acinis and frequent lymphoepithelial lesions in the glandular epithelium or conjunctiva. The infiltrate is composed of small B cells with irregular nuclei and inconspicuous nucleoli, sometimes with monocytoid features or plasmacytic differentiation with Dutcher bodies. Most marginal zone B-cell lymphomas arise within pre-existing chronic inflammation, in the context of a lymphoepithelial sialadenitis or associated with Hashimoto's thyroiditis [2].

Immunohistochemistry There are no specific markers for MALT lymphoma. Neoplastic cells express pan B-cell markers and BCL2 and are negative for CD5, CD10 and CD23. Follicular dendritic cell markers reveal an expanded meshwork that may correspond to colonized follicles (Fig. 13.16).

Genetics Trisomy 18 and trisomy 3 are frequent cytogenetic abnormalities found in MALT lymphomas, although translocation involving t(11;18)(API2-MALT1) and t(14;18)(IGH-MALT1) has also been found, especially in salivary gland and ocular adnexa. The t(3;14)(p14.1;q32) involving *IGH* and *FOXP1* is a recurrent translocation in MALT lymphoma affecting the thyroid and ocular adnexa, while it is not found in salivary gland involvement [129, 135–137].

Differential diagnosis The main differential diagnosis is with reactive lymphoid hyperplasia and inflammatory pseudotumour. In some cases of MALT lymphoma with overt follicular colonization it may be difficult to distinguish from follicular lymphomas. In these cases, negativity for CD10,

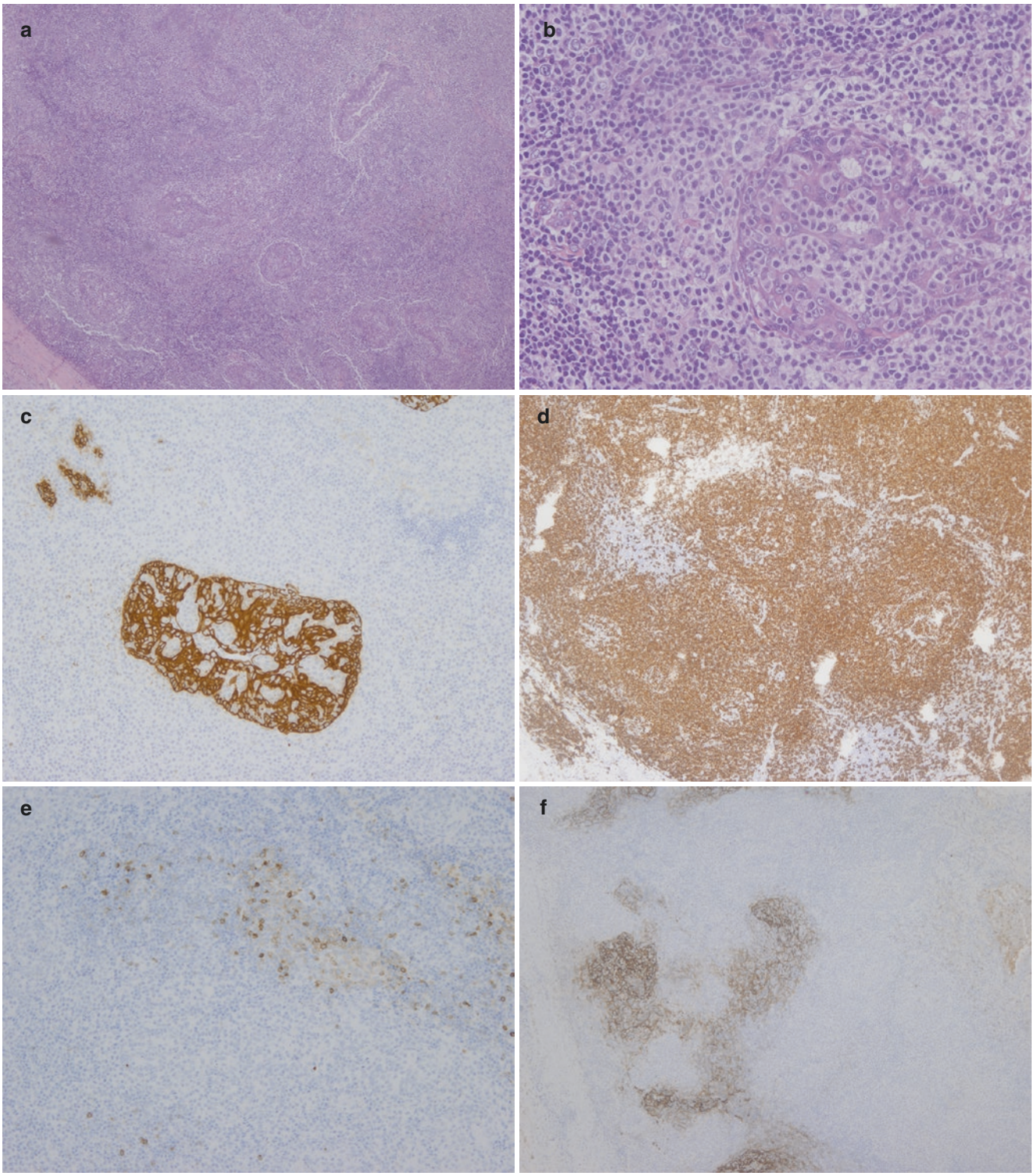

Fig. 13.16 (**a**) Marginal zone lymphoma in parotid gland. Several lymphoepithelial lesions are present within a dense lymphoid infiltrate. (**b**) Lymphoepithelial lesions are characterized by permeation of neoplastic lymphocytes. (**c**) Cytokeratin staining demonstrates the permeation of neoplastic cells into the epithelium. (**d**) The infiltrate corresponds to B cells, positive for CD20. (**e**) CD10 staining shows that neoplastic cells are negative and reveals scattered positive cells corresponding to residual germinal centre cells. (**f**) The presence of germinal centres is better identified with CD21

BCL6 and other germinal centre markers such as LMO2 or HGAL favours the diagnosis of MALT lymphoma.

Treatment and prognosis The clinical course of this lymphoma is indolent and usually remains localized, although transformation to diffuse large B-cell lymphomas sometimes occurs [138]. Patients can be treated with surgery, chemotherapy or local radiotherapy, depending of clinical status and grade of extension of the disease. There is evidence of variable association between *Chlamydia psittaci* and ocular adnexa lymphomas in different countries, and some patients respond to antibiotic therapy with doxycycline[139–141].

13.5 Craniofacial Bones

13.5.1 Plasma Cell Neoplasms

Definition The plasma cell neoplasms are clonal proliferations of plasma cells or lymphocytes that produce a single class of immunoglobulin detectable on serum or urine as a monoclonal protein (M protein).

Clinical aspects Most plasma cell neoplasms arise in the bone marrow, but may have extramedullary presentations in 5 % of cases. Plasma cell myeloma is a multifocal neoplasm with disseminated marrow involvement. Other organs are rarely affected. The diagnosis is based on the combination of clinical, morphologic, immunologic and radiographic features. Solitary plasmacytoma of the bone is morphologically similar to plasma cell myeloma, but it is localized and lacks the additional clinical features typical of plasma cell myeloma. The skull is frequently involved by these neoplasms.

Extramedullary plasmacytomas are localized plasma cell tumors that arise in tissues outside of the bone marrow. Approximately 75 % of them occur in the upper respiratory tract, particularly in the nasal cavity, sinuses, oropharynx and larynx, as well as the parotid and thyroid gland. Regional lymph nodes are involved in approximately 15 % of cases. Extramedullary plasmacytomas occur in adults and two thirds of patients are male. Clinically they present as solitary tumors with no bone marrow involvement [2].

Microscopy The cytological features may be variable depending on the amount of immature plasmablastic cells. Mature tumors are composed of plasma cells usually indistinguishable from normal plasma cells, whereas plasmablastic plasmacytomas contain high number of plasmablasts admixed with more proplasma cells and mature plasma cells [90]. Mature plasma cells are oval, show eccentric nuclei with 'spoke wheel' chromatin and abundant basophilic cytoplasm with a perinuclear hof (Fig. 13.17). The plasmablasts show an immunoblastic morphology, with a large and eosinophilic central nucleoli, and a similar cytoplasm to plasma cells. Multinucleated and pleomorphic cells may be prominent in some cases.

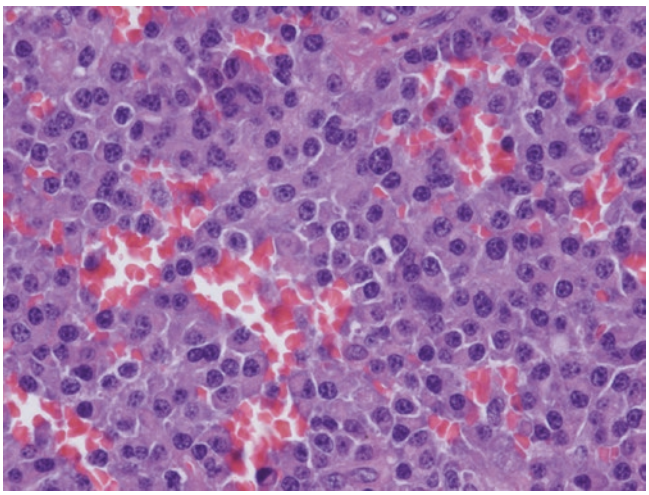

Fig. 13.17 Plasmocytoma. Neoplasm composed of almost entirely mature appearing plasma cells

Immunohistochemistry The immunophenotype shows light chain restriction associated with plasma cell phenotype. The expression of CD19, CD20 and CD79 is absent or weak, and CD38, CD138 and the transcription factor IRF4/MUM1 are positive. EMA may be expressed in lymphomas with plasma cell differentiation. CD56 is positive in approximately 80 % of plasma cell myelomas, and its negativity has been associated with aggressive behaviour and primary or secondary plasma cell leukaemia [142, 143]. In our experience, a similar proportion of extramedullary plasmacytomas maintain CD56 expression [90]. Cyclin D1 may be expressed in few cases.

Differential diagnosis It includes low-grade and high-grade lymphomas with plasma cell differentiation arising in the head and neck area such as extranodal marginal zone lymphoma, lymphoplasmacytic lymphoma and diffuse large cell lymphoma, in particular plasmablastic lymphoma. Reactive conditions may be difficult to distinguish because of the large amount of plasma cells residing in this area, but the pattern of immunoglobulin light chains should help in these cases.

Treatment and prognosis The treatment of extramedullary plasmacytomas is surgical, usually accompanied with radiotherapy. In 25 % of patients, there is local recurrence or spread to regional lymph nodes, and about 15 % may progress to plasma cell myeloma. The behaviour of extramedul-

lary plasmacytomas that are not a progression of medullary plasma cell myeloma is usually indolent. On the contrary, extramedullary spread of a medullary plasma cell myeloma is associated with poor prognosis [144].

13.5.2 Myeloid Sarcoma

Definition Myeloid sarcoma (MS) is a proliferation of myeloid blasts with or without maturation that involves extramedullary sites. MS can be associated with a concurrent myeloid neoplasm involving the bone marrow, but in some cases, the bone marrow is negative. Tissue infiltration by myeloid blasts in patients with overt leukaemia is not considered as MS. The disease also can occur in association with myelodysplastic syndromes, myelodysplastic/myeloproliferative neoplasms and myeloproliferative neoplasms as manifestation of a blastic transformation [2, 145].

Clinical aspects Myeloid sarcoma shows variability in presentation and almost any site may be involved [2]. The most common areas is the skin, followed by central nervous system, lymph nodes, bones, gonads and other internal organs. The orbit, skull bones, and paranasal sinuses are the most common sites involved in the head and neck location. A common presentation in paediatric patients is unilateral exophthalmos due to orbit involvement. In this population, MS is more often diagnosed during the initial course of acute myeloid leukaemia or during remission; approximately one quarter of children with acute myeloid leukaemia develop myeloid sarcoma [146]. In adults, the oral cavity is a commonly involved site, and in these patients, the clinical presentation is nonspecific and MS may mimic inflammatory diseases or other types of malignancies [147].

Microscopy The histology of MS is characterized by sheets of mononuclear cells that efface the tissue architecture. In the lymph nodes, an interfollicular pattern may be observed. The blast cells may be monotonous or have admixed maturing cells. The blasts may have round to folded nuclei, usually with fine nuclear chromatin.

Immunohistochemistry Immunophenotyping identifies myeloid or myelomonocytic markers such as CD13, CD33, myeloperoxidase, CD14, CD64, CD43, lysozyme and CD68. Approximately 50 % of cases are CD34+, but CD117 expression is more common. B- or T-cell markers are negative [2].

Treatment and prognosis The clinical significance of myeloid sarcoma remains unclear, and it is not clear whether myeloid sarcoma confers specific prognosis. Patients should be treated with chemotherapy regimens similar to acute myeloid leukaemia.

13.5.3 Langerhans Cell Histiocytosis

Definition Langerhans cell histiocytosis (LCH) is a disease with several clinical manifestations characterized by the accumulation of cells similar to cutaneous Langerhans cells that express CD1a, langerin and S-100 protein and may contain Birbeck granules detected by ultrastructural microscopy [2, 148].

Epidemiology LCH occurs most commonly in childhood to elderly, with a peak incidence from 1 to 3 years. In adults, the disease tends to be more chronic than in children.

Clinical aspects The clinical presentation of LCH may be as a localized (solitary), multifocal or disseminated (multisystem) disease. Localized disease may involve the bone, presenting as lytic lesions, and less frequently the lymph nodes, skin and lung, where the disease presents as enlarged lymph nodes. Cervical, as well as inguinal, axillary, mediastinal, or retroperitoneal lymph nodes may be involved. The thyroid is not an unusual site of localized LCH [149] and the diagnosis may be obtained by aspiration cytology or biopsy. Multifocal disease occurs in older children and adults, and bone and adjacent soft tissues are usually involved. Multisystem disease occurs most frequently in infants, and the skin, bone, liver, spleen and bone marrow are the most common sites involved. LCH is very uncommon in the upper respiratory tract and only isolated cases have been reported.

Microscopy On morphological grounds, the lesions are composed by a monotonous population of Langerhans-like cells admixed with variable proportions of histiocytes and eosinophils. LCH cells have characteristic nuclear features with oval, bland and folded morphology. The oval shape of LCH cells and the absence of cytoplasmic extensions are the morphological differences with Langerhans cells of the skin.

Immunohistochemistry LCH express CD1a, langerin and nuclear and cytoplasm S-100 (Fig. 13.18). Birbeck granules are identifiable by ultrastructural studies.

Genetics Activating *BRAF* mutations in 57 % of LCH has been recently described [148].

Treatment and prognosis No histologic features predict outcome of the disease, but cytologic atypia and atypical mitoses may suggest Langerhans cell sarcoma. The prognosis of the disease is related to the stage at presentation [2].

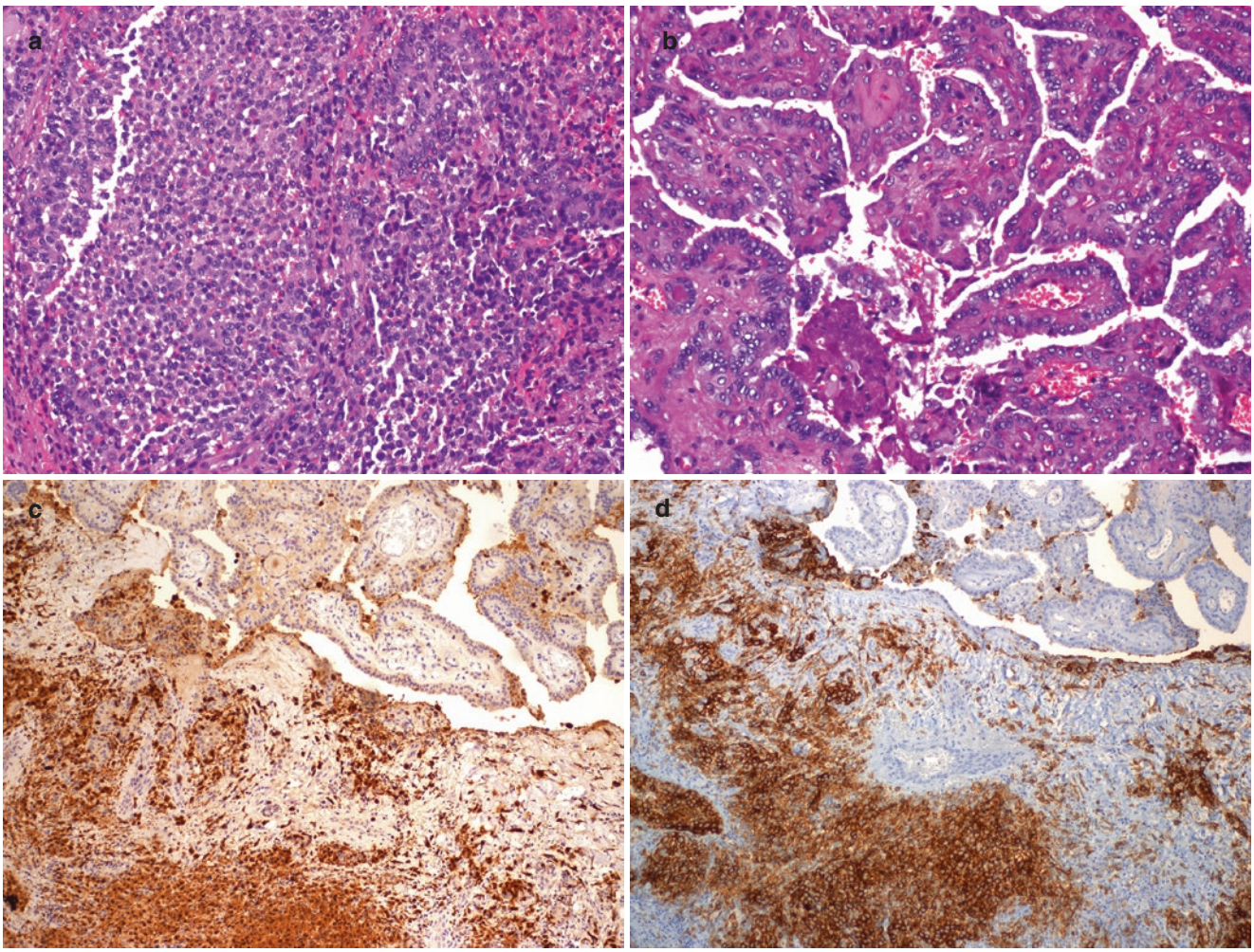

Fig. 13.18 (**a**) Langerhans cell histiocytosis in thyroid. Atypical population in the thyroid composed of medium to large cells with oval, bland and indented nuclei. (**b**) These cells are close to foci of papillary thyroid carcinoma. (**c**) The LCH cells are positive for S100. (**d**) The LCH cells are positive for CD1a. Unlike Langerhans cell histiocytosis, papillary thyroid carcinoma cells are negative for S100 and CD1a

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Thyroid and Parathyroid Glands

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Catarina Eloy, Paula Soares, Valdemar Máximo,
and Manuel Sobrinho-Simões

Contents

C. Eloy, MD, PhD (✉) • P. Soares, BSc, MSc, PhD
V. Máximo, BSc, MSc, PhD
IPATIMUP (Institute of Molecular Pathology and Immunology of the University of Porto), Rua Júlio Amaral de Carvalho, 45, 4200-319 Porto, Portugal

Pathology and Oncology Department, Medical Faculty of the University of Porto, Alameda Prof. Hernâni Monteiro, 4200-135 Porto, Portugal
e-mail: celoy@ipatimup.pt; psoares@ipatimup.pt; vmaximo@ipatimup.pt

M. Sobrinho-Simões, MD, PhD
IPATIMUP (Institute of Molecular Pathology and Immunology of the University of Porto), Rua Júlio Amaral de Carvalho, 45, 4200-135 Porto, Portugal

Pathology and Oncology Department, Medical Faculty of the University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal

Anatomic Pathology Service, Centro Hospitalar de São João, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal
e-mail: ssimoes@ipatimup.pt

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14.1 Thyroid Development and Normal Histology

The thyroid gland is an endocrine organ located in the neck that is responsible for the production and controlled release of thyroid hormones. The thyroid hormones are essential to fetal development and regulation of many metabolic processes.

The thyroid median *primordium* derives from the endoderm and appears at the foramen *cecum* of the tongue by the 26th day of fetal life. From this cephalic location, the median *primordium* descends to its final position anteriorly to the second to fourth tracheal ring and expands laterally to form the lateral lobes. At this stage, by the ninth to tenth week of fetal life, the thyroid parenchyma is organized into solid and trabecular structures with small *lumina* where thyroglobulin-positive colloid starts to be produced (Fig. 14.1a, b). The well-formed follicles that constitute the functional units of the thyroid appear 2 weeks later. The follicles are composed by cuboid follicular cells that face colloid-filled lumina and are surrounded by a basement membrane.

Concurrently, at sixth to seventh week of fetal life, the two thyroid lateral *primordia* derived from the ultimobranchial bodies (fourth to fifth branchial pouches complex) are integrated in the lateral lobes of the thyroid carrying the neural crest-derived C cells and the endoderm-derived main cells. The C cells are neuroendocrine cells responsible for the production of calcitonin – a protein involved in the phospho-calcium homeostasis – and are difficult to distinguish from follicular cells as they often integrate the follicles. The main cells can be detected in the so-called solid cell nests (Fig. 14.2). The solid cell nests are normal elements of the adult thyroid occurring in up to 68 % of adult thyroids [1] that can harbor C cells together with the main cells. The solid cell nests can become hyperplastic [2] and are thought to represent nests of putative thyroid stem cells [3]. Other intrathyroidal tissues, presumably of branchial pouch origin, include islands of mature cartilage and ectopic thymus, parathyroid, and salivary glands [4–6].

The normal adult thyroid with all its components is organized in lobules separated by fibrous septa conveying nervous bundles and vessels, including a rich lymphatic network that coalesces in the perithyroid soft tissue condensation and drains to the cervical and mediastinic lymph nodes (Fig. 14.3).

The follicular cell, under a hostile environment like chronic inflammation and/or in senescent *status*, may acquire particular phenotypes. Examples of these phenotypes include the oncocytic transformation and the squamous and ductal metaplasia of the follicular epithelium [7]. The oncocytic phenotype results from the accumulation in the cytoplasm of numerous and abnormal mitochondria and can also occur in both benign and malignant tumor cells [8]. Similarly, with age, the colloid tends to coalesce in globular formations and to accumulate calcium oxalate crystals.

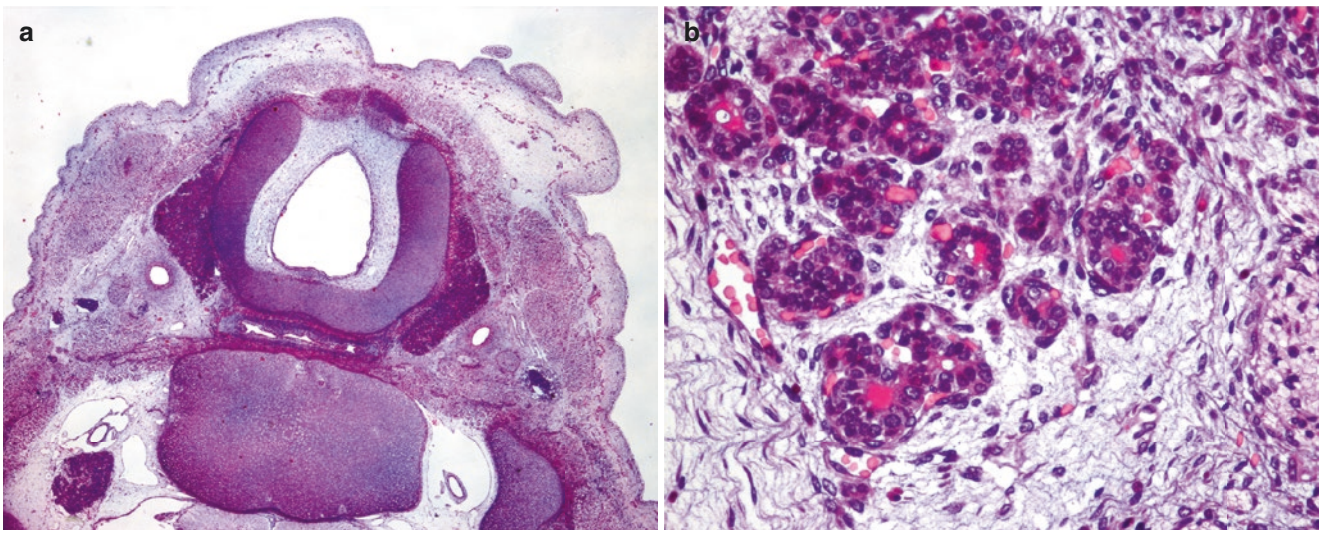

Fig. 14.1 (**a**) Thyroid aspect at 12th gestation week, at low magnification. (**b**) Thyroid aspect at 12th gestation week at high magnification

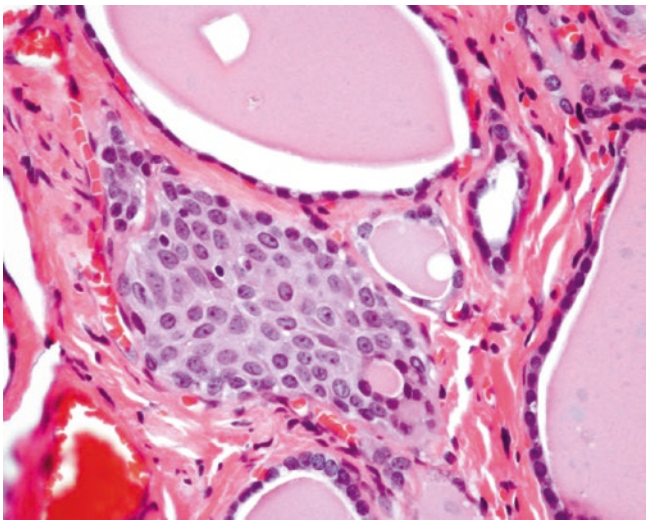

Fig. 14.2 Solid cell nest in an adult thyroid

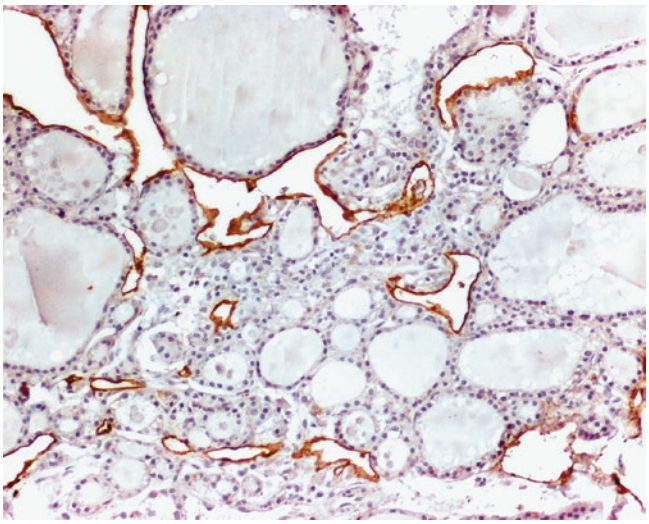

Fig. 14.3 Rich lymphatic network in an adult thyroid highlighted by the D2-40 immunostaining

14.2 Thyroid Tissue in Abnormal Locations

14.2.1 Thyroglossal Duct Remnants

Definition, etiology, and pathogenesis A persistent thyroglossal duct can manifest as a sinus tract, connected either to the foramen cecum or to the suprasternal notch, or in the form of a blunted end tubular structure which occurs in the region of the hyoid bone.

Epidemiology It occurs in nearly 7 % of the population and can harbor malignancy in 1 % of the cases [9]. The persistence of the thyroglossal duct along the cervical midline can result in anomalies that become present in childhood or, less frequently, later in life.

Macroscopy The cystic change of these tracts or tubular structures is designated as thyroglossal duct cyst and constitutes the most frequent congenital cervical anomaly [9] (Fig. 14.4).

Microscopy The lining of these abnormal cysts is a pseudostratified ciliated, columnar, transitional, and/or squamous epithelium, often displaying erosions (Fig. 14.5). The underlying stroma may be infiltrated by inflammatory cells and usually discloses mucous glands and thyroid follicles.

Treatment and prognosis The neoplastic transformation of thyroglossal cyst duct structures can affect the epithelial lining of the cyst manifesting as a squamous cell carcinoma or affect the thyroid tissue usually in the form of papillary thyroid carcinoma (PTC) [10]. The treatment of these malformative lesions with or without neoplastic transformation is complete surgical removal of the tract, along with the middle third of the hyoid bone, to minimize recurrence [11].

14.2.2 Heterotopic Tissue

Definition and etiology Heterotopic thyroid tissue consists in thyroid tissue located outside its normal location that can be found anywhere along the course of the thyroglossal duct, in the so-called Wolfler area that is a triangle with the base located at the edge of the mandible and the apex in the concavity of the aortic arch [12]. The most frequent location of heterotopic thyroid tissue is the base of the tongue, but it can also be found in the anterior tongue, submandibular region, larynx, trachea, mediastinum, retroperitoneum, heart, and *sella turcica* [13–16]. It is likely that most of the mediastinic-located thyroid tissues with nodular adenomatous transformation result from the gravity effect over a previously located voluminous goiter.

Heterotopic locations of thyroid tissue distant from the tract of the thyroglossal duct include the wall of duodenum, gallbladder, *porta hepatis*, adrenal gland, fallopian tube, and vagina [17–19].

Epidemiology Lingual thyroid has been found in 10 % of normal individuals without symptoms. In nearly 70 % of patients with grossly evident heterotopic thyroid, there is absence of normal thyroid.

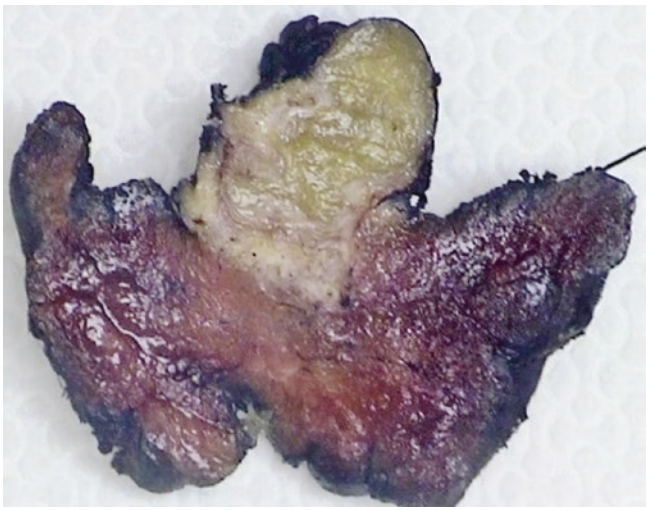

Fig. 14.4 Macroscopic appearance of thyroglossal duct cyst with a colloid-type content attached to the thyroid

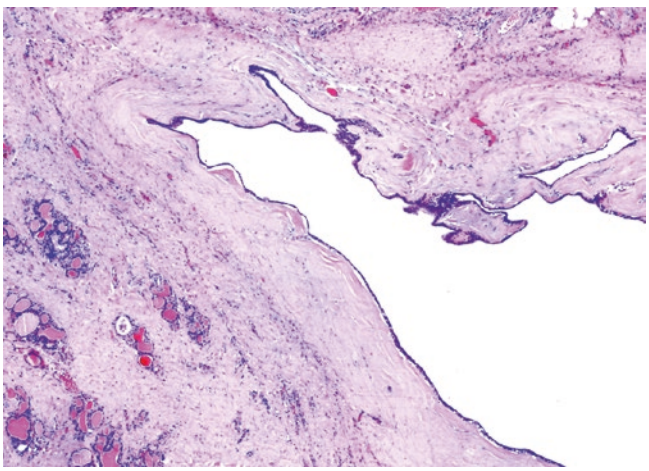

Fig. 14.5 Microscopic aspect of thyroglossal duct cyst with squamous lining and adjacent thyroid follicles

Clinical aspects Lingual thyroid can become clinically evident due to mass effect, resulting in dysphagia and respiratory obstruction [20].

Microscopy Heterotopic thyroid follicles usually have a normal appearance. The irregular interface between the heterotopic follicles and the surrounding soft tissues must not be confused with malignancy.

Differential diagnosis Heterotopic thyroid may be difficult to distinguish from the rare mechanic implantation of thyroid tissue in the neck, as a result of surgical intervention or accidental trauma. The iatrogenic origin of the tissue is usually supported by its coexistence with suture material.

Treatment and prognosis Thyroid tissue in heterotopic locations can develop diseases similar to those that occur in the main gland. Removal of the heterotopic thyroid tissue may lead to hypothyroidism.

14.2.3 Parasitic Nodules

Definition and macroscopy Parasitic nodules, also called sequestered nodules or accessory nodules [21], are the expression of adenomatous goiter, Hashimoto thyroiditis, or Graves' disease [22] in which one or more of the most peripherally located nodules are anatomically separated from the main gland. While in some cases a thin pedicle may be found joining the nodule to the main gland, in other cases the connection with the thyroid may be lost (Fig. 14.6).

Differential diagnosis Parasitic nodules may be found in the neck or mediastinum and should not be confused with heterotopically located thyroid, mechanical implants, or with malignant neoplasms.

When the nodule is detected in the setting of Hashimoto thyroiditis, it can be difficult to distinguish from a metastatic lymph node due to the exuberance of the lymphoid component.

14.2.4 Inclusions in Cervical Lymph Nodes

Definition and pathogenesis The existence of inclusions of normal thyroid follicles within a cervical lymph nodes, also called lateral aberrant thyroid, is still a matter of controversy in the literature [21, 23].

Differential diagnosis Some authors deny the occurrence of nodal inclusions of normal thyroid tissue claiming that these "inclusions" represent metastatic foci from occult PTC [24]. In case multiple lymph nodes are affected or the cytoarchitectural features of the intranodal follicles are typical of PTC, the possibility of metastasis should be advanced. In case the thyroid gland has no signs of malignancy and the intranodal thyroid tissue consists of scant, normal-appearing peripherally located follicles, the possibility of an inclusion has to be considered.

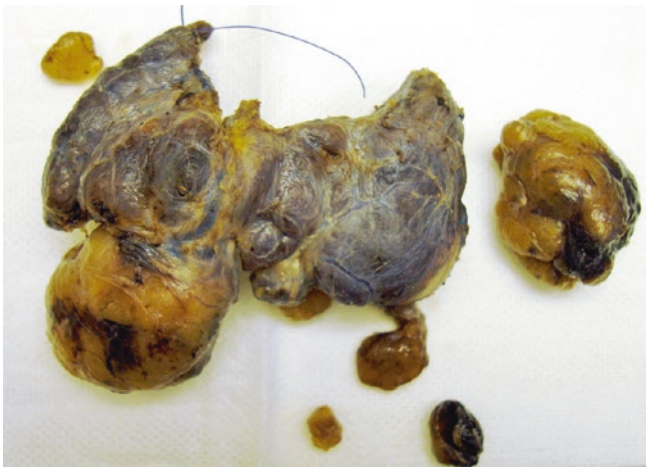

Fig. 14.6 Parasitic nodules in the setting of adenomatous goiter

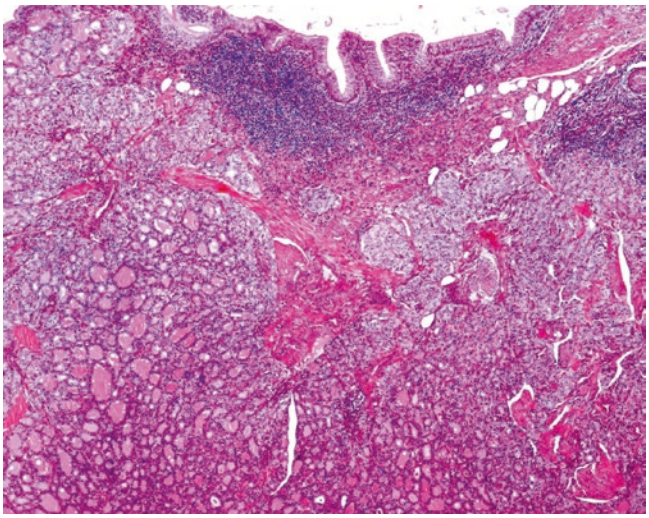

Fig. 14.7 Normal-appearing thyroid tissue as a component of ovarian teratoma

14.2.5 Thyroid as a Component of Teratoma

Definition and epidemiology Thyroid tissue is a relatively common component of teratomas, mainly ovarian teratomas. When the thyroid tissue is the predominant or single component of an ovarian teratoma, the designation *struma ovarii* is used (Fig. 14.7).

Clinical aspects It can be associated with hyperthyroidism.

Microscopy In the ovarian location, the thyroid tissue may appear normal, may display Hashimoto thyroiditis changes, or may exhibit adenomatous alterations.

Microscopy The sequestered nodule is subject to the same morphological alterations as the main gland.

Differential diagnosis *Struma ovarii* should be distinguished from the extremely rare thyroid carcinoma that metastasizes to the ovary [25]. In case the ovarian located thyroid tissue coexists with a well-differentiated neuroendocrine tumor or the carcinoid type, it is designated as *struma carcinoid*.

Genetics The study of the genetic alterations present in *struma ovarii* shows the existence of a genotype-phenotype association similar to the one present in cervical thyroid tumors.

The majority of the benign *struma ovarii* are negative for genetic alterations (only one case has been reported harboring a *RET/PTC* rearrangement). In the malignant *struma ovarii* cases reported to date, the prevalence of *RET/PTC* rearrangements is high and frequently associated with the occurrence of follicular variant of PTC and *RAS* mutations; $BRAF^{K601E}$ and $BRAF^{TV599\text{-}600M}$ and *PAX8/PPARγ* alterations have also been described in this setting [26]. The most frequent alteration in cases of malignant *struma ovarii* presenting as classic PTC is the $BRAF^{V600E}$ mutation.

Treatment and prognosis The treatment is surgical excision. Malignant neoplasms developing in this setting have been described, namely, PTC.

14.3 Thyroiditis

14.3.1 Acute Thyroiditis

Definition Acute thyroiditis is an acute inflammation of the thyroid.

Epidemiology Occurs mainly in elderly and immune-compromised patients but has been reported to occur also in children.

Etiology and pathogenesis Acute thyroiditis is usually due to thyroid infection by bacteria; fungi, such as *Pneumocystis spp.*; or viruses [27–32]. It may occur after respiratory tract infections, sepsis, or open wounds in the neck region. A case

of a patient who developed a thyroid abscess after a fish bone injury in the throat has been reported [33].

Clinical aspects The ultrasound findings include the observation of a hypoechoic lesion that can be restricted to the thyroid or involve the tissues around the gland and, in more severe cases, destruction of the thyroid lobe and abscess formation in the neck [34]. Ultrasound examination of the thyroid associated with fine needle aspiration biopsy (FNAB) and cytology can assist in confirming the early diagnosis of acute suppurative thyroiditis [35].

Microscopy Acute thyroiditis is characterized by inflammatory infiltrates composed by neutrophils, tissue necrosis, and abscess formation (Fig. 14.8a, b).

Treatment and prognosis Acute suppurative thyroiditis has usually an excellent prognosis and is treated with antibiotics and, if necessary, surgical drainage of the abscess [33]. It can be complicated by potential life-threatening thyrotoxicosis [28, 29], sepsis [36], compression of large vessels of the cervical and mediastinal regions [36], pyriform sinus tract fistula [37], and recurrent nerve palsy [38].

14.3.2 Subacute Thyroiditis

Definition and etiology Subacute thyroiditis, also known as de Quervain thyroiditis or granulomatous thyroiditis, is an inflammatory disease with unknown etiology. Some authors proposed a viral origin for subacute thyroiditis occurring after respiratory tract infections and for cases occurring in members of the same family [32, 39, 40].

Epidemiology Occurs mainly in middle-aged women.

Clinical aspects Manifests as a sore throat, odynophagia, and pain on palpation of the neck, often associated with fever [40, 41]. The involvement of the thyroid is usually bilateral and diffuse and manifests as a diffuse goiter or as a unilateral enlargement of the gland, followed by hypothyroidism in nearly 30 % of cases [42].

The diagnosis of subacute thyroiditis is based on clinical and ultrasound data that may be supported by FNAB findings if necessary.

Macroscopy Subacute thyroiditis is characterized by an enlargement of the gland without adherences to the surrounding tissues.

Microscopy The thyroid parenchyma is distorted by fibrosis, lymphocytic infiltration, and multiple granulomas containing foreign body-type giant cells that are follicle centered (Fig. 14.9a). Images of colloid phagocytosis are common, while caseous necrosis is absent (Fig. 14.9b). In the FNAB setting, the observation of multinucleated giant cells in the background of small lymphocytes leads to the diagnosis and prevent unnecessary surgery.

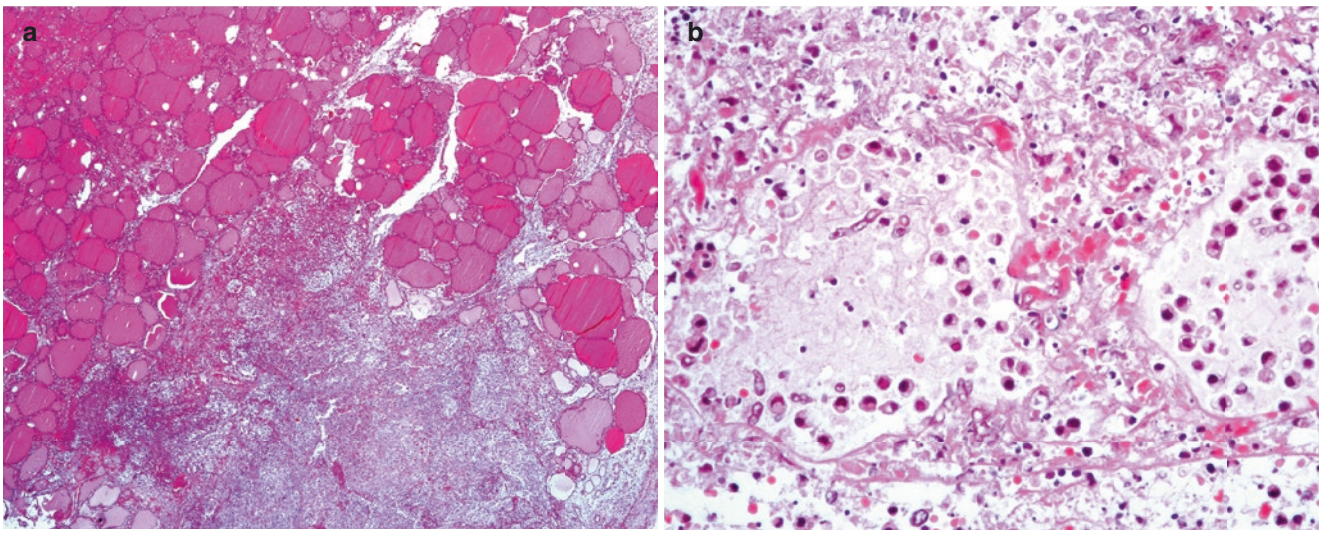

Fig. 14.8 (**a**) Microscopic aspect of thyroid abscess due to *Aspergillus spp.* infection, at low magnification. (**b**) Microscopic aspect of thyroid abscess due to *Aspergillus spp.* infection at high magnification

Differential diagnosis Subacute thyroiditis must be distinguished from Riedel thyroiditis that is a paucicellular lesion with strong adherences to the surrounding neck tissues. Both Graves-Basedow's disease and Hashimoto thyroiditis may be difficult to distinguish from subacute thyroiditis and rarely may coexist with it [43, 44]. Other granulomatous lesions that may be confused with subacute thyroiditis include palpation thyroiditis, tuberculosis [45], sarcoidosis [46], sarcoid-like granulomas in the setting of FNAB [47], and Wegener granulomatosis [48] (Table 14.1).

Treatment and prognosis The initial treatment of subacute thyroiditis is based upon corticoid therapy that, in most cases, is sufficient to achieve complete resolution [41].

14.3.3 Palpation Thyroiditis

Definition and etiology Palpation thyroiditis, also known as multifocal granulomatous folliculitis, is thought to result from manipulation during neck inspection at clinical examination or during surgery.

Pathogenesis It is a histological finding rather than a true lesion.

Clinical aspects Palpation thyroiditis is usually clinically insignificant although it can manifest as hyperthyroidism and atrial fibrillation [49].

Microscopy Consists of foci of lymphocytes, foam cells, and multinucleated giant cells into the lumina of follicular structures or surrounding them (Fig. 14.10).

Differential diagnosis It should not be confused with the diffuse lesions observed in subacute thyroiditis nor with other granulomatous diseases.

14.3.4 Tuberculosis

Definition Thyroid involvement by tuberculosis.

Epidemiology It is a rare condition in the thyroid.

Pathogenesis and macroscopy Manifests in two forms: milliary spread as part of a generalized dissemination and as focal caseous tuberculosis that can mimic carcinoma [50, 51]. Often, the focal involvement of the thyroid results from the local spread from a nodal or laryngeal tuberculosis.

Microscopy The microscopic features of cytological or histological specimens of thyroid with tuberculosis are similar

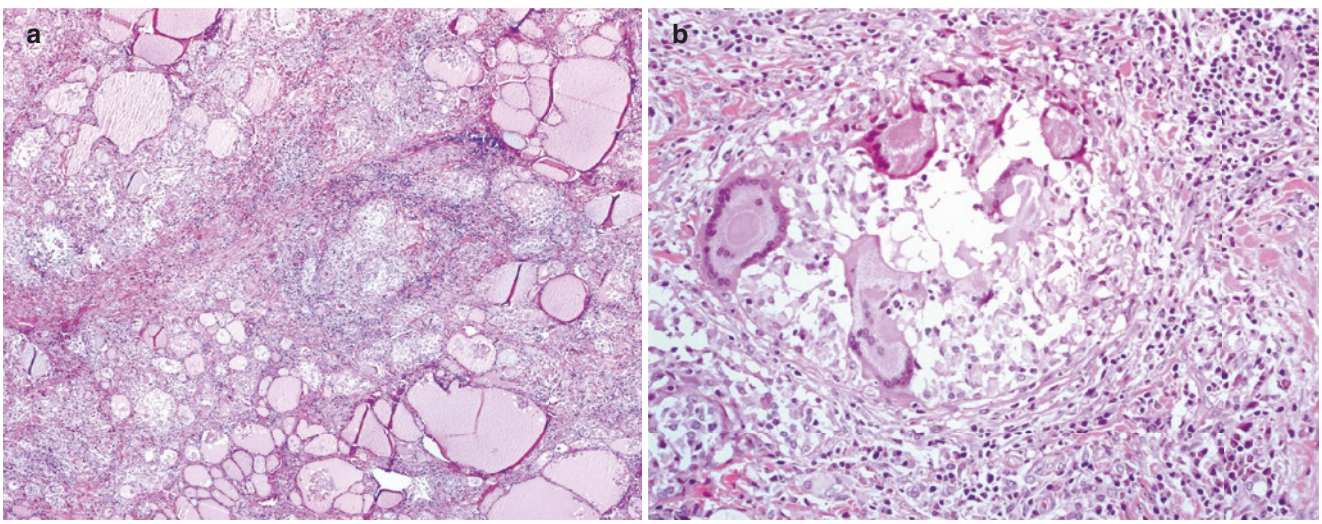

Fig. 14.9 (**a**) Subacute thyroiditis destroying the normal thyroid architecture. (**b**) Giant cell reaction with colloid phagocytosis in subacute thyroiditis

Table 14.1 Causes of granulomatous inflammation in the thyroid

Subacute thyroiditis
Palpation thyroiditis
Infection (such as tuberculosis)
Sarcoidosis
Wegener granulomatosis
Sarcoid-like granulomas in the setting of FNAB
Foreign body reaction to suture material after surgery
Drug-induced inflammation (such as amiodarone, minocycline)
Percutaneous laser ablation of thyroid nodules

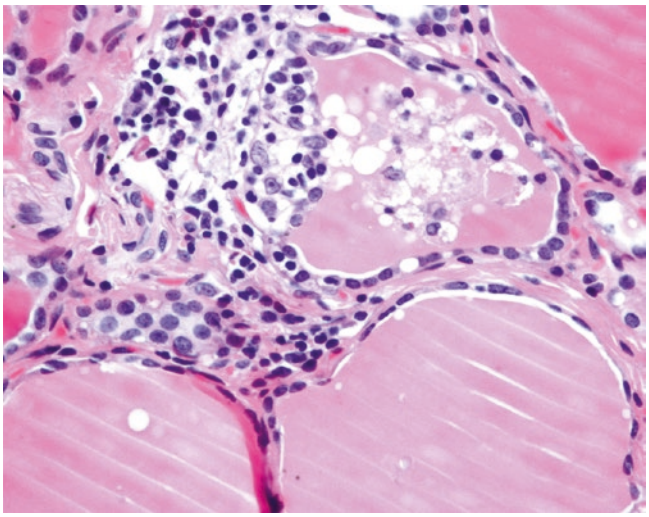

Fig. 14.10 Palpation thyroiditis

to those of tuberculosis infection elsewhere or may appear as an abscess [51]. The identification of acid-fast bacilli using Ziehl-Neelsen staining may be difficult.

14.3.5 Sarcoidosis

Definition Involvement of the thyroid by systemic sarcoidosis.

Clinical aspects May mimic the appearance of a nontoxic multinodular goiter [46].

Microscopy Non-necrotizing, granulomatous focal process [52] (Fig. 14.11).

Differential diagnosis Must be distinguished from focal sarcoid-like changes probably secondary to FNAB in patients who do not have the systemic manifestations of sarcoidosis [47].

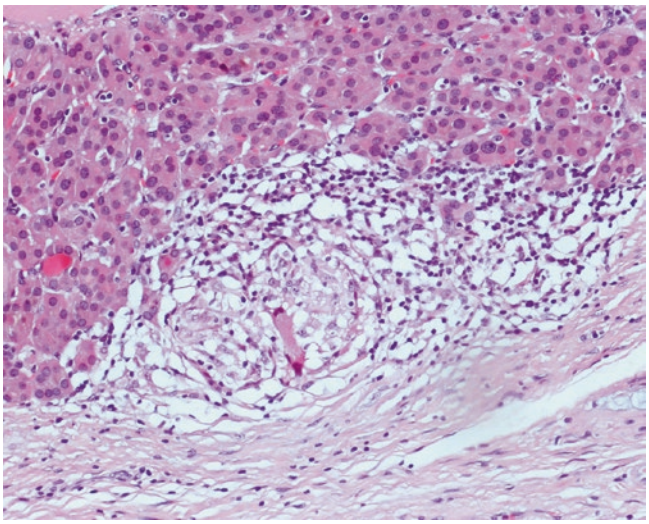

Fig. 14.11 Granulomatous lesion in a patient with sarcoidosis

14.3.6 Autoimmune Thyroiditis: Lymphocytic Thyroiditis

Definition Multifactorial disorder characterized by the production of autoantibodies that modify thyroid function. Autoimmune thyroiditis includes lymphocytic thyroiditis and Hashimoto thyroiditis which are thought to be different phases of the same disease.

Epidemiology Autoimmune thyroiditis occurs in patients with concurrent autoimmune disorders and affects patients within the same family, following a pattern of heritance with variable penetrance. Lymphocytic thyroiditis or juvenile form of autoimmune thyroiditis occurs mainly in children and young patients.

Etiology and pathogenesis Autoantibodies include anti-thyroglobulin, anti-thyroperoxidase, and anti-thyrotrophin receptor (TSHR) antibodies. Other mechanisms that have been implicated in the pathogenesis of autoimmune thyroiditis are the defect on suppressor T lymphocytes [53] and the expression of aberrant HLA-DR antigen in the membrane of follicular cells [53]. Some authors stated that the initiation of the inflammatory events is probably the result of viral or bacterial infection or reflects the injury to the thyroid cells from iodine [54].

Clinical aspects The patients present with an enlarged gland of short duration that is usually accompanied by altered thyroid function tests.

Macroscopy The thyroid is diffusely enlarged and discloses a whitish compact cut surface.

Microscopy The whitish aspect of the thyroid is mainly due to infiltration of the interfollicular stroma by aggregates of mature lymphocytes with germinal centers. The follicular epithelium is predominantly preserved; however, focally, it may be atrophic or show mild oncocytic changes (Fig. 14.12). The oncocytic transformation does not attain the threshold observed in Hashimoto thyroiditis, although the clinicolaboratory data in young patients with lymphocytic thyroiditis are indistinguishable from those of patients with Hashimoto thyroiditis (see below). Papillary hyperplasia of the follicular epithelium can occasionally be found.

Differential diagnosis Lymphocytic thyroiditis must be distinguished from the so-called focal, nonspecific thyroiditis that is a frequent finding in surgical specimens of older patients without thyroiditis-related symptoms and low levels of autoantibodies [55]. Focal, nonspecific thyroiditis consists in focal aggregates of lymphocytes with occasional germinal centers in fibrous septa of the gland that have no impact on the macroscopic aspect of the gland. Another entity that must be separated from lymphocytic thyroiditis is subacute lymphocytic thyroiditis or silent thyroiditis. Subacute lymphocytic thyroiditis is also an autoimmune-based disorder of the thyroid that occurs mainly in women in the *postpartum* period and is characterized by a diffuse goiter characterized by an patchy lymphocytic infiltrate that results in follicular disruption and reactive hyperplastic changes.

Treatment and prognosis Consists usually in the control of the hormone imbalance; surgery is only used for cases with associated malignancy, esthetic problems, and/or compressive symptoms.

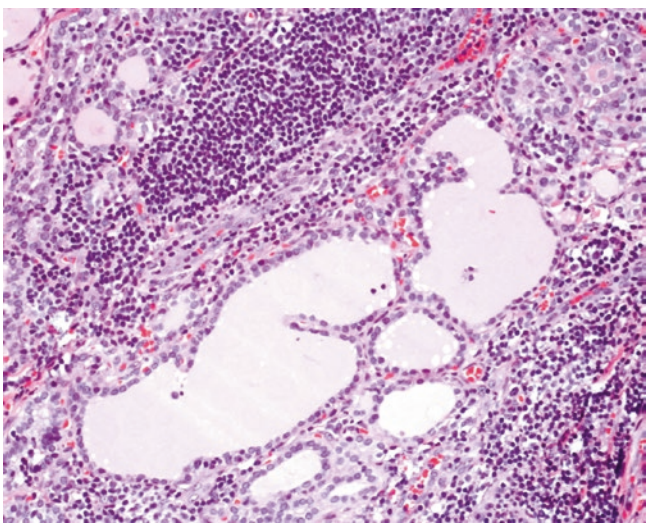

Fig. 14.12 Lymphocytic thyroiditis in a 17-year-old male

14.3.7 Autoimmune Thyroiditis: Hashimoto Thyroiditis

Definition Multifactorial disorder characterized by the production of autoantibodies that modify thyroid function (see above). Hashimoto thyroiditis, also known as *struma lymphomatosa*, is the adult form of autoimmune thyroiditis.

Epidemiology Occur in patients with concurrent autoimmune disorders and affects patients within the same family, following a pattern of heritance with variable penetrance. It can also coexist with Graves' disease, another autoimmune disorder of the thyroid, under the designation of hashitoxicosis [56]. It is more common in middle-aged women. Occurs in up to 2 % of the general population [54].

Etiology and pathogenesis Autoantibodies include antithyroglobulin, anti-thyroperoxidase, and anti-thyrotrophin receptor (TSHR) antibodies. Other mechanisms that have been implicated in the pathogenesis of autoimmune thyroiditis are the defect on suppressor T lymphocytes [53] and the expression of aberrant HLA-DR antigen in the membrane of follicular cells. Some authors stated that the initiation of the inflammatory events is probably the result of viral or bacterial infection or reflects the injury to the thyroid cells from iodine [54].

Clinical aspects Patients with Hashimoto thyroiditis present with a diffuse or slightly nodular painless goiter with or without compressive symptoms. It is usually accompanied by hypothyroidism. Normal function or hyperthyroidism may occur at the early stages of the disease.

Macroscopy The gland is diffusely enlarged and has a smooth and lobulated surface. Fibrous bands attaching thyroid to the surrounding tissues, such as those observed in Riedel thyroiditis, are consistently absent. On cut surface, the thyroid is whitish and compact, similar to the cut surface of glands with lymphocytic thyroiditis, and may be multinodular (Fig. 14.13).

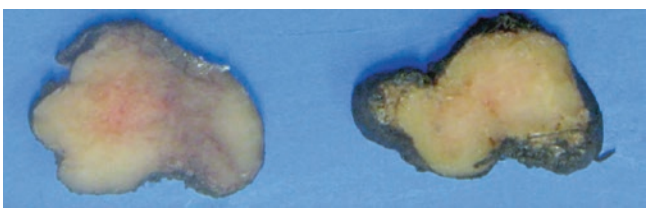

Fig. 14.13 Macroscopic aspect of the cut surface of a thyroid gland with Hashimoto thyroiditis

Microscopy The parenchyma is variably infiltrated by mature mononucleated inflammatory cells with germinal centers that surround foci of atrophic follicular epithelium with oncocytic changes (Fig. 14.14). Reactive hyperplastic papillary growth may also be observed. The inflammatory cells are mainly lymphocytes with a predominant T cell phenotype and less frequent B lymphocytes, plasma cells, histiocytes, and multinucleated giant cells [54]. Fibrosis ranges from mild to intense; in the latter setting, there is a prominent fibrosclerotic background that occurs mainly at the late stages of the disease. Necrosis and calcifications are usually absent. The presence of apparently isolated psammoma bodies in the setting of Hashimoto thyroiditis points to the need of search for an occult PTC. Squamous metaplasia (Fig. 14.15), ductal metaplasia, and C cell hyperplasia may occur in a thyroid with Hashimoto thyroiditis [7] and must not be confused with incidentally found papillary microcarcinomas. Reminiscent of branchial cleft cysts lined by squamous epithelium and bordered by a row of lymphoid follicles can rarely appear in thyroids with or without Hashimoto thyroiditis [57] (Fig. 14.16). The nuclei of the follicular cells entrapped in the inflammatory infiltrate can simulate the PTC-type nuclei and lead to a wrong diagnosis of malignancy, mainly in cytological sampling [58] (Fig. 14.17).
Hashimoto thyroiditis encompasses the fibrous variant, the fibrous atrophic variant, and the recently described IgG4-related thyroiditis [54, 59, 60].

The fibrous variant is characterized by an extensive paucicellular fibrosis of the thyroid that obliterates more than 30 % of the parenchyma [54] and usually coexists

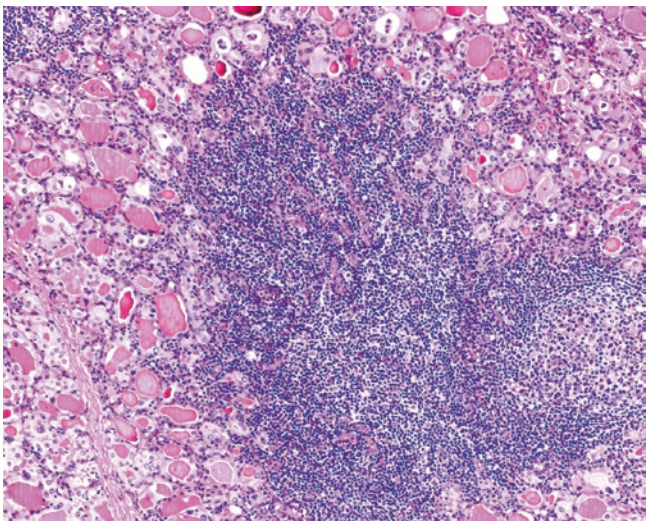

Fig. 14.14 Hashimoto thyroiditis with prominent oncocytic changes

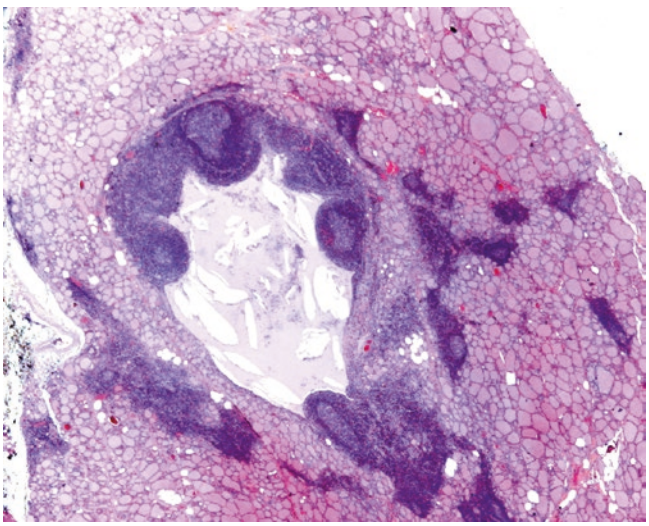

Fig. 14.16 Branchial cleft cyst

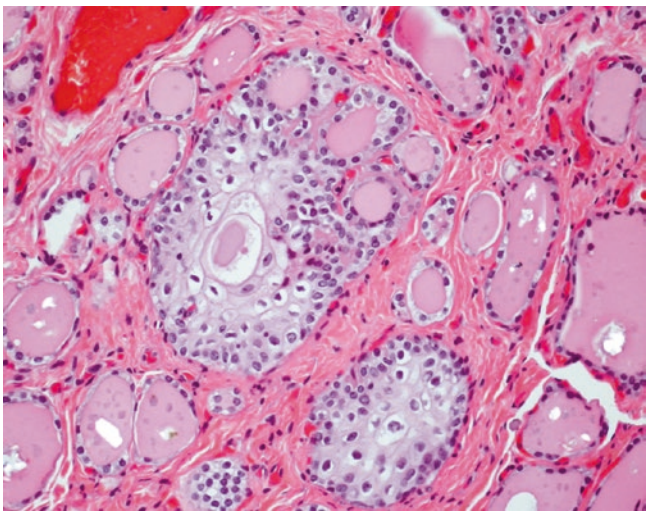

Fig. 14.15 Squamous metaplasia in a long-standing Hashimoto thyroiditis

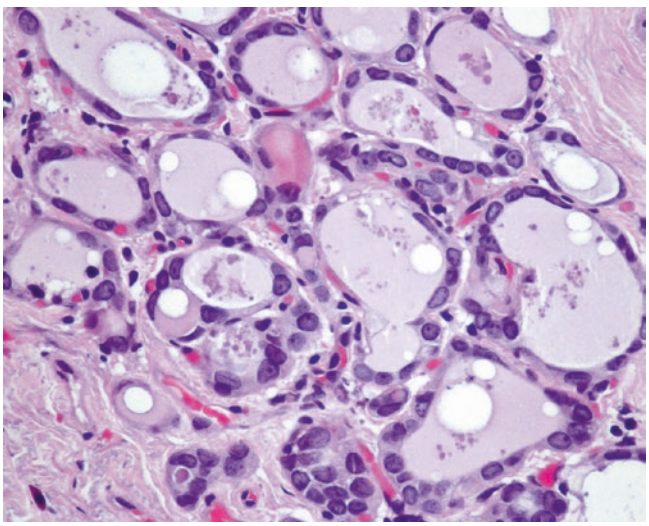

Fig. 14.17 Nuclear alterations in Hashimoto thyroiditis mimicking papillary thyroid carcinoma-type nuclei

with multifocal squamous metaplasia (Fig. 14.18). It is more common in patients with a long-standing disease displaying pressure symptoms and elevated circulation of autoantibodies [54].

The fibrous atrophic variant of Hashimoto thyroiditis is probably the end stage of the disease. It presents as a very small thyroid with less than 10 g and is almost totally replaced by hyaline fibrosis that surrounds small reminiscent islands of atrophic follicular epithelium (Fig. 14.19).

IgG4-related thyroiditis has been considered to be part of the group of IgG4-related sclerosing diseases. It is characterized by an inflammatory infiltrate rich in lymphocytes with germinal center formation and plasma cells. Immunostaining reveals increased IgG4 plasma cells with an IgG4/IgG ratio higher than 30–40 % [54].

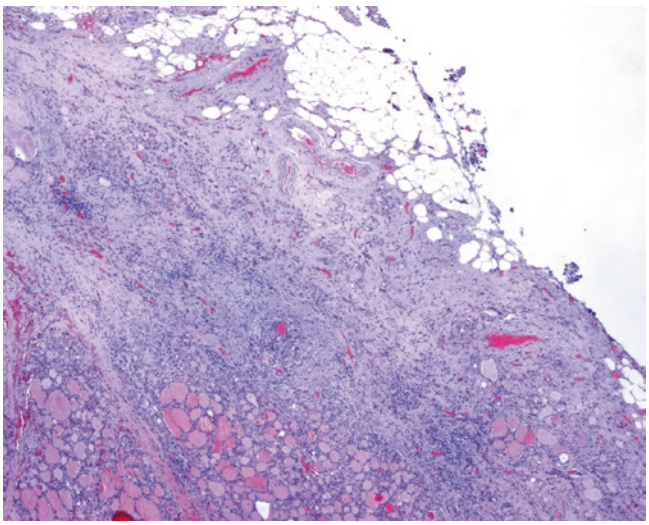

Fig. 14.18 Fibrous variant of Hashimoto thyroiditis

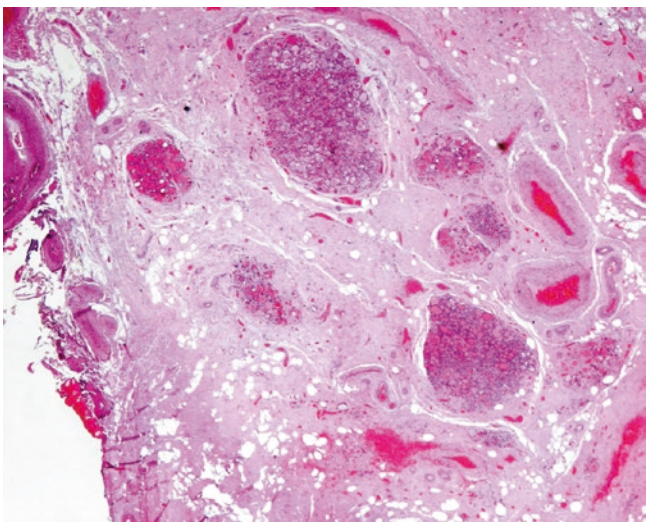

Fig. 14.19 Fibrous atrophy variant of Hashimoto thyroiditis

Differential diagnosis The nuclei of the follicular cells entrapped in the inflammatory infiltrate can simulate the PTC-type nuclei and lead to the diagnosis of PTC (see below the differential diagnosis of Graves' disease). PTC can also develop in patients with Hashimoto thyroiditis being significantly associated to this disease [61].

Exuberant Hashimoto thyroiditis can be confused with low-grade lymphoma such as marginal B-cell lymphoma in which lymphoepithelial images can be found (see below). The distinction between these two entities is based upon the observation of the destruction of the gland architecture in lymphomas together with the presence of atypical lymphocytes and a predominant B-cell phenotype of the cells with light chain restriction that can be evaluated by immunohistochemistry [62]. Both marginal B-cell lymphoma of the MALT type and diffuse large B-cell lymphoma can develop in the setting of Hashimoto thyroiditis. Whenever Hashimoto thyroiditis presents as a multinodular goiter, the so-called nodular transformation of Hashimoto thyroiditis, the nodules are usually hyperplastic mimicking an adenomatous goiter [61].

The fibrous variant must be distinguished from Riedel thyroiditis that usually discloses a proliferative type of fibrosis and is strongly adherent to perithyroid tissues [63].

Genetics *RET/PTC* rearrangements were described as being a frequent genetic alteration in Hashimoto thyroiditis [64], being present in up to 95 % of cases including those associated with PTC. These findings reinforce the suggestion that Hashimoto thyroiditis induces epithelial alterations that may serve as a precursor lesion of PTC. More recently, analysis by FISH and careful microscopic examination reported *RET/PTC* in rare follicular cells displaying PTC-like nuclear alterations in thyroid glands with Hashimoto thyroiditis [65].

IgG4-related thyroiditis is plasma cell-rich variant of Hashimoto thyroiditis that must be distinguished from Riedel thyroiditis, plasmacytoma, and plasma cell granuloma.

Treatment and prognosis Consists usually in the control of the hormone imbalance; surgery is only used for cases with associated malignancy, esthetic problems, and/or compressive symptoms.

14.3.8 Graves-Basedow's Disease

Definition and etiology Graves' disease, also known as Basedow's disease, thyrotoxicosis, and diffuse toxic goiter, is an immunologically driven disease. Graves' disease is thought to be due to circulating autoantibodies directed against the TSHR as well as to antithyroid peroxidase autoantibodies [66].

Epidemiology Affects mainly adult females and constitutes the most frequent cause of childhood hyperthyroidism [67].

Clinical aspects The patients present with goiter and hyperthyroidism symptoms such as tachycardia, weight loss and appetite increase, irritability, and anxiety [68]. The most striking features of the disease are exophthalmia that can be present in up to half of the patients [69, 70] and the uncommon pretibial myxedema, a condition characterized by swelling and clubbing of the extremities.

Macroscopy The gland is diffusely enlarged and discloses a glistening reddish surface (Fig. 14.20). The cut surface is homogeneous but may also have a vaguely nodular appearance.

Microscopy Graves' disease is characterized by hyperplastic changes of the follicular epithelium, with prominent papillary growths into the follicles lumina filled with pale scalloped colloid (Fig. 14.21). The follicles are lined by columnar-shaped cells with small dark nuclei that can occasionally be larger and clear, superficially resembling PTC-like nuclei. The interfollicular stroma shows a variably prominent inflammatory infiltrate rich in lymphocytes with germinal center formation and scant fibrosis [71]. Extensive fibrosis, follicular atrophy, and oncocytic changes are not common, except in long-term disease. In patients previously treated with antithyroid drugs or radioactive iodine, the findings may be less obvious or, alternatively, be more impressive displaying nodular transformation of the parenchyma and prominent nuclear abnormalities such as anisokaryosis (Fig. 14.22).

The putative association between thyroid carcinoma and Graves' disease has been examined over many years, but the issue still remains controversial [72]. Patients with Graves' disease and thyroid nodules appear to be at higher risk to develop thyroid cancer, namely, papillary thyroid carcinoma, in comparison with patients with diffuse adenomatous goiter. PTCs, particularly incidental papillary microcarcinomas, constitute up to 88.0 % of all cancers detected in Graves' disease. In this setting, PTCs are usually aggressive and more metastatic to regional lymph nodes, even when the primary tumor is small, than in cases of PTC occurring in euthyroid patients [72].

Differential diagnosis The most difficult differential diagnosis is with PTC due to the PTC-like appearance of the nuclei in Graves' disease. The best way to approach this problem is to rule out the unlikely hypothesis of a diffuse PTC involving the whole gland. The same approach should also be used whenever facing the differential diagnosis between Hashimoto thyroiditis and PTC.

Treatment and prognosis Death related-Graves' disease may rarely occur in patients that develop arrhythmic heart disturbances due to severe and uncontrolled hyperthyroidism [73].

14.3.9 Riedel Thyroiditis

Definition and pathogenesis Riedel thyroiditis, also known as Riedel *struma* and invasive fibrous thyroiditis, is an

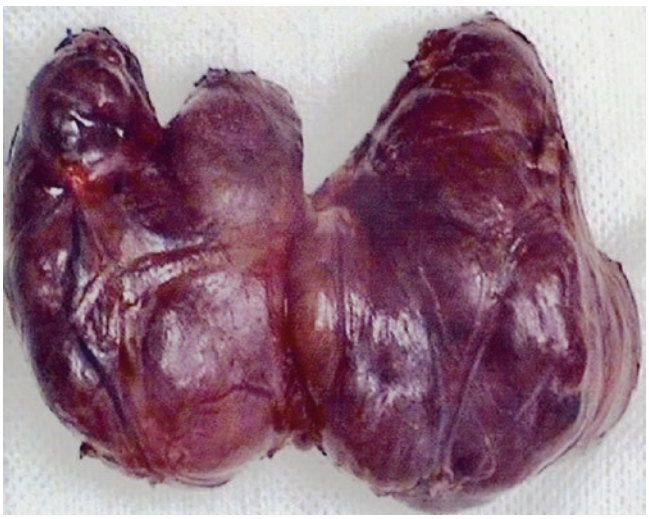

Fig. 14.20 Total thyroidectomy specimen of a patient with Graves' disease

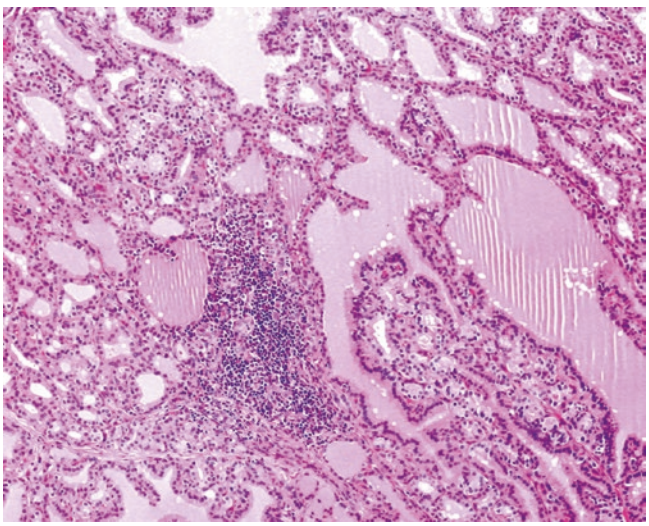

Fig. 14.21 Papillary hyperplasia of the follicular epithelium and focal inflammatory infiltrate rich in lymphocytes in a patient with Graves' disease

inflammatory disease that is thought to be part of the IgG4-related sclerosing diseases [74, 75].

Epidemiology Rare disease that occurs most frequently in elderly women.

Clinical aspects Present with an ill-defined enlargement of the thyroid associated to compressive symptoms. On physical examination, the thyroid is stony hard and painless, suggesting a diagnosis of malignancy. The involvement of the thyroid is often multifocal and asymmetric and extends into the adjacent muscles. Riedel thyroiditis may co-exist with other IgG4-related sclerosing diseases in the same patient [76].

Macroscopy The thyroidectomy specimen may be fragmented due to surgical difficulties in separating the thyroid from other neck structures.

Microscopy The affected areas are mainly composed by hyalinized fibrous tissue that obliterates the thyroid parenchyma. There is focal infiltration by lymphocytes, plasma cells, and eosinophils. Occlusive phlebitis is a typical finding. The plasma cells are mainly IgG4-producing cells, a feature that is common to other IgG4-related sclerosing diseases [74, 75].

Differential diagnosis Riedel thyroiditis can be confused with paucicellular anaplastic thyroid carcinoma (ATC) that frequently discloses expression of cytokeratins in the spindle-shaped neoplastic cells and does not exhibit a prominent IgG4 plasma cell-producing population. It can be distinguished from subacute thyroiditis due to the lack of granulomas and giant cells and from Hashimoto thyroiditis due to the lack of oncocytic change and lymphoid aggregates. The separation of Riedel thyroiditis from IgG4-related thyroiditis which some authors consider as a subtype of Hashimoto thyroiditis is controversial [60, 74]. Both subacute and Hashimoto thyroiditis are typically limited to the thyroid parenchyma, at variance with Riedel thyroiditis. There are reports describing the coexistence of Graves' disease and Hashimoto thyroiditis with Riedel thyroiditis [77, 78].

Treatment and prognosis Riedel thyroiditis is treated with corticoids or by surgery in medically refractory cases.

14.3.10 Multifocal Sclerosing Thyroiditis

Definition and microscopy Multifocal sclerosing thyroiditis (MST) is a recently proposed term by Juan Rosai [12] to designate a condition characterized by multiple foci of fibrosis that can be seen throughout the gland, with a tendency to concentrate in its periphery, often displaying a radial configuration [79] (Fig. 14.23).

Etiology and pathogenesis These lesions that are thought to be similar to the radial scars observed in the breast. Both the clinical significance and the pathogenesis of MST are unknown.

Differential diagnosis Must be distinguished from papillary microcarcinoma using the identification of typical PTC-type

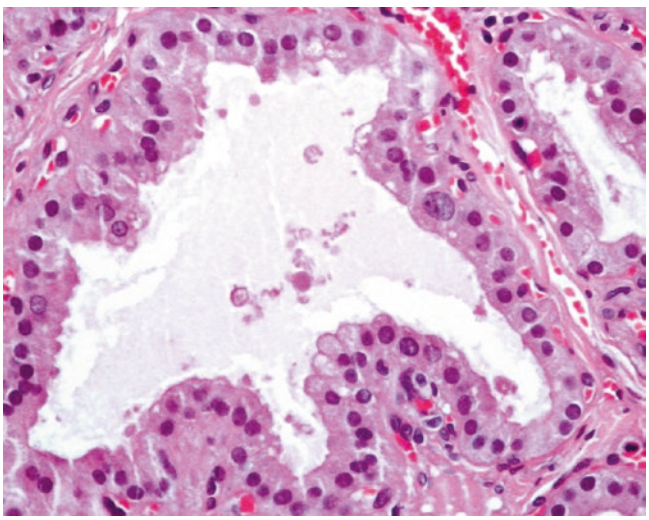

Fig. 14.22 Anisokaryosis of the follicular epithelium in a patient with Graves' disease previously treated with antithyroid drugs

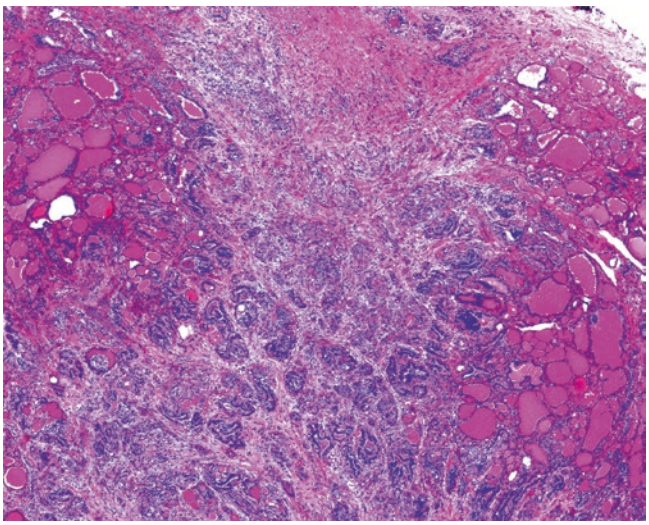

Fig. 14.23 Multifocal sclerosing thyroiditis

nuclei in the epithelial cells. Whenever we see several MST lesions in the thyroid, the diagnosis of papillary microcarcinoma is very unlikely.

14.3.11 Therapeutically Induced Inflammation

14.3.11.1 Therapeutically Induced Inflammation: Drug Related

Definition Inflammation of the thyroid due to drugs.

Etiology and pathogenesis The most frequent drugs that may induce thyroid inflammation include amiodarone and tetracycline derivatives, such as minocycline. Thyroid inflammation may also be caused by lithium salts [80] and diphenylhydantoin [81]. Recently, thyroid dysfunction (mainly hypothyroidism subsequent to follicular cell damage) was reported as a frequent adverse effect in patients under targeted therapies for cancer (tyrosine kinase inhibitors such as sunitinib, sorafenib, axitinib).

Epidemiology Amiodarone is an antiarrhythmic drug that has a high iodine content and triggers thyroid dysfunction in up to 25 % of patients [82].

Clinical aspects Amiodarone can cause thyrotoxicosis or, less often, hypothyroidism [82]. While type I amiodarone-associated thyrotoxicosis occurs in patients with goiter, type II amiodarone-induced thyrotoxicosis is associated with thyroiditis in iodine-deficient populations and tends to be severe [82].

Microscopy The thyroid involved by type II amiodarone-induced thyrotoxicosis is characterized by degenerative changes with destruction of colloid-filled follicles. The follicular cells display a swelled appearance with granular vacuolated cytoplasm. Multinucleated giant cells, foci of fibrosis, and chronic inflammatory infiltrates are usually present [82].

Minocycline is associated to a condition designated black thyroid due to the black-colored aspect of the thyroid caused by the lysosomal accumulation of lipofuscin-like pigment [83]. This accumulation is often associated to a significant inflammatory component made of lymphocytes and fibrosis that may lead to the development of hypothyroidism.

Differential diagnosis Other thyroiditis.

Treatment and prognosis Type II amiodarone-induced thyrotoxicosis is refractory to standard therapies, thus requiring surgical excision of the gland.

14.3.11.2 Therapeutically Induced Inflammation: Radiation Related

Definition Inflammation of the thyroid due to radiation.

Etiology and pathogenesis External beam radiation can initially induce decrease of radioiodine uptake and of the serum levels of thyroxin, as well as cytological alterations.

Clinical aspects Patients can develop hypothyroidism and thyroid atrophy.

Microscopy Cytological alterations can be appreciated in FNAB samples and include vacuolization of the cytoplasm of follicular cells and abundant macrophages [84]. Some patients treated with radioactive iodine and submitted to FNAB present follicular cells with cytoplasmatic vacuolization and minimal degenerative changes [85]. Chronic changes induced by external beam radiation or radioactive iodine include atrophy of the gland that becomes substituted by fibrosis [86, 87].

Differential diagnosis Other thyroiditis.

14.3.11.3 Therapeutically Induced Inflammation: Laser Related

Definition Inflammation of the thyroid due to laser treatments. Percutaneous laser ablation of thyroid nodules (PLATN) is considered an alternative to surgery in patients with compressive symptoms due to a clinically and cytologically benign nodule [88].

Microscopy The early histological findings in patients submitted to PLATN include cavity formation with a dark amorphous and necrotic content and inflammatory infiltrates composed by neutrophils, lymphocytes, and macrophages [88]. If the surgical removal of the nodule occurs more than 18 months after PLATN, the histological findings consist in a well-defined area surrounded by a fibrous capsule, filled in by a dark amorphous material. Foreign body-type granulomatous inflammation can be observed [88].

Differential diagnosis Other thyroiditis.

14.3.12 Rosai-Dorfman Disease

Definition and etiology Rosai-Dorfman disease (RDD), also known as sinus histiocytosis with massive lymphadenopathy, rarely involves the thyroid either as an isolated event or as part of systemic involvement. The association between chronic lymphocytic thyroiditis with RDD in the thyroid raises the possibility of a common pathogenesis of both entities [89].

Epidemiology RDD is a rare disease of unknown etiology that occurs in adult patients and has been documented only in women [89].

Clinical aspects Thyroid involvement by RDD can manifest as a thyroid mass that may mimic a subacute thyroiditis or an ATC.

Microscopy The histological features of RDD in the thyroid include nodules of histiocyte-like cells disclosing images of emperipolesis of lymphocytes, usually in the background of chronic lymphocytic thyroiditis [23, 24, 89].

Treatment and prognosis Whenever the involvement of the thyroid by RDD disease is an isolated event, it usually remains at a local stage and carries an excellent prognosis [23, 24, 89].

14.4 Tumor-Like Conditions of the Thyroid

14.4.1 Dyshormonogenetic Goiter

Definition and etiology Dyshormonogenetic goiters result from defects in thyroid hormone synthesis [90] including lack of responsiveness to TSH, defects in iodide transport and organification, defects in coupling, abnormalities of thyroglobulin synthesis and secretion, defects in deiodinase, abnormalities in the transport of thyroid hormone, and others [12, 91]. Some of those defects have been found to be genetically determined [90] and include inactivating mutations of genes encoding thyroglobulin, thyroid peroxidase, thyroid oxidase, Na/iodide symporter (NIS), and pendrin (an ion channel involved in iodide transport) [12, 92].

Epidemiology Dyshormonogenetic goiter is a rare condition which is usually clinically evident during childhood.

Clinical aspects It manifests as a diffuse goiter associated to hypothyroidism.

Macroscopy The thyroid gland is macroscopically enlarged and multinodular, weighing up to 600 g [90].

Microscopy The thyroid is totally abnormal displaying multiple hypercellular nodules that exhibit predominantly solid and/or microfollicular pattern of growth with scant colloid, surrounded by fibrosis [90]. The follicular cells of the nodules, as well as those of the surrounding thyroid parenchyma, present marked nuclear atypia and mitoses that may lead to confusion with cancer (Fig. 14.24a, b) [90]. Very rare cases of thyroid carcinoma, mainly follicular thyroid carcinoma (FTC), have been reported in patients with dyshormonogenetic goiter [93].

Differential diagnosis Dyshormonogenetic goiter must be distinguished from iatrogenic goiter resulting from the administration of antithyroidal agents. The dyshormonogenetic goiter associated to loss of pendrin function defines Pendred syndrome that is characterized by goiter and early hearing loss in children (Fig. 14.25).

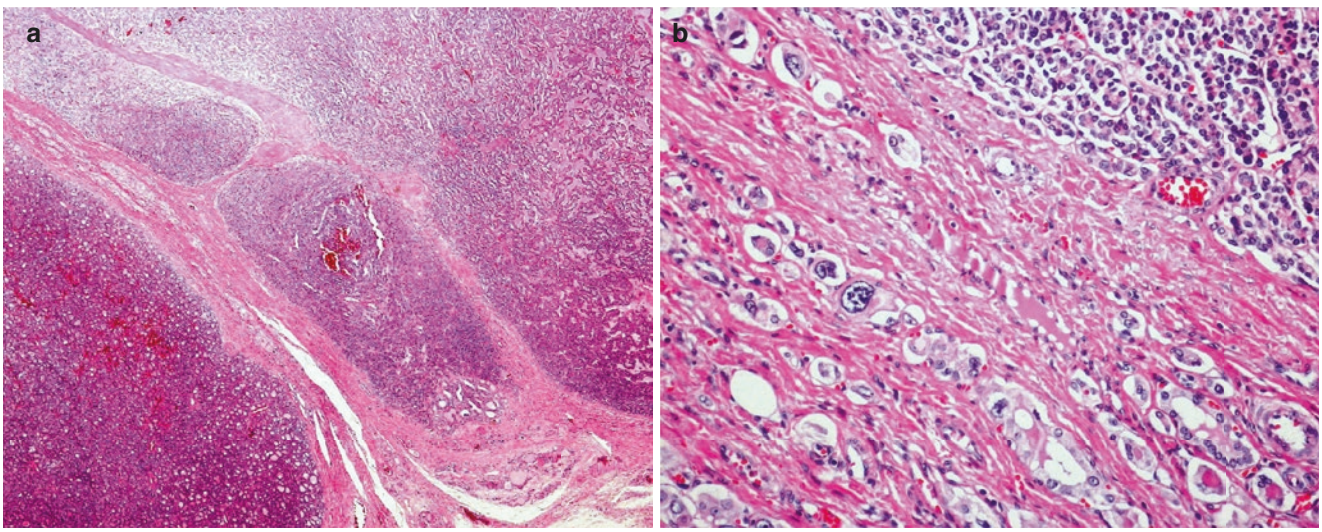

Fig. 14.24 (**a**) Multinodular aspect of a dyshormonogenetic goiter in a 24-year-old female. (**b**) Marked nuclear atypia of the follicular cells throughout the whole gland in a patient with dyshormonogenetic goiter

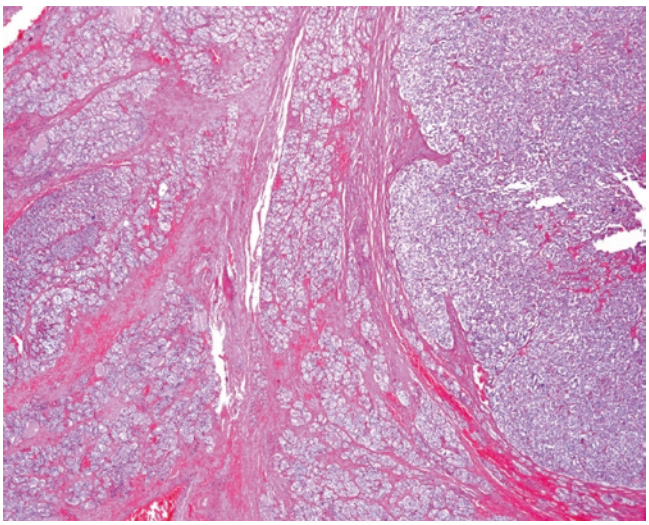

Fig. 14.25 Histological aspect of the thyroid in a young patient with Pendred syndrome

14.4.2 Adenomatous Goiter

Definition and epidemiology Adenomatous goiter, also known as nodular hyperplasia, multinodular goiter, or adenomatous hyperplasia, is the most frequent disease of the thyroid. It presents mainly as a sporadic disease with unknown pathogenesis or as an endemic disease in iodine-deficient areas.

Etiology and pathogenesis The low levels of iodine intake impair the production of adequate levels of thyroid hormone leading to increased TSH secretion and consequently to overstimulation of thyroid parenchyma.

Clinical aspects Most patients have normal thyroid function tests but, rarely, may present with hyperthyroidism symptoms – the so-called toxic adenomatous goiter.

Some cases of adenomatous goiter are associated with lymphocytic or Hashimoto thyroiditis [61]. Patients with adenomatous goiter have an increased thyroid volume that may cause compressive symptoms and esthetic problems. Hemorrhage within a nodule can cause sudden enlargement and pain. The extension of the goiter into the mediastinum may occur and be confused with a thoracic neoplasm.

Macroscopy The thyroid with adenomatous goiter is enlarged and distorted by multiple nodules that occasionally occur as masses separated from the main gland [22]. On cross section, multiple nodules are seen, some surrounded by a partial or complete capsule. Hemorrhage, calcification, and cystic degeneration are common (Fig. 14.26a).

Microscopy The nodules are composed by large follicles lined by flattened epithelium; others are extremely cellular displaying (micro)follicles or a solid growth pattern as one sees in follicular adenoma (FA) (see below). Oncocytic transformation may be observed.

Some of the large follicles have an aggregate of small follicles at one pole (the so-called Sanderson's polster), while others have papillary projections into the lumina, mimicking the structure of PTC (Fig. 14.26b). The nuclei of the follicular cells in adenomatous goiter are small and hyperchromatic, distinct from those observed in PTC; sometimes, however, the nuclei may be focally large and clear and may also display some atypical features, usually in patients previously submitted to medical treatment or radioactive iodine.

The large follicles observed in adenomatous goiter may rupture and lead to a granulomatous reaction with recruitment of histiocytes and foreign body-type giant cells. Areas of hemorrhage, chronic inflammatory infiltrates and fibrous septa with dystrophic calcification, and even ossification can be observed. Another interesting secondary feature observed in adenomatous goiter is the reactive endothelial hyperplasia that is occasionally observed in the rich vascular network that accompanies the adenomatous nodules and may be confused with vascular invasion by epithelial cells [94].

Differential diagnosis Adenomatous goiters are usually composed of multiple nodules, but they can also consist of a single nodule or a large dominant nodule that can be confused with a neoplasm. The differential diagnosis between a dominant nodule in the setting of adenomatous goiter and a true adenoma is based upon arbitrary criteria and is often impossible. The FA is usually a single lesion surrounded by a capsule, displaying a pattern of growth that is different from that of the remaining parenchyma; typically, the FA compresses the adjacent tissue and is composed mainly of follicles that are smaller than those of the normal gland. At variance, the adenomatous nodule is almost always one of several nodules with incomplete capsule, with variable follicular size, that does usually cause major compression of the adjacent parenchyma. To address this differential diagnosis problems, the clonality of hyperplastic nodules has been evaluated using X chromosome inactivation patterns since it was thought that clonality studies could distinguish adenomatous goiters from adenomas. Nodules from adenomatous goiters are frequently polyclonal, although monoclonal neoplasms also frequently arise within multinodular glands [95], thus turning impossible to use this characteristic to distinguish adenomatous nodules from FA that present mainly a monoclonal pattern [95].

The risk of malignant transformation, in the form either of FTC or PTC, exists but is low, both in nodules of adenomatous goiters and FAs. The risk of malignant transformation is

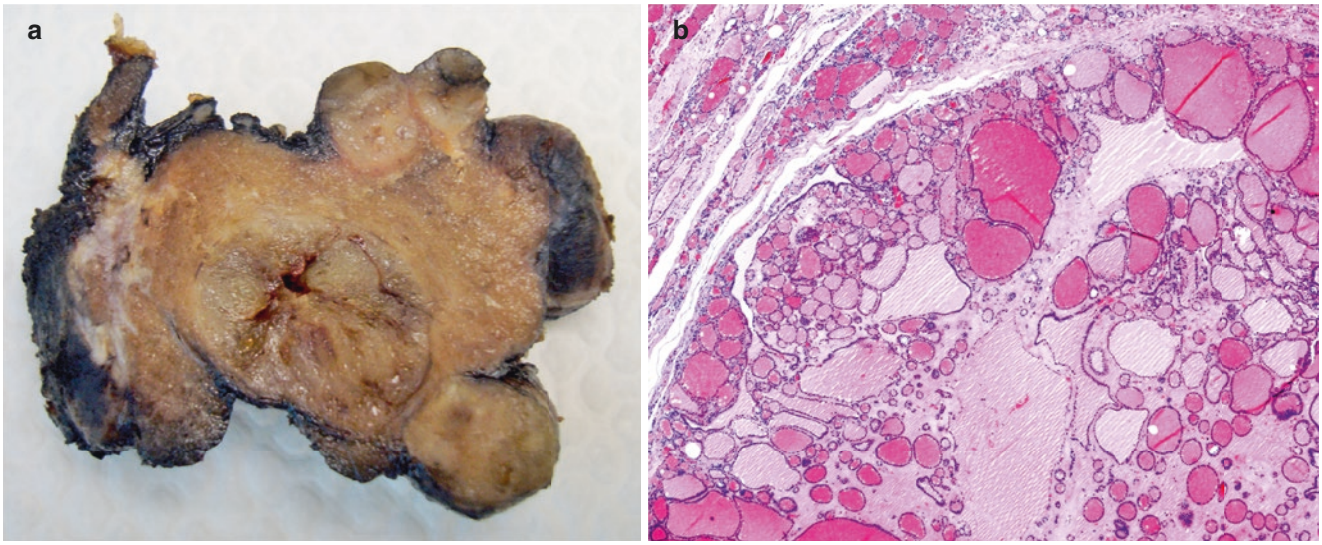

Fig. 14.26 (**a**) Cut surface of a multinodular adenomatous goiter with areas of hemorrhage and fibrosis. (**b**) Sanderson's polster and macrofollicles in adenomatous goiter

nearly zero in hyperfunctioning nodules of adenomatous goiter and in hyperfunctioning FAs.

Genetics A locus in chromosome 14 (*MNG1*) has been implicated in some familial forms of adenomatous goiter although the gene has not been identified. Other candidate genes for familial forms of adenomatous goiter have been indicated, namely, mutations in *DICER 1* and *KEAP1*.

Treatment and prognosis Treatment of adenomatous goiter is recommended whenever hyperthyroidism symptoms develop and if the enlargement causes disfigurement and/or pressure symptoms develop.

14.4.3 Amyloid Goiter

Definition and epidemiology Amyloid goiter is an uncommon disease characterized by the increased size of the thyroid due to the presence of amyloid substance, usually of the AA type, throughout the gland.

Etiology and pathogenesis It can be primary or secondary, being part of a systemic disease such as an inflammatory chronic condition or multiple myeloma [96].

Clinical aspects The increased size of the thyroid may cause compressive symptoms.

Macroscopy Diffusely enlarged thyroid or enlarged single lobe of the gland that is typically orange colored in cut surface.

Microscopy Atrophic follicular parenchyma composed mainly by large follicles without atypical cells, amyloid deposition in the interstitium, and abundant mature adipose tissue (Fig. 14.27). FNAB can be performed to exclude a primary malignant lesion and to do immunohistochemical studies that allow the characterization of the amyloid deposits [97].

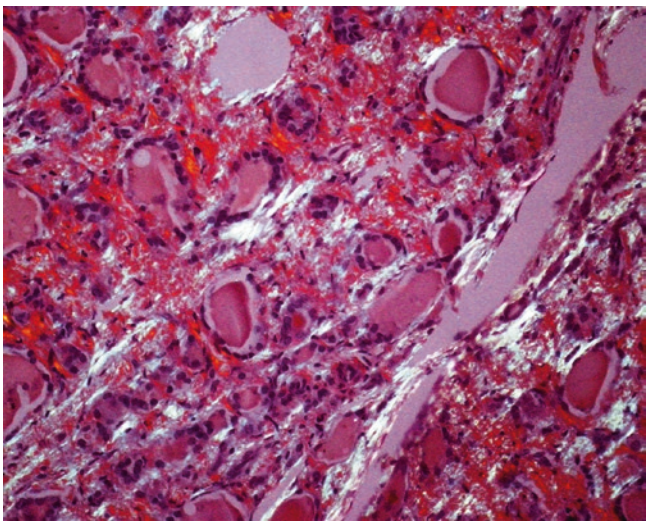

Fig. 14.27 Amyloid goiter (Congo red and polarized light)

Differential diagnosis The abundance of adipose tissue can lead to confusion with thyroid lipomatosis that is a very rare condition displaying abundant adipose tissue but no amyloid deposition. Amyloid deposition can be found in thyroid gland in other conditions such as medullary thyroid carcinoma (MTC) and, in extremely rare situations, PTC [96].

Treatment and prognosis Patients with compressive symptoms are treated with total surgical excision of the gland.

14.4.4 Diffuse Lipomatosis

Definition and epidemiology Thyroid diffuse lipomatosis or thyrolipomatosis is a rare condition characterized by diffuse infiltration of an otherwise normal thyroid by mature adipose tissue without evidence of encapsulation [98]. It can occur at any age, including children with congenital goiter.

Etiology and pathogenesis The pathogenesis of this disease is unknown.

Clinical aspects Presents as a diffuse or nodular goiter, with or without compressive symptoms. The patients may be euthyroid, hypothyroid, or hyperthyroid [99].

Macroscopy The thyroid is soft, nodular, or diffusely enlarged and yellow in cut surface (Fig. 14.28a).

Microscopy The thyroid is diffusely infiltrated by mature adipose tissue and may disclose stromal fibrosis and small aggregates of lymphocytes (Fig. 14.28b).

Differential diagnosis Thyroid diffuse lipomatosis must be distinguished from other lesions with adipose tissue content (Table 14.2).

14.4.5 Plasma Cell Granuloma

Definition and etiology Plasma cell granuloma (PCG) is a form of pseudotumor of unknown etiology.

Epidemiology and clinical aspects Is a rare lesion that affects mainly adult women and presents as a diffuse thyroid enlargement or a thyroid mass. The patients with PCG have Hashimoto thyroiditis in nearly half of cases and polyclonal hypergammaglobulinemia in 10 % of cases [100, 101].

Microscopy PCG is characterized by a prominent polyclonal plasma cell infiltrate embedded in a more or less dense fibrous stroma.

Differential diagnosis The differential diagnosis includes other plasma cell disorders, infectious diseases, inflammatory

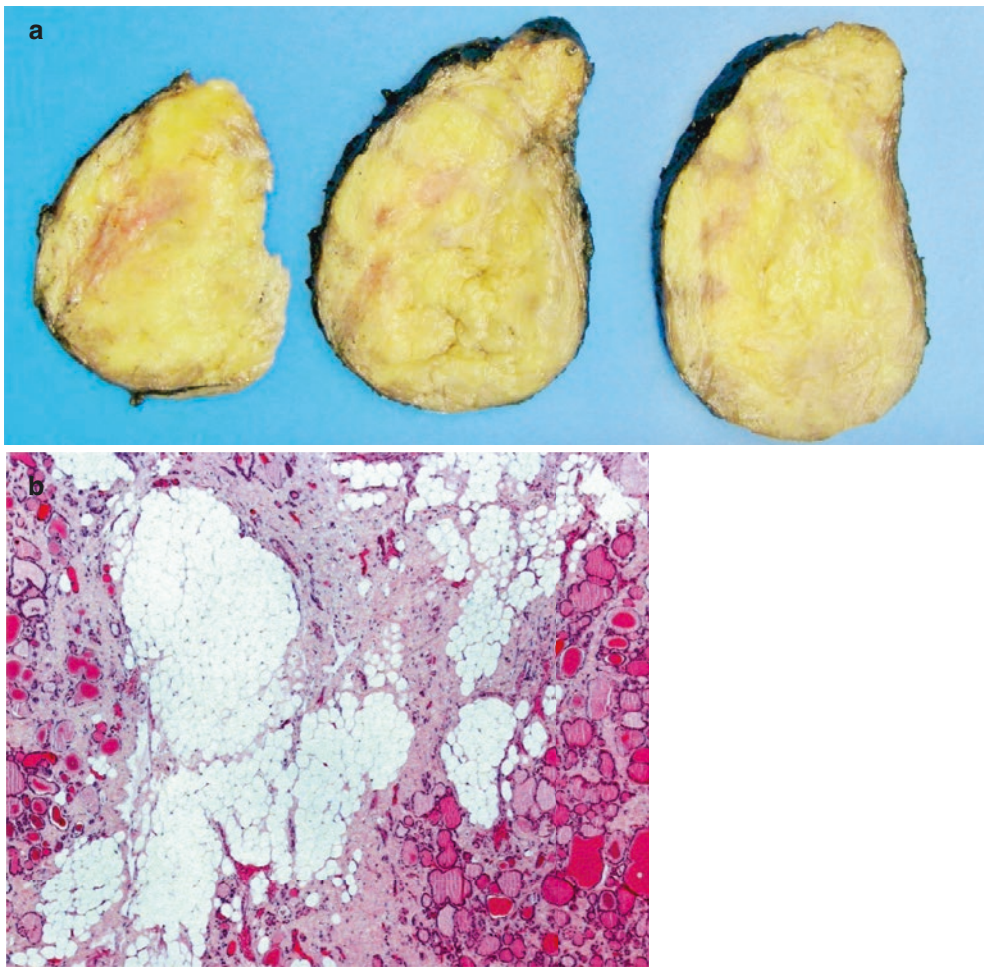

Fig. 14.28 (**a**) Diffuse lipomatosis of the thyroid, macroscopic aspect. (**b**) Diffuse lipomatosis of the thyroid, microscopic aspect

Table 14.2 Thyroid lesions (always or occasionally) displaying adipose tissue

Adipose metaplasia/infiltration of the interfollicular stroma
Adenolipoma/thyrolipoma
Diffuse lipomatosis
Amyloid goiter
Papillary thyroid carcinoma with adipose metaplasia
Benign or malignant adipocytic tumor
Intraparenchymatous parathyroid gland with adipose stroma

myofibloblastic tumor, Hashimoto thyroiditis, and IgG4-related sclerosing (see above) [102].

14.5 Thyroid Tumors

14.5.1 Follicular Adenoma

Definition Follicular adenoma (FA) is defined as a benign, encapsulated tumor that shows evidence of follicular cell differentiation. It lacks capsular invasion, vascular invasion, and the nuclear features typical of PTC.

Epidemiology and etiology FA is the most common thyroid neoplasm. FAs tend to be more frequent in women living in iodine-deficient regions. The frequency of FA in autopsy series is up to 4.3 % [103, 104].

Clinical aspects Most patients are asymptomatic, euthyroid adults who present with a usually "cold" and, only rarely, "hot" nodule in the thyroid that is usually solid and well circumscribed by ultrasound. Hyperfunctioning FAs, which are scintigraphically "hot," are sometimes associated with hyperthyroidism, being also designated toxic adenomas or Plummer adenomas. Spontaneous hemorrhage into a FA can cause acute neck pain associated to enlargement of the nodule.

Macroscopy FA is usually a solitary nodule surrounded by a complete fibrous capsule that varies in thickness, with a size ranging from less than 1 cm to over 10 cm. Multiple FAs can be observed in genetically determined syndromes such as Cowden syndrome and Carney complex. On cut surface, FAs are usually solid, homogeneous tumors, but hemorrhage and cystic degeneration are not uncommon (Fig. 14.29).

Microscopy FA cytoarchitectural features are different from those of the surrounding gland which usually shows signs of compression. FAs may exhibit a variety of patterns, singly or mixed, without apparent clinical significance: normofollicular, macrofollicular (colloid), microfollicular (fetal), and trabecular/solid (embryonal) (Fig. 14.30a). The FA cells have small, hyperchromatic, uniform, and round nuclei with scant mitoses (Fig. 14.30b). Secondary changes such as cystic degeneration, hemorrhage, edema, fibrosis, calcification, and bone formation are common in larger tumors.

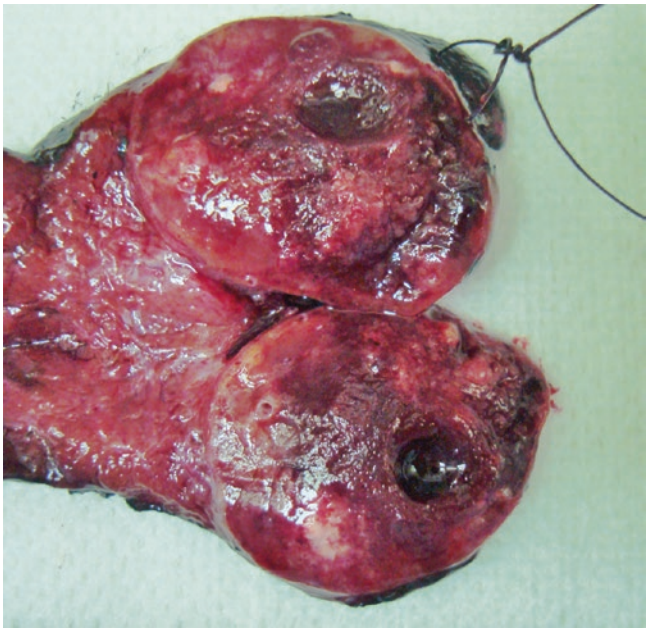

Fig. 14.29 Follicular adenoma cut surface with hemorrhage and cystic change

On the basis of microscopic features, several variants have been described, including oncocytic FA (Hürthle cell FA) (Fig. 14.31), FA with papillary hyperplasia, FA with clear cell change (Fig. 14.32), FA with bizarre nuclei (Fig. 14.33), signet-ring cell FA (Fig. 14.34), lipoadenoma (rich in mature adipocytes), adenochondroma (with condroid metaplasia of the stroma), spindle cell FA, and black FA that results from the deposition of black pigment following minocycline therapy [104]. The utilization of the designation atypical FA, that has been proposed for FAs with pronounced cellular proliferation and cytoarchitectural atypia, lacking evidence of capsular or blood vessel invasion, is not recommended.

Differential diagnosis The differential diagnosis of FA may be very difficult and in the majority of cases includes a single nodule of adenomatous goiter, minimally invasive FTC, and follicular variant of PTC.

As discussed above, the distinction between FA and nodule of adenomatous goiter is not clear-cut in most instances of single nodules (in both instances, the tumors are, or may be, monoclonal) and has no practical implications since the risk of recurrence after partial

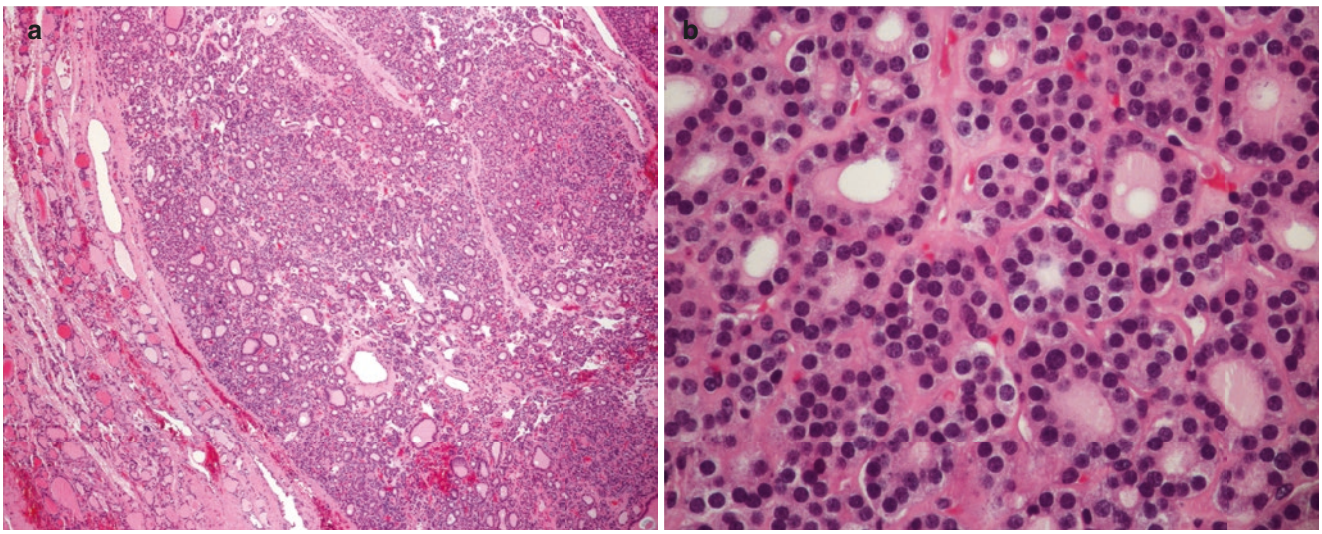

Fig. 14.30 (**a**) Follicular adenoma displaying a microfollicular growth pattern. (**b**) Follicular adenoma with fairly typical small and hyperchromatic nuclei

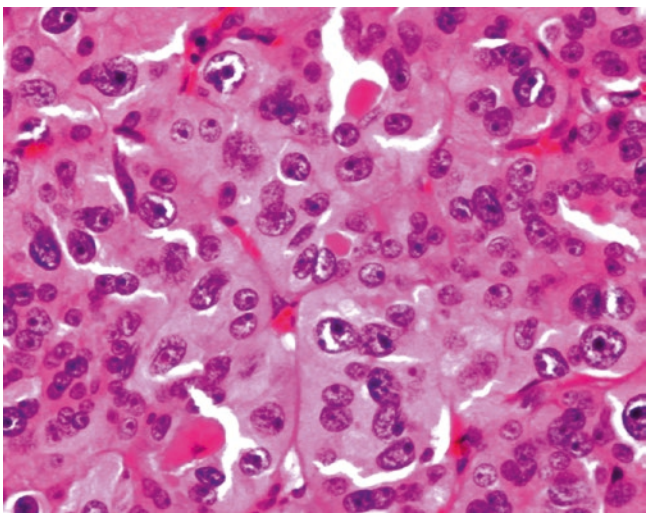

Fig. 14.31 Oncocytic follicular adenoma composed of large, granular, and eosinophilic cells with round nuclei, often with focal anisokaryosis, and prominent pinkish nucleoli that often surround follicular lumina with dense colloid

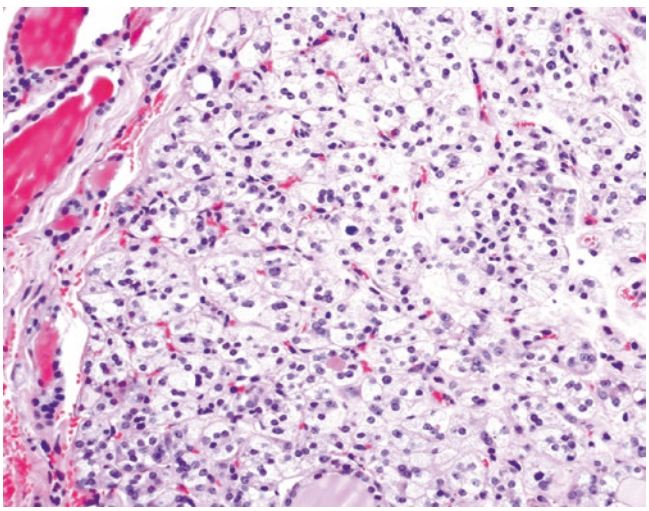

Fig. 14.32 Follicular adenoma with clear cells that may result from mitochondria ballooning or from the cytoplasmic accumulation of lipids or glycogen or thyroglobulin. In this case the clearing of the cytoplasm was due to mitochondria ballooning

thyroidectomy and the risk of malignant transformation are similar.

Papillae can be rarely observed in FA, a feature that may lead to confusion with classic PTC.

The distinction between follicular variant of PTC and FA is based upon the presence of typical PTC-type nuclei in the former. If PTC-type nuclei occur in dispersed cells and/or are not particularly characteristic, the differential diagnosis between benign and malignant tumor can be very difficult. In this situation, it may be advisable to classify lesion that does not display invasive features as well-differentiated tumor of uncertain malignant potential (WDTUMP) [105]. If one is not sure about the presence of invasive features and the nuclei are of the follicular type, thus turning impossible to separate FTC from FA, the use of follicular tumor of uncertain malignant potential (FTUMP) is advisable [105] (Table 14.3).

The diagnosis of FA is basically a diagnosis of exclusion that can only be made in a precise manner after the complete analysis of the tumor capsule. This turns impossible its diagnosis in the preoperative setting, following FNAB, as the capsular and vascular invasion images are not assessed by this method, and also extremely difficult to identify in most frozen section examinations.

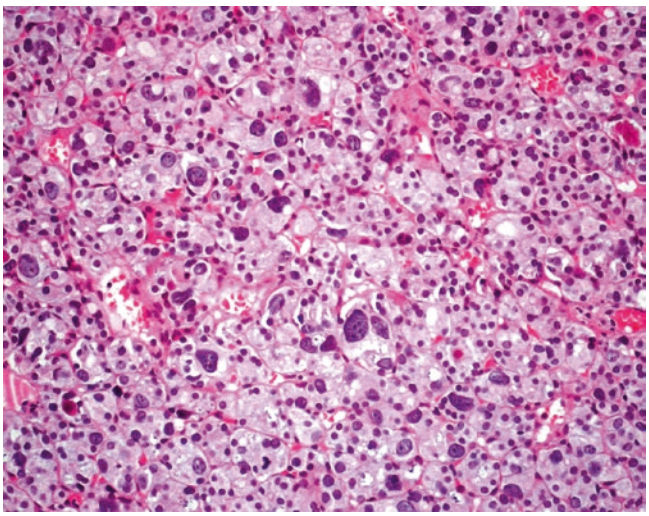

Fig. 14.33 Follicular adenoma with bizarre nuclei is a tumor with a benign behavior that is characterized by the presence of huge hyperchromatic nuclei, usually in clusters, unaccompanied by other features associated to malignancy

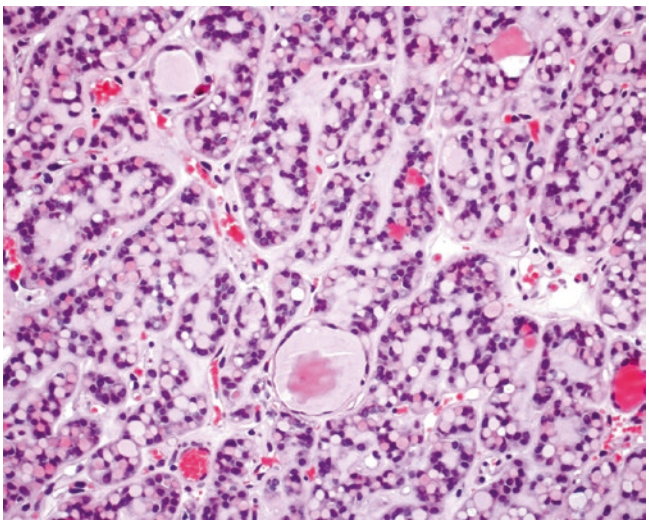

Fig. 14.34 Signet-ring cell follicular adenoma which may be confused with metastatic adenocarcinoma of the stomach

Table 14.3 Differential diagnosis of encapsulated follicular-patterned lesions

		Vascular and/or capsular invasion		
PTC-type nuclei		Present	Doubtful	Absent
	Present	PTC	PTC	PTC
	Doubtful	Well-differentiated carcinoma, NOS	WDTUMP	WDTUMP
	Absent	Follicular carcinoma	FTUMP	Follicular adenoma

PTC Papillary thyroid carcinoma, *WDTUMP* Well-differentiated tumor of uncertain malignant potential, *FTUMP* Follicular tumor of uncertain malignant potential

The distinction between FA and other well-differentiated follicular tumors cannot be based upon immunohistochemical features as all the lesions show reactivity for low molecular weight keratins and thyroglobulin in the cytoplasm and for TTF-1 in the nuclei.

The predominance of solid/trabecular areas in a FA should alert to the possibility of poorly differentiated thyroid carcinoma (PDTC) or MTC and lead to the search for invasive features and, in MTC, to the immunohistochemical detection of calcitonin.

Genetics It is useless to search for molecular biomarkers to solve the aforementioned differential diagnoses since the molecular alterations frequently detected in FTC and follicular variant of PTC (*RAS* mutations or *PAX8/PPARγ* rearrangements) are present, at similar prevalence, in FA [106]. RET rearrangements were also detected in high frequency (45 %) in FAs of patients with a history of external irradiation [107]. "Hot" nodules (toxic FA or toxic adenomatous goiter) display a different pattern of genetic alterations from "cold" nodules, in particular mutations in Gs protein (GSP oncogene) and constitutively activating mutations of TSH receptor. There are also Gs and TSH receptor mutation-negative "hot" nodules which show a monoclonal pattern thus suggesting that further candidate genes may be involved in the etiopathogenesis of "hot" tumors [106].

Treatment and prognosis The standard therapy for FA is removal by lobectomy.

14.5.2 Hyalinizing Trabecular Tumor

Definition Hyalinizing trabecular tumor (HTT) is the term coined by Carney for a follicular cell neoplasm exhibiting a trabecular arrangement and a prominent hyaline appearance both in the cytoplasm of tumor cells and in the stroma (Fig. 14.35) [108].

Epidemiology HTT develops mainly in adult women.

Clinical aspects Single "cold" nodule, otherwise asymptomatic.

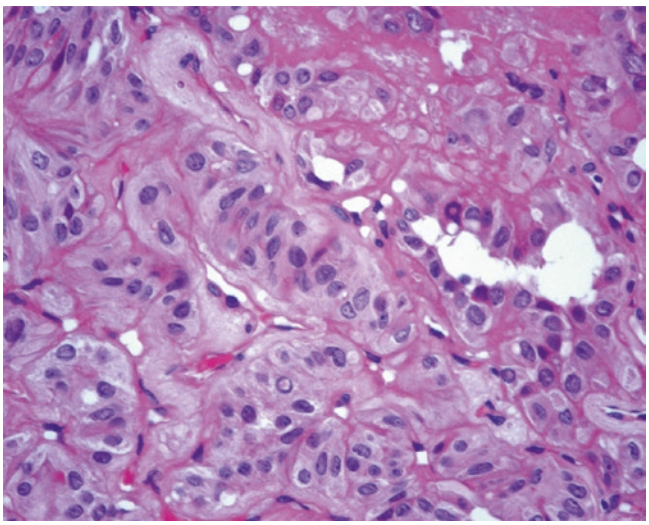

Fig. 14.35 Hyalinizing trabecular tumor

Macroscopy HTT presents as a well-circumscribed, yellowish, and single nodule.

Microscopy Composed by trabeculae and nests of elongated cells disposed perpendicularly to the long axis of the trabeculae and surrounded by a deeply hyalinized stroma. The cells disclose irregularly shaped nuclei, clarified, with grooves and abundant pseudoinclusions, looking like PTC-like nuclei. A pale yellow cytoplasmatic inclusion in paranuclear location can often be observed.

Differential diagnosis and pathogenesis The pattern of growth may simulate that of paraganglioma and MTC, thus justifying the alternative designation of this lesion: paraganglioma-like adenoma of the thyroid [109].

On the other hand, the presence of nuclear grooves and nuclear pseudoinclusions indicates that this entity is probably related to PTC and may be difficult to distinguish from the trabecular variant of PTC.

The distinction between true HTT and other thyroid tumors displaying foci of trabecular-patterned areas with increased hyalinization such as adenomatous nodules or PTC may be impossible to achieve in H&E slides [110, 111]. Immunohistochemically, HTT is negative for calcitonin and expresses thyroglobulin and TTF-1; it may express galectin-3 [112], NSE, and neurotensin [113]. Monoclonal antibodies used to detect MIB1 are characteristically expressed in the cytoplasm and cell membrane of HTT cells [114]; this finding is sometimes difficult to reproduce since it depends from technical conditions. The extracellular and intracellular deposits of hyalinized substance, including nuclear pseudoinclusions, express type IV collagen.

It has been suggested that HTT may constitute a variant of PTC [109, 115] due to the morphological nuclear resemblance, expression of similar types of stratified epithelial-type keratins [115], and the occurrence of *RET/PTC* rearrangement with a frequency similar to that observed in PTC [116].

Prognosis From the practical standpoint, a thyroid tumor with the morphologic criteria described by Carney [108] will behave in a benign fashion virtually always [117]. However, extremely rare cases of HTT with malignant behavior have been reported [117–119].

14.5.3 Papillary Thyroid Carcinoma

Definition Papillary thyroid carcinoma (PTC) is a malignant tumor that constitutes, together with follicular thyroid carcinoma, the group of the well-differentiated thyroid carcinomas disclosing follicular cell differentiation. The diagnosis of PTC relies on the distinctive nuclear features.

Epidemiology and etiology PTC is the most common type of thyroid malignancy. Although it can occur in a wide range of ages, PTC is more frequent in middle-aged women, predominantly as a sporadic disease, with only 5 % of cases developing in a familial setting. Familial PTC is a heterogeneous disorder caused by more than one susceptibility gene [120]. Regarding the influence of environmental factors, the exposure to radiation in general and to radioactive iodine in particular appears to be the factor most closely related to the occurrence of PTC, as it is illustrated by the striking incidence of PTC in children exposed to radioactive fallout [121]. The relative frequency of PTC is also greater in regions of adequate or high dietary iodine intake compared to regions of iodine deficiency. There is evidence supporting an increase of incidence of PTC in Hashimoto thyroiditis, and the putative higher incidence of PTC in Graves' disease remains controversial.

Clinical aspects PTC usually presents as a cold nodule or mass in the thyroid or as a cervical adenopathy. Additionally, numerous clinically silent, incidentally found small or very small PTCs are detected in ultrasound and other image studies of the neck performed by other reasons, at autopsy or in surgically removed glands for benign conditions (Fig. 14.36) [122].

Macroscopy In the surgical specimen, PTC may assume the appearance of an infiltrative whitish mass (Fig. 14.37) or mimic a FA with a well-circumscribed, occasionally encapsulated periphery (Fig. 14.38). PTCs can be multicentric and are often very small, measuring less than 1 cm, becoming difficult to identify (Fig. 14.39). Cystic changes and papillary formations can be macroscopically evident.

Microscopy and prognosis The diagnosis of PTC relies on the distinctive nuclear features of tumor cells that include enlargement, elongation and overlapping, clear-empty

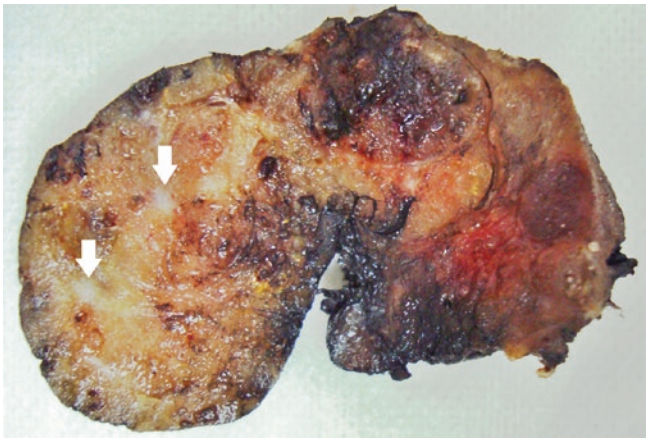

Fig. 14.36 Papillary microcarcinomas (*arrows*) incidentally found in a thyroid specimen removed for nodular goiter

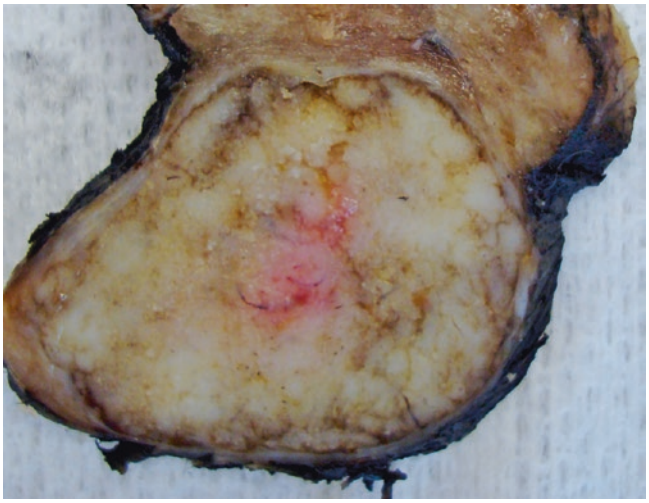

Fig. 14.38 Well-circumscribed and encapsulated papillary carcinoma

Fig. 14.37 Papillary microcarcinoma assuming the appearance of a whitish infiltrative nodule

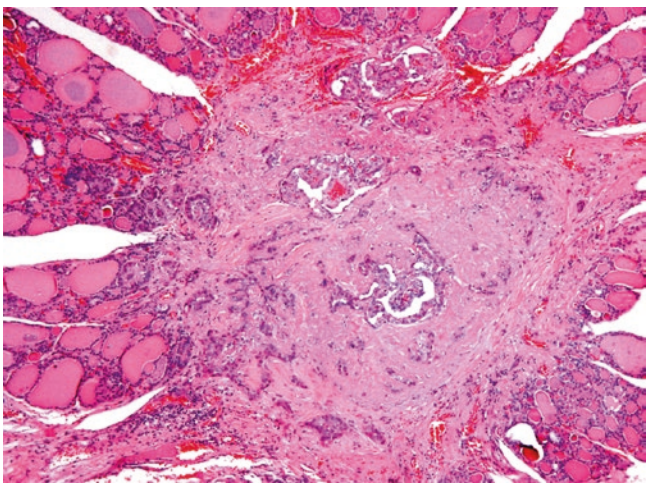

Fig. 14.39 Papillary microcarcinoma with infiltrative growth pattern and abundant sclerotic stroma

appearance (ground glass), irregularity and thickening of the nuclear membrane, and prominence of nuclear grooves and nuclear pseudoinclusions (Fig. 14.40). The nucleolus is usually inconspicuous, located near the nuclear membrane, and mitotic figures are rare or absent.

Ultrastructurally, PTC cells disclose an extremely convoluted nuclear membrane displaying very few nuclear pores and forming cytoplasmatic pseudoinclusions that contain phagolysosomal bodies and constitute a sort of autophagic vacuoles [123]. The cytoplasm is rich in intermediate filaments, and the apical surface exhibits numerous and long microvilli. Abnormal deposition of basement membrane material is also typical of PTC [124, 125].

The architectural pattern of PTC is variable as is illustrated by its numerous variants. If one excludes the incidentally found papillary microcarcinoma which by definition measures 1 cm or less than 1 cm, the classic PTC (CPTC) and the follicular variant of PTC (FVPTC) are the most frequently diagnosed PTCs.

Microscopically, CPTC discloses usually an infiltrative often multicentric, growth pattern, occasional cystic formations, and psammoma bodies in the papillary stalks (Fig. 14.41). Some tumors present sharply outlined bands of more or less cellular fibrosis. CPTC contains numerous papillae that are usually branching with a central fibrovascular core and a lining of cuboidal to elongated cells that may display hobnail features (Fig. 14.42). The stroma of the papillae is often edematous and may contain lymphocytes, foamy macrophages, and nests of adipose tissue. Together with the papillae, there are often a variable amount of follicles lined by neoplastic cells and occasionally solid areas, spindle cell areas, and foci of squamous metaplasia. Scattered lymphocytes and multinucleated giant cells may be present in the lumina of the follicles.

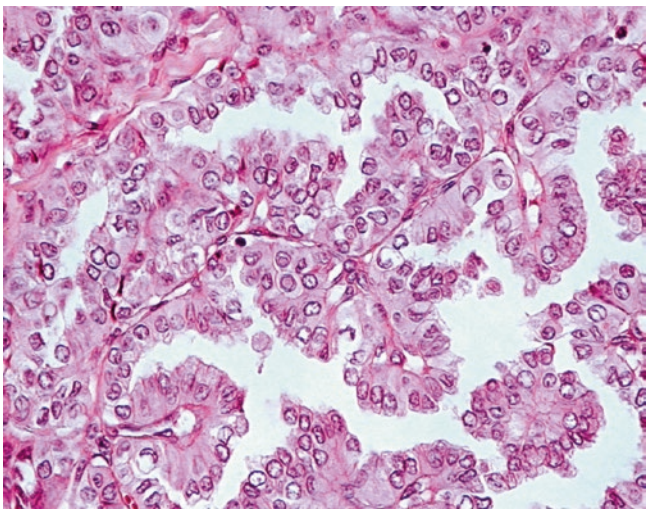

Fig. 14.40 Typical papillary carcinoma nuclear features

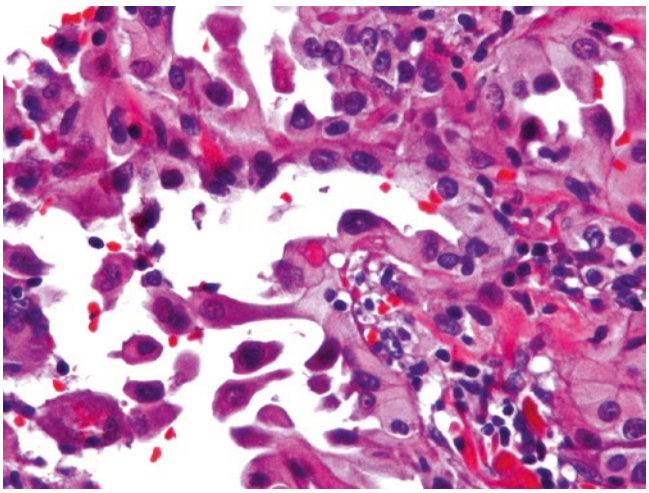

Fig. 14.42 Hobnail features in a papillary carcinoma

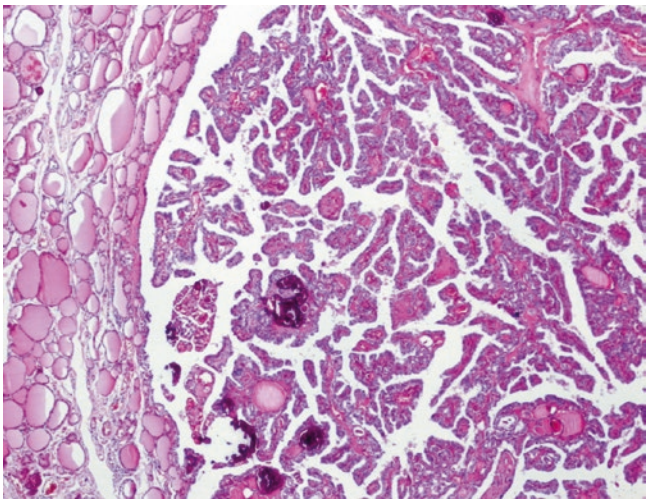

Fig. 14.41 Papillary structures with psammoma bodies in a classic papillary carcinoma

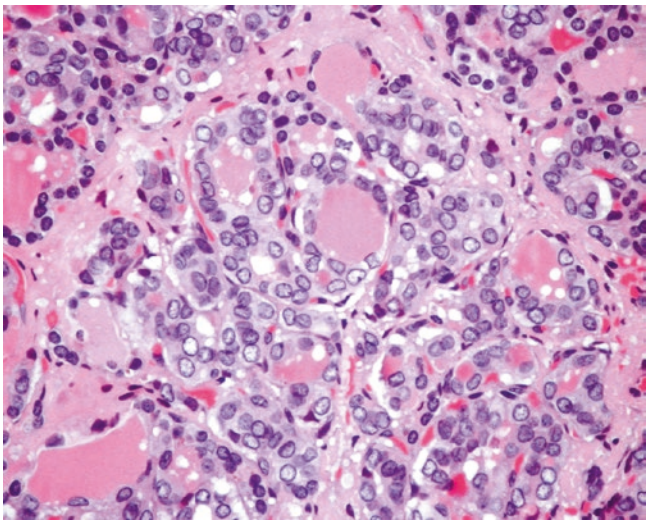

Fig. 14.43 Follicular variant of papillary carcinoma

FVPTC discloses either an expansive or an infiltrative growth pattern and is characterized by an exclusive or almost exclusive (more than 95 %) follicular architecture (Fig. 14.43). The follicles are small to medium sized and irregularly shaped, are frequently displaying an elongated, tubular structure, and are lined by cuboid follicular cells with the abovementioned typical nuclear features. There is peripheral scalloping of the colloid and intraluminal crystalloids. When compared with the CPTC, the FVPTC is more often encapsulated and unicentric, and almost never exhibits psammoma bodies. The behavior of FVPTC, whenever invasive, is analogous to that of CPTC.

The encapsulated subtype of FVPTC is completely surrounded by a capsule and virtually never gives rise to distant metastases or patients' death. This subtype of tumor, previously designated as Lindsay tumor, is a source of controversy and disagreement among pathologists as its diagnosis relies only in the subjective observation of the typical PTC-type nuclei in a tumor that may not disclose any signs of capsular or vascular invasion. This subtype should be distinguished from the adenoma(tous) nodule with or without papillary growth that, instead, discloses follicular cells with basally located, hyperchromatic nuclei. The evaluation of the nuclear features, as well as the consequent distinction between benignity and malignancy, may be extremely difficult if the nuclear changes are focal or not fully developed. The utilization of immunohistochemistry does not usually help in difficult cases but may be used to confirm almost typical cases (Figs. 14.43 and 14.44).

The macrofollicular variant is a rare variant of PTC that implies the recognition of the typical PTC-type nuclei in cells that line large follicles, resembling a benign follicular lesion in low magnification [126]. The prognosis of patients

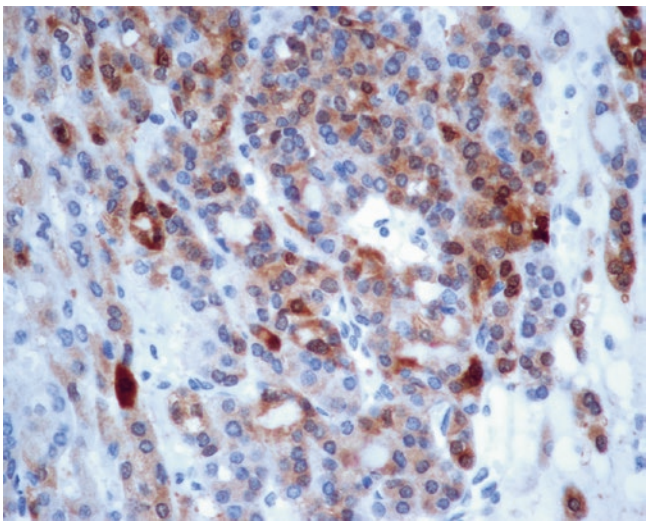

Fig. 14.44 Galectin 3 expression in follicular variant of papillary carcinoma

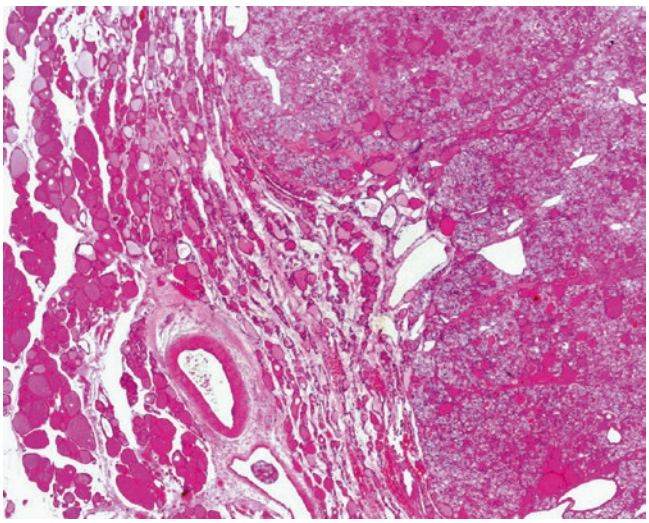

Fig. 14.45 Multinodular subtype of the follicular variant of papillary carcinoma

Table 14.4 Prognosis associated to PTC variants

Very good/excellent prognosis	Guarded prognosis
Papillary microcarcinoma	Diffuse sclerosing PTC
Encapsulated non-invasive FVPTC	Diffuse (multinodular) subtype of FVPTC
Macrofollicular subtype of FVPTC	Tall cell PTC
Cystic, non-invasive PTC	Columnar cell PTC
	Micropapillary (hobnail) PTC

Adapted from Asioli et al. [127]

with the macrofollicular variant of PTC falls in the group of very good outcome (Table 14.4).

The diffuse (multinodular) subtype of FVPTC is an unusual subtype of FVPTC that is composed by poorly defined nodules that occupy a lobe or both thyroid lobes. This subtype of FVPTC occurs in young women, mimics a multinodular adenomatous goiter, shows prominent vascular invasion, and besides nodal metastases gives rise to distant metastases to lung and/or bones in virtually all the cases (Fig. 14.45) [128].

A PTC measuring 1 cm or less in largest dimension is designated as papillary microcarcinoma, regardless of the growth pattern. This PTC variant is exceedingly frequent, occurring as an incidental finding in autopsies and thyroid specimens removed by other reasons. They may, however, be identified by ultrasound examination and diagnosed by FNAB. Most clinical guidelines do not advocate to perform FNA in infracentimetric thyroid nodules.

Most papillary microcarcinomas disclose an infiltrative growth pattern associated to extensive sclerosis of the stroma, but some are well circumscribed or even encapsulated.

The prognosis of papillary microcarcinoma is generally excellent, even when accompanied by regional lymph node metastases. Distant metastases are rare. Knowing that papillary microcarcinoma carries an extremely favorable prognosis, the term papillary microtumor was advanced by Rosai et al. – the so-called Porto proposal – in order to avoid the term carcinoma in incidentally found minute neoplasms occurring in adult patients [129].

The solid variant of PTC is more frequent in children. These tumors are composed by solid sheets of cells that disclose the typical PTC-type nuclei, a feature that distinguishes them from the more aggressive PDTC with solid growth pattern (Fig. 14.46) [64].

The diffuse sclerosing variant is also more frequent in young patients. It is a PTC variant that diffusely involves one or both thyroid lobes, clinically mimicking Hashimoto thyroiditis. This variant is characterized by dense sclerotic stroma involving nests of squamoid, solid, spindled, and papillary-arranged cells. Abundant psammoma bodies, lymphocytic infiltration, and extensive lymph vessel invasion are also present (Fig. 14.47). Lymph node metastases are frequent as well as lung metastases. The lymph node metastases may be difficult to diagnose since they may be thyroglobulin and TTF1 negative. The disease-free survival rate is lower than that of patients with CPTC.

PTCs can harbor a variable quantity of cells rich in mitochondria that frequently display abnormal morphology – the so-called oncocytic cells [130]. The oncocytic cells disclose an abundant, granular, eosinophilic cytoplasm, and round to bizarre nuclei with prominent eosinophilic nucleoli. In PTC, the oncocytic cells disclose PTC-type nuclei and can occur in tumors with mixed papillary-follicular growth pattern. The diagnosis of oncocytic variant of PTC (Hürthle cell or oxyphil PTC) must be considered whenever oncocytic cells comprehend the majority of the tumor cells [8]. Intraluminal foci of inspissated secretion are often present in

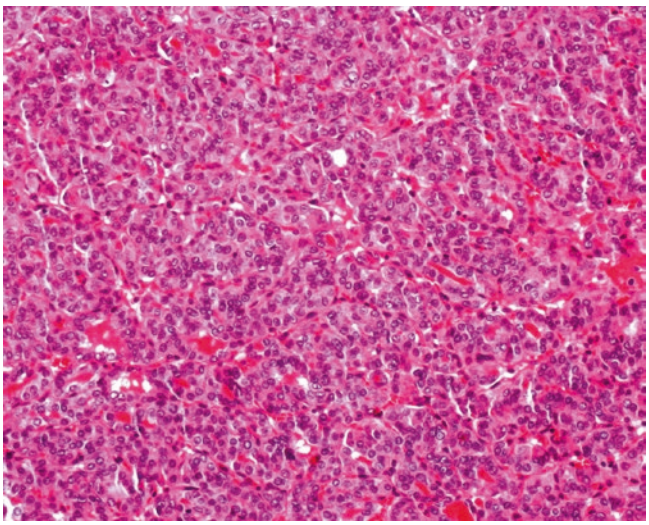

Fig. 14.46 Solid variant of papillary carcinoma

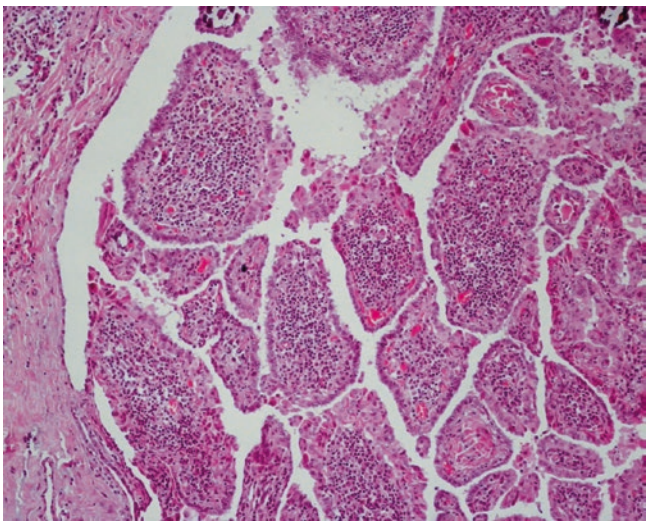

Fig. 14.48 Warthin-like (oncocytic) variant of papillary carcinoma

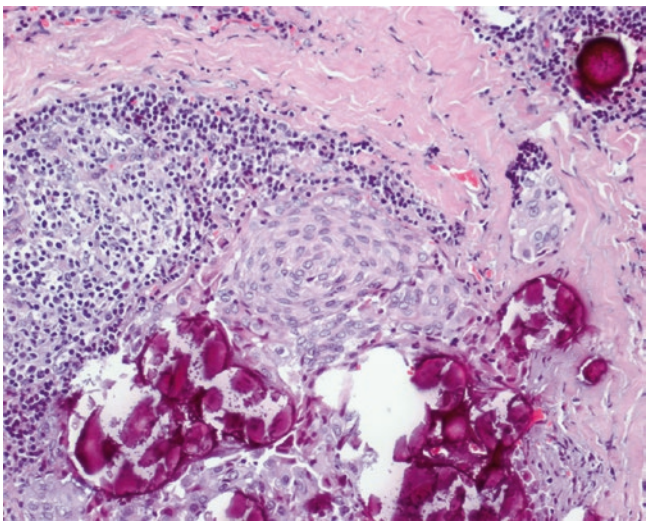

Fig. 14.47 Diffuse sclerosing variant of papillary carcinoma

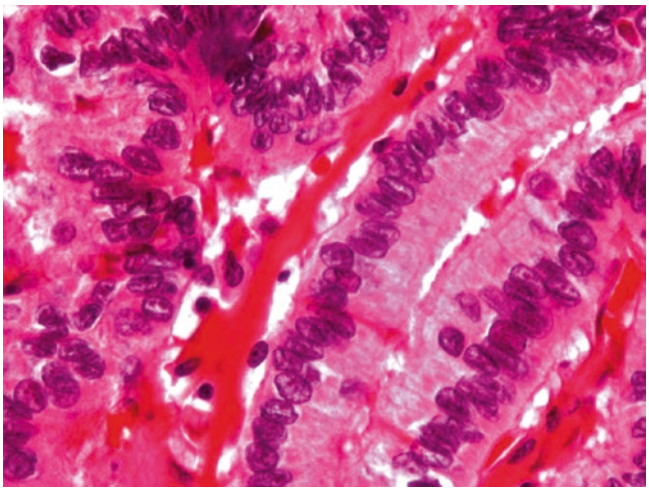

Fig. 14.49 Tall cell variant of papillary carcinoma

this variant and should not be confused with psammoma bodies. A variation on the theme is the Warthin-like oncocytic tumor of the thyroid that has morphologic similarities with Warthin tumor of the salivary glands. The Warthin-like oncocytic PTC is a tumor with papillary architecture, constituted by cells with PTC-type nuclei and abundant, granular, eosinophilic cytoplasm, infiltrated, and surrounded by abundant lymphocytes (Fig. 14.48).

It tends to be associated with an excellent prognosis provided there are not overtly invasive features.

The tall cell variant of PTC is a tumor composed by tall cells (at least half of the cells have a height that is three times larger than the width) (Fig. 14.49). These cells are arranged in a single row that lines papillary growths and are characterized by an abundant eosinophilic cytoplasm and PTC-type nuclei that often harbor prominent pseudoinclusions. The tall cell variant of PTC affects older patients and carries a worse prognosis than CPTC usually due to the development of extrathyroidal extension and resistance to radioactive iodine [131].

The columnar cell variant of PTC can be difficult to separate from the tall cell variant of PTC, not only due to the morphological resemblance but also due to the occasional coexistence of tall cell and columnar cell patterns in the same tumor. The columnar cell variants are usually arranged in well-formed papillae frequently associated with glandular structures [132]. The cytoplasm of the neoplastic cells is often clear and vacuolated. The nuclei lack the typical PTC-type features and disclose a high mitotic index and elongated and pseudostratified, thus resembling endometrial, nuclei (Fig. 14.50). The tumors have a high Ki67 labeling index. Thyroglobulin expression is often weak and focal. Regardless of considering columnar cell carcinomas as a variant of PTC

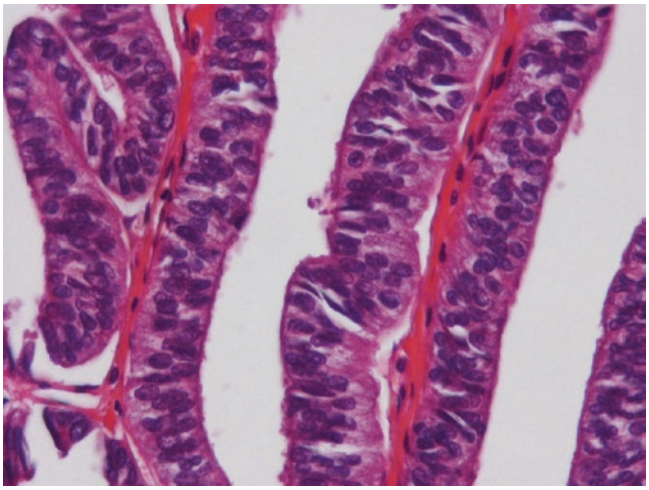

Fig. 14.50 Columnar cell variant of papillary carcinoma

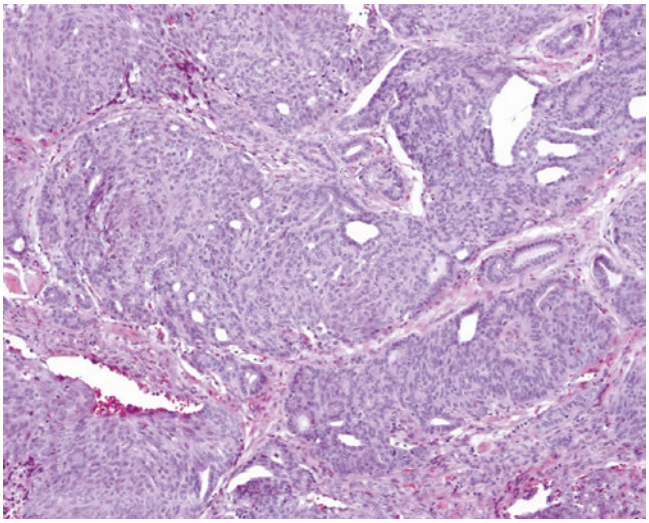

Fig. 14.51 Cribriform-morular variant of papillary carcinoma

or a thyroid carcinoma histotype per se, every author agrees that its clinical behavior is similar to that of other PTC variants carrying guarded prognosis (Table 14.4), giving rise to regional lymph node metastases and blood-borne distant metastases.

The cribriform-morular variant is, like the columnar cell variant, a type of tumor that lacks both the typical PTC-type nuclei and thyroglobulin diffuse expression. For these reasons, some authors do not consider this carcinoma as a variant of PTC. The cribriform-morular variant of PTC is characterized by areas of cribriform aspect and morular formation (Fig. 14.51), as well as variable quantities of solid areas, spindle cell areas, and cysts. This PTC variant occurs almost exclusively in women either sporadically or in a familial context of patients with colonic adenomatous polyposis. It carries a good prognosis, but aggressive forms have been reported [133, 134]. ß-Catenin is strongly expressed in the nuclei of the tumor cells of this PTC variant in contrast to the cell membrane staining of normal thyroid and other PTC variants.

Less frequent variants such as clear cell variant, PTC with nodular fasciitis-like stroma, PTC with focal PDTC component, PTC with squamous cell or mucoepidermoid carcinoma, PTC with spindle and giant cell carcinoma, and combined PTC and MTC have been described.

The majority of PTC cases evolve favorably, and the patients have a 10-year survival up to 98 % [135]. Despite the generally good prognosis, PTCs are frequently accompanied by cervical lymph node metastases and may give rise to recurrences as well as to distant metastases to the lungs and bones, sometimes many years after diagnosis. Cervical lymph node metastases tend to be cystic and may be the first manifestation of the disease particularly in very small primary tumors. The presence of lymph node metastases is not associated to an increased mortality rate but predicts an increased risk of subsequent locoregional recurrence. On the other hand, the occurrence of distant metastases, advanced age at time of diagnosis, male gender, tumor size above 1 cm, extrathyroidal extension, multicentricity, absence of total encapsulation, vascular invasion, and coexistence of poorly differentiated or anaplastic foci are some of the clinicopathological prognostic factors associated to poor prognosis. There are systems such as AMES, AGES, and MACIS [136] that provide good, and frequently equivalent, prognostic information on patients with PTC. The most controversial item considered in some of these systems is tumor grading due to the difficulty in using the usual classification in grade I, II, and III to "frame" the peculiar characteristics of PTC nuclei. Incidentally, the revision of PTC cases classified as grade III (or high grade) was found to correspond mainly to PDTC. In an attempt to solve this difficulty, it has been suggested to grade PTC using vascular invasion and tumor necrosis together with nuclear atypia; this approach provides valuable prognostic information but goes beyond the usual parameters of a grading system (e.g., vascular invasion is a staging feature) [137].

Differential diagnosis In the preoperative setting, the ultrasound examination of the thyroid nodule followed by FNAB constitutes the most effective procedure to diagnose PTC and to separate it from benign thyroid nodules that do not need surgery. The determinant nature of the typical nuclear features of PTC underlies the success of the cytological diagnosis by FNAB.

The cells of PTC express low and high molecular weight cytokeratins including cytokeratin 7 and 19, but do not express cytokeratin 20. There is also reactivity for thyroglobulin (except in some of the abovementioned variants), TTF-1, TTF-2, PAX8, HBME-1, galectin-3, EMA, and CEA.

The expression of galectin-3, HBME-1, and cytokeratin 19 has been used to help in the differential diagnosis between encapsulated FVPTC and FA, but these immunohistochemical expressions, in tumors with focal or not fully developed nuclear changes, are variable and not conclusive. For some authors, the aforementioned lesions with borderline nuclear features and no unequivocal signs of invasion should be designated as well-differentiated tumors of uncertain malignant potential and treated conservatively [105].

Oncocytic cells are frequently observed not only in PTC but also in benign (FA) and other malignant thyroid tumors (FTC and MTC) as well as in hyperplastic conditions (e.g., multinodular adenomatous goiter) and in chronic inflammatory conditions (e.g., Hashimoto thyroiditis) of the thyroid [138]. In contrast with this, oncocytic transformation of solid cell nests is extremely rare [139]. The criteria used in the diagnosis of the oncocytic variants of PTC, FTC, and PDTC are those used in the diagnosis of their non-oncocytic counterparts: nuclear characteristics of the PTC-type, signs of capsular and/or vascular invasion, and the morphologic features of the so-called Turin algorithm for diagnosing PDTC, respectively. In all these instances, the oncocytic appearance is thought to represent a phenotype that is superimposed on the genotypic and conventional histopathologic features of the tumors.

Genetics Classic PTC epitomizes the dissociation between proliferation which is very low in most PTCs and local invasion and lymph node metastization, which is thought to be partly mediated by downregulation of E-cadherin [140].

BRAF mutation is the most prevalent genetic alteration ascertained to PTC. A point mutation in codon 600 (*BRAFV600E*) of *BRAF* gene is present in, at least, 30 % of PTC, being more prevalent in conventional PTC (~45 %) than in FVPTC (<15 %) and particularly common in rare subtypes of PTC like the tall cell variant and Warthin-like PTC (~75 %). Others, much less frequent types of *BRAF* mutation, are detected in the FVPTC (*BRAF K601E*) [141] and in the solid variant of PTC (*BRAFVK600-1E*) [142]. In the setting of radiation-induced PTC (e.g., post Chernobyl), a *BRAF* rearrangement (*AKAP9-BRAF*) has been occasionally detected [143]. The detection of *BRAF* mutation seems to have some diagnostic utility since it is present only in malignant lesions. "Unfortunately" it is more prevalent in CPTC that is the easiest to diagnose by FNAB. The prognostic significance of *BRAF* mutation in PTC remains disputable, although the presence of this genetic alteration has been associated with downregulation of iodine-metabolizing proteins. This fact explains, at least in part, the higher recurrence and the refractoriness to iodine treatment of patients harboring PTC with *BRAF* mutations [144, 145]. Recently, our group shown that the coexistence of *BRAF* mutation together with the *TERT* promoter mutation identifies the group of PTCs with guarded prognosis [146, 147].

RET/PTC rearrangements are also relatively prevalent in sporadic PTC (20–60 %), in particular in PTC from children and young adults. A less frequent *RET* rearrangement (*RET/PTC3*) appears to be prevalent in the solid variant of PTC [148]. In populations subjected to accidental or therapeutic irradiation, the rearrangements can be present in up to 80 % of the associated PTC [149]. *RET/PTC* rearrangements have also been described in other benign and malignant thyroid lesions, lowering its utility as a diagnostic marker [144].

Similar prevalence of *RET/PTC* rearrangements and *BRAF V600E* mutation in oncocytic and non-oncocytic variants of PTC has been observed. The coexistence of oncocytic phenotype and *RET* mutation can also be found in the oncocytic variant of MTC [150]. Oncocytic tumors differ from their non-oncocytic counterparts regarding the prevalence of large deletions of mitochondrial DNA, mutations of mitochondrial DNA genes coding for OXPHOS proteins (namely, mutations of complex I subunits genes), and mutations of nuclear genes coding for mitochondrial OXPHOS proteins [151].

RAS mutations are less frequent in PTC and associated with neoplasms with follicular pattern being detected in 17–43 % of FVPTC and in 0–15 % of conventional PTC [152]. There is no robust data linking *RAS* mutations with the prognosis of patients with *RAS* mutated PTC [144].

TERT promoter mutations, although not very frequent in PTC (present in about 7,5 % of the cases), seem to cluster in tumors with bad prognosis features (older age, larger tumors, higher stage) and confer a poor outcome and shorter survival to the patients [146, 147].

Treatment and prognosis Most PTCs are treated by surgical excision of the whole thyroid together or not with regional lymph node dissection of the cervical regions, followed by treatment with radioactive iodine, except in patients with tumors following into one of the categories of excellent prognosis. The two controversial topics at the moment concern to stop at the lobectomy or lobectomy plus isthmectomy level and, the other end of the spectrum, when (and how far) to do systematic lymphadenectomy (Table 14.4).

14.5.4 Follicular Thyroid Carcinoma

Definition Follicular thyroid carcinoma (FTC) is a malignant thyroid tumor exhibiting follicular cell differentiation in the absence of the typical PTC-type nuclear features.

Epidemiology FTC accounts for less than 10 % of clinically evident thyroid malignancies and occurs mainly in middle-aged women, being very rare in children [93].

Etiology and pathogenesis Both radiation and iodine deficiency have been associated with the development of FTC.

Clinical aspects This tumor is rarely occult and manifests as a cold single nodule or as a multinodular mass with or without compressive symptoms, seldom accompanied by lymphadenopathies.

Macroscopy Minimally invasive FTC is macroscopically similar to a FA. It is surrounded by a well-defined thick fibrous capsule and discloses a gray to tan cut surface. Widely invasive FTC discloses macroscopically evident capsular penetration and invades the "normal" adjacent parenchyma. Typically, oncocytic FTC has a mahogany brown cut surface and tends to develop necrotic, hemorrhagic, and cystic areas spontaneously or after FNAB.

Microscopy Typically, FTC presents images of capsular invasion (Fig. 14.52) as well as vascular invasion of the vessels of the capsule (Fig. 14.53). The architecture of FTC is based on the follicle, assuming aspects that oscillate between medium size (rarely macrofollicles) and microfollicles, areas of solid/trabecular growth patterns are frequently present, whereas spindle and glomeruloid aspects can be occasionally detected [153, 154]. The tumor cells resemble those of a FA with small, dark, and regular nuclei, disclosing scant mitotic figures (Fig. 14.54). This resemblance underlies the similitude between FTC and FA in cytological aspirates. Necrosis may be present, while psammoma bodies are not found. Multicentricity is unusual.

The cytoplasm of FTC cells can show clear cell change or oncocytic change that, whenever present in more than 75 % of the cells, lead to the separation of FTC in clear cell variant and oncocytic (Hürthle cell or oxyphil) variant, respectively. The oncocytic variant is diagnosed a decade later than the classic FTC and discloses lymph node metastases in 30 % of cases, at variance with classic FTC that almost never gives rise to nodal metastases. Microscopically, the growth pattern of oncocytic variant FTC includes follicular to solid/trabecular architecture and less frequently also focal papillary structures. The nuclei are often pleomorphic with focal bizarre aspects, and the cytoplasm is eosinophilic and finely granular (Fig. 14.55).

FTCs have been separated into minimally invasive and widely invasive subtypes [155].

Minimally invasive FTC discloses a thick capsule with limited or focal invasion and may present or not vascular invasion. Widely invasive FTC shows widespread capsular and vascular invasion that, in advanced cases, assumes the aspect of a multinodular growth that grows beyond a preexisting capsule. Minimally invasive FTCs that disclose prominent vascular invasion should be individualized as angioinvasive FTCs as their behavior approaches that of widely invasive FTCs and should be treated accordingly (see below).

Differential diagnosis Only the tumor penetration through the capsule, often in a mushroomlike fashion, is considered a true image of capsular invasion, and only the vessels located outside the tumor are considered appropriate for vascular invasion evaluation. Images of capsular invasion should be separated from the capsular rupture after FNAB that is usually accompanied by an exuberant stromal reaction and from tumor herniation caused by manipulation of the specimen during clinical examination or surgery. Capsular invasion is not always easy to appreciate and can pass undetected if the section misses

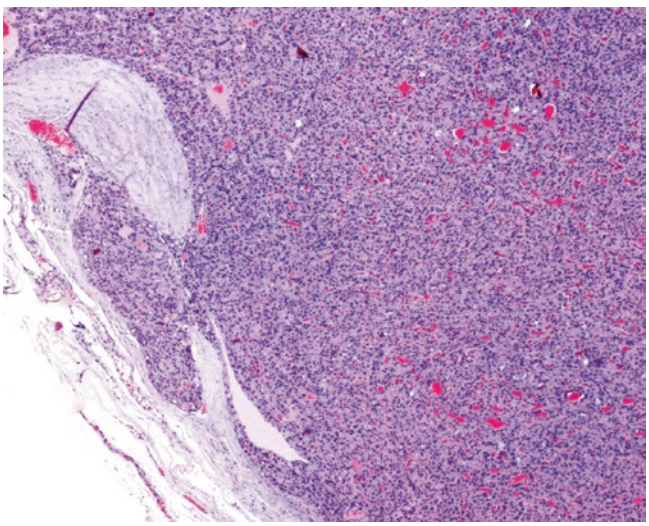

Fig. 14.52 Mushroomlike capsular invasion in minimally invasive follicular carcinoma

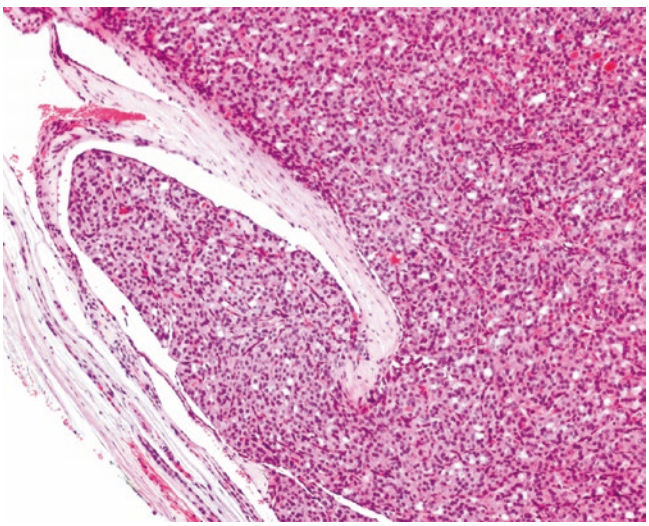

Fig. 14.53 Invasion of the vessels of the capsule in angioinvasive follicular carcinoma

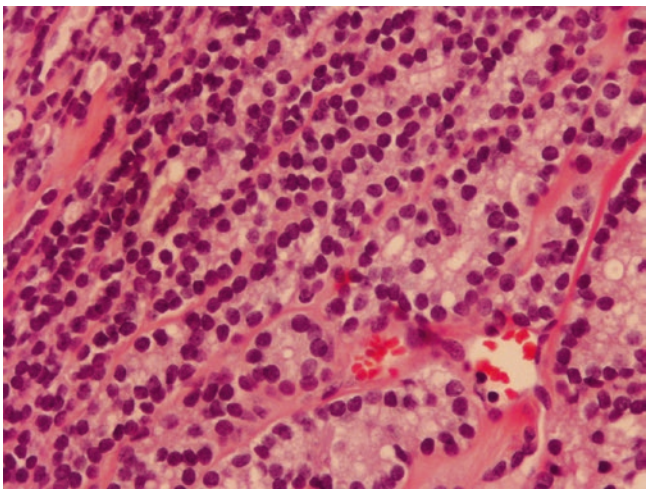

Fig. 14.54 Tumor cells arranged in small follicles in a follicular carcinoma

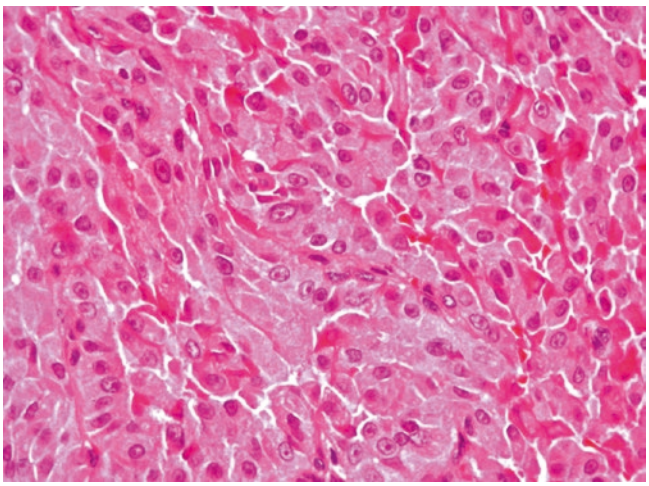

Fig. 14.55 Oncocytic cells in an oncocytic variant of follicular carcinoma

the penetration area, thus requiring the complete observation of the tumor capsule and consecutive sections. The requirement of a complete capsule sampling turns impossible the distinction, in most cases, between minimally invasive FTC and FA in the perioperatory frozen section procedure. Vascular markers such as CD31 and CD34 have been used to highlight the vessels lining in order to help in the identification of vascular invasion that can also be difficult to evaluate (see Table 14.3). Most authors only rely, however, in the demonstration of unequivocal invasion of vessels that can be identified in H&E-stained sections. A mimic of vascular invasion is the hyperplasia of endothelial and smooth muscle cells of capsular vessels.

The utilization of the aforementioned strict criteria for recognizing capsular and vascular invasion and the increased number of follicular-patterned tumors classified by their nuclear characteristics, as FVPTC, have led to a progressive reduction of the percentage of FTC among well-differentiated thyroid carcinomas.

FTC cells express thyroglobulin, TTF-1, low molecular weight keratin, and EMA together with the above, the expression of Bcl-2 and p27, in the absence of p53 expression, constitutes the general profile of FTC.

Immunohistochemistry, morphometry, ploidy analysis, and cytogenetic and molecular markers have failed to provide reliable information concerning the distinction between FTC and FA. Tumors showing a predominantly solid/trabecular/nesting pattern of growth should be evaluated for mitoses and necrosis in order to rule out the diagnosis of PDTC [64].

Genetics As referred above, none of the classical molecular markers assigned to FA or FTC allow the distinction between benign and malignant follicular tumors, the most important issue from a clinical standpoint. *RAS* mutations are relatively frequent in FA (24.53 %) and in FTC (40–53 %) as well as *PAX8/PPARγ* rearrangement (present in up to 50 % of the FA and 65 % of FTC), that is also present in other sporadic follicular-patterned tumors (e.g., FVPTC) [141, 153, 156]. Neither *RAS* nor *PAX8/PPARγ* seem to carry a clear prognostic meaning in FTC.

RET/PTC rearrangements are rare in follicular-patterned lesions [157]. Nevertheless, the presence of this rearrangement in oncocytic FVPTC with a solid growth pattern raises the possibility of its utilization as predictive indicator for using *RET* inhibitors therapy in patients with those tumors.

TERT promoter mutations are present in 17 % of the FTC (and absent in FA) and were found to be associated with clinicopathological characteristics of guarded prognosis in FTC (older age and presence of distant metastases). Preliminary data also indicate that patients harboring FTC with *TERT* promoter mutations show poorer outcome and shorter survival [147].

Treatment and prognosis Metastases are usually blood borne, including lungs (Fig. 14.56) and bones, rather than to regional lymph nodes. Such distant metastases are relatively common in the widely invasive subtype. Non-angioinvasive, minimally invasive FTCs almost never develop metastases. The treatment of widely invasive FTC (as well as of all angioinvasive FTC) is total thyroidectomy followed by radioactive iodine. The oncocytic variant usually responds less well to the radioactive iodine therapy, a feature that may justify the worse clinical outcome of patients with the oncocytic variant of FTC in comparison with those with classic FTC. Recent evidence points to the adequacy of a conservative approach in the treatment of patients with minimally invasive FTC, provided the tumors do not display vascular invasion, as they only exceptionally

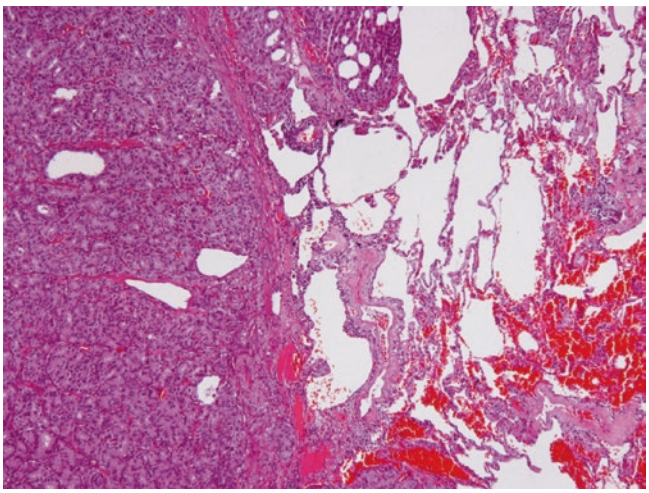

Fig. 14.56 Lung metastasis of a follicular carcinoma

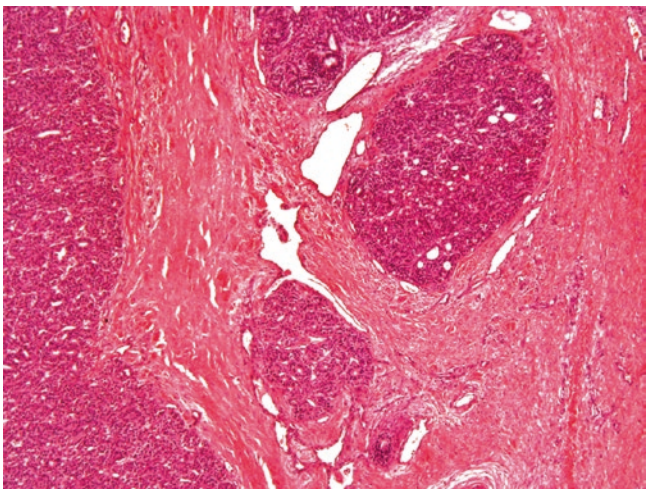

Fig. 14.57 Poorly differentiated carcinoma with solid pattern invading beyond the tumor capsule and displaying images of vascular invasion

give rise to distant metastases after lobectomy or lobectomy plus isthmectomy.

14.5.5 Poorly Differentiated Thyroid Carcinoma

Definition Poorly differentiated carcinoma (PDTC), previously designated as insular carcinoma, is a neoplasm with limited evidence of follicular cell differentiation whose behavior and morphology fall in between well-differentiated carcinomas and ATC.

Epidemiology Like other thyroid neoplasms, PDTC is more frequent in women presenting with a long-standing nodular thyroid, after the sixth decade of life, and comprehends nearly 4 % of clinically evident thyroid carcinomas.

Clinical aspects PDTC is an invasive neoplasm that usually manifests as a cold, solitary, and locally aggressive mass with associated compressive symptoms. Enlarged lymph nodes and distant metastases to lungs and bones can also be detected at the time of diagnosis. Exceptional cases of encapsulated, angioinvasive PDTC have been reported [106].

Macroscopy PDTC presents as a large uninodular or multinodular mass with expansive growth that frequently discloses an invasive front growing beyond the tumor capsule and into extrathyroidal tissues.

Microscopy and pathogenesis The diagnostic criteria of PDTC have varied in the last years until the recognition of PDTC as an entity in the WHO booklet in 2004. This recognition has been consolidated in the Turin proposal in 2007 [64]. Microscopically, PDTC usually discloses a thick capsule with widespread images of capsular and vascular invasion (Fig. 14.57). It is a tumor with insular (Fig. 14.58), solid/trabecular, or microfollicular growth pattern. The insular growth is characterized by nests of tumor cells with a peripheral palisade that are separated from the surrounding stroma by artifactual clefts. PDTC is constituted by monotonous tumor cells without the typical PTC-type nuclei. The nuclear features are those either of the so-called intermediate type or darker and smaller fitting the so-called convoluted or raisin-like nuclei. The mitotic index is usually higher than 3 mitoses *per* 10 high-power fields, and necrosis, that is often present, contributes to the frequent peritheliomatous growth pattern.

PDTC cells may assume an oncocytic (Hürthe cell or oxyphil) phenotype that, in this setting, is characterized by smaller cells than those observed in the oncocytic variants of well-differentiated carcinomas. The oncocytic variant of PDTC can be partially constituted by cells with rhabdoid features and behaves as a classic PDTC.

The cytologic features by FNAB include cellular smears with follicular cells disclosing a low grade of atypia, isolated or organized in nests, trabeculae, and microfollicles in a necrotic background.

The coexistence of PDTC with a well-differentiated thyroid carcinoma component, either PTC or FTC, has been reported in more than half of cases (Fig. 14.59). The well-differentiated component has been assumed to give rise to the poorly differentiated component, even in cases where PDTC coexists with a PTC variant associated to favorable prognosis [158].

Differential diagnosis The distinction between PDTC and widely invasive FTC can be difficult to accomplish as most of the Turin criteria are present in both types of tumors. The

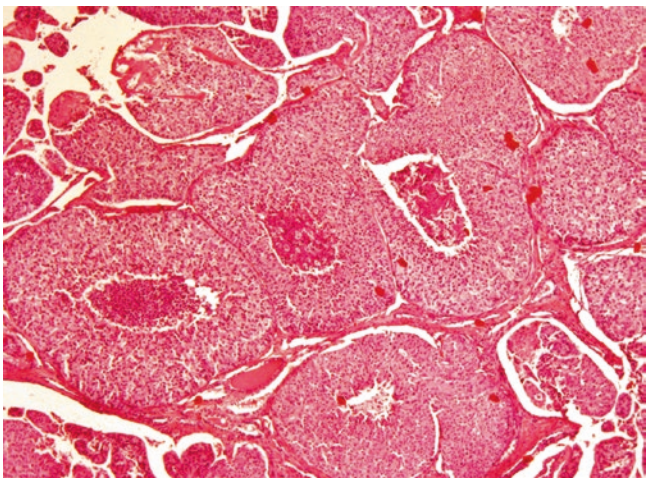

Fig. 14.58 Insular growth pattern with comedo-type necrosis in poorly differentiated carcinoma

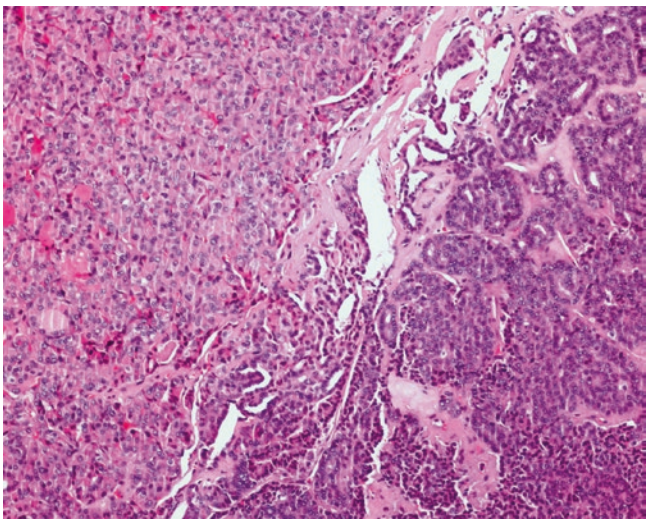

Fig. 14.59 Poorly differentiated carcinoma (*bottom right*) coexisting with follicular variant of papillary thyroid carcinoma (*upper left*)

finding of insular pattern; limited evidence of follicular cell differentiation, p53, and/or beta-catenin nuclear expression; and high Ki-67 labeling index favor the diagnosis of PDTC. The solid/trabecular growth pattern in a thyroid primary malignancy is not a synonym of PDTC as besides FTC, PTC can also display this growth pattern being composed by cells with PTC-type nuclei. PDTC can be mistaken for MTC due to the insular pattern or for a metastatic disease; the immunohistochemical profile of PDTC provides useful differential diagnostic markers since PDTC expresses thyroglobulin and TTF-1 and no calcitonin. Expression of neuroendocrine markers other than calcitonin has been described in some PDTC [133].

Genetics The morphologic heterogeneity of tumors that are often included under the designation of PDTC explains in part the quite different percentages of molecular alterations reported in different series of PDTC (e.g., *BRAF* mutations, *RET/PTC* rearrangement, and beta-catenin mutations), probably reflecting the discrepancies in the classification. Assuming that PDTC (and ATC) may derive from well-differentiated thyroid carcinomas, it is expected that some PDTC and ATC will harbor genetic alterations that are typical of PTC and FTC [159]. This is the case for some molecular markers (*BRAF* and *NRAS*) that are present in well-differentiated thyroid carcinomas and PDTC. Other genes, namely, *P53*, are almost exclusively found mutated in PDTC and ATC. Thyroid-specific rearrangements, namely, *RET/PTC* and *PAX8/PPAR*, on the other hand, are rarely found in PDTC.

No *BRAF* mutations were detected in PDTC exclusively composed of insular and insular-like tumors [160]. *BRAF* mutations were nevertheless described in PDTC with PTC-like nuclei, as well as in PDTC coexisting with foci of PTC [161]. In the clinical setting *BRAF* mutations are more frequent in PDTC refractory to radioactive iodine treatment (39 %), than in non-refractory PDTC (12 %) [145].

The prevalence of *RAS* mutations in PDTC ranges between 18 % and 55 % [159] and has been advanced as the most common genetic alteration and a negative prognostic parameter [162]. P53 mutations have been also reported in approximately 26 % of PDTC [159].

Recently, mutations in the promoter of telomerase gene *hTERT* have been described in thyroid tumors. These mutations are associated with clinicopathological features associated to poor prognosis and are more frequent in less differentiated tumors like [147].

Treatment and prognosis The treatment of PDTC is similar to that of widely invasive FTC.

14.5.6 Anaplastic Thyroid Carcinoma

Definition Anaplastic thyroid carcinoma (ATC), also known as undifferentiated thyroid carcinoma, sarcomatoid carcinoma, carcinosarcoma, metaplastic carcinoma, and dedifferentiated carcinoma.

Epidemiology and etiology Is a lethal neoplasm that represents much less than 1 % of thyroid malignant tumors. ATC occurs in elderly patients over the sixth to seventh decade of life, often preceded by a history of a long-standing nodular goiter.

Clinical aspects Manifests as a rapidly growing, fixed, and hard neck mass with compressive symptoms that is locally

very aggressive. It may be bilateral and involve both local lymph nodes and distant sites such as lungs and bones. The mean survival time after diagnosis is 6 months.

Macroscopy ATC presents as a large, hard mass, usually with necrotic, hemorrhagic, and solid fleshy areas that replaces most of the thyroid and invades beyond the thyroid limits, encasing cervical organs.

Microscopy The microscopic aspect of ATC cells is variable ranging from epithelioid to giant and spindle cells that can be present in variable amounts in the same tumor (Fig. 14.60). The cells are poorly cohesive and invade the adjacent tissues and the vessel walls leading to venous thrombosis. The epithelioid cells can disclose squamous differentiation with keratin formation and lymphoepithelioma-like features. The spindle cell areas can display a fascicular or storiform pattern of growth as well as foci of bone formation, skeletal muscle differentiation, and angiosarcoma-like areas. Overall, the nuclei are atypical, pleomorphic, and bizarre and have a very high mitotic index. Inflammatory infiltrates and reactive osteoclast-like giant cells are frequent.
Ultrastructural examination highlights the existence of epithelial differentiation signs in most of the cases raising differential diagnostic problems.

Like PDTC, ATC can coexist with a well-differentiated thyroid carcinoma component, either PTC or FTC, as well as with a PDTC component. In these cases, the ATC component tends to overgrow the better differentiated components and is the major determinant of prognosis.

Differential diagnosis ATC with prominent collagenous and hyalinized matrix and scant spindle neoplastic cells configures the so-called paucicellular variant of ATC that can be easily mistaken for Riedel thyroiditis or other collagenous-rich lesion. ATC must be distinguished from metastatic disease and from true sarcomas of the neck region involving the thyroid.

ATC typically expresses vimentin and usually, only weak and focal, expression cytokeratins (Fig. 14.61). Some ATCs do not express any cytokeratins at all, and the diagnosis, in these cases, is based upon the positivity for PAX8 or on the clinicopathological context and the absence of a better alternative diagnosis. Thyroglobulin, calcitonin, and TTF1 are usually not expressed, whereas PAX8 has been found to be expressed in up to 79 % of ATCs [163]. Ki-67 labeling index is typically very high (Fig. 14.62).

Genetics Mutations in *TP53* are the most prevalent genetic alteration in ATC being present in more than 60 % of the cases in the majority of the series [159]. Mutations are mainly located in the known hotspots (exons 5–9), being codon 273 most frequently affected. The prognosis is similar in ATC with and without *TP53* mutations. Taking in consideration the data on the progression of well-differentiated thyroid carcinomas (no *TP53* mutations) to ATCs which frequently harbor *TP53* inactivation, gross genetic alterations, and aneuploidy, it is likely that *TP53* inactivation ("suppressor pathway") represents the most frequent mechanism of proliferation deregulation and chromosome instability in thyroid cancer [159].

BRAF mutations have also been described in 10–44 % of ATC [159]; in *BRAF*-mutated ATC, the mutations are frequently detected in adjacent foci of PTC supporting the idea that ATC may progress from *BRAF*-mutated PTC through the acquisition of an additional *TP53* mutation [164].

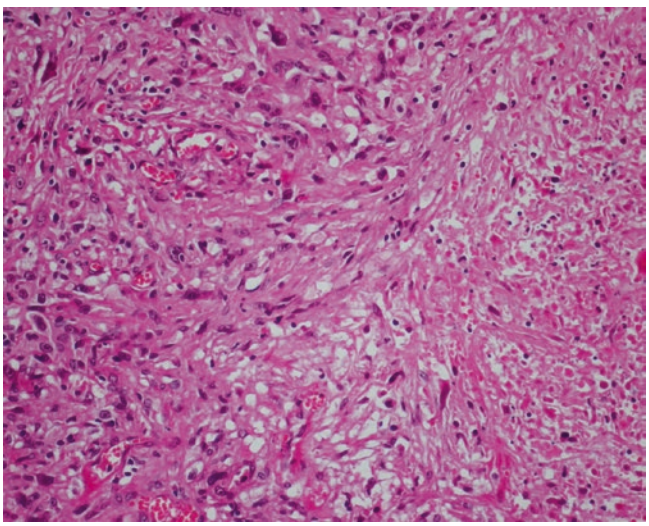

Fig. 14.60 Spindle cell anaplastic carcinoma with foci of necrosis (*right*)

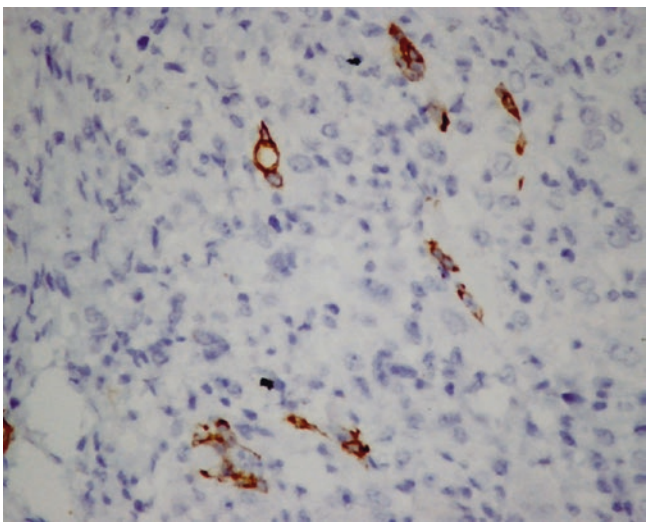

Fig. 14.61 Focal expression of pan-cytokeratins in anaplastic carcinoma (AE1.AE3)

The prevalence of *RAS* mutations varies from series to series of ATC from 4 % to 60 % without carrying apparent prognostic meaning.

Copy number gains and point mutations in *PI3K/Akt* pathway genes were also reported as being frequent in ATC [165].

As in PDTC, *RET/PTC* and/or *PAX8/PPARγ* rearrangements are virtually absent in ATC.

TERT promoter mutations which associate with poor prognosis characteristics and less differentiated tumors seem to be a frequent event in ATC (ranging from 30 % to 70 % of the cases) [147]. In accordance, telomerase activation/expression seems to be more frequent in ATC being detected in about 78 % of the cases [11, 159]; this finding fits with the assumption that thyroid cancers with telomerase promoter mutations have a more aggressive clinical behavior [147].

Treatment and prognosis ATC carcinoma has no proved efficient treatment. Very limited results have been obtained with a combination of surgery followed by radio and chemotherapy.

14.5.7 Medullary Thyroid Carcinoma and C Cell Hyperplasia

Definition Medullary thyroid carcinoma (MTC), also known as C cell carcinoma and solid carcinoma with amyloid stroma, is a malignant tumor of the thyroid showing C cell (parafollicular cell) differentiation. Medullary adenoma is not a recognized entity.

Epidemiology MTC represents about 5 % of clinically evident thyroid malignancies and occurs in both sporadic and hereditary setting.

Etiology and pathogenesis The sporadic cases occur mainly at or after the sixth decade and are usually unicentric tumors, while hereditary cases may develop in early childhood, are usually multicentric, and frequently coexist with C cell hyperplasia. Hereditary MTCs developed in the setting of multiple endocrine neoplasia syndrome 2A, 2B, and familial MTC comprehend nearly 25 % of all MTC cases [120].

Clinical aspects MTC manifests as a cold, painless nodule typically located in the middle third of the gland accompanied or not (usually not) by compressive symptoms: MTCs give frequently rise to metastases in cervical and mediastinal lymph nodes and less often to metastases in the lungs and bones. Recurrences and paraneoplastic syndromes such as Cushing syndrome may develop in patients with MTC [148].

Macroscopy MTCs are well-circumscribed, non-encapsulated, solid tumors with yellowish cut surface (Fig. 14.63).

Microscopy and differential diagnosis MTC is a tumor with a predominant by expansive growth that may exceptionally be limited by a thin capsule. MTC is usually constituted by round, plasmacytoid to spindle cells with granular and amphophilic cytoplasm (Figs. 14.64 and 14.65). The nuclei are round with focal pleomorphism and are devoid of the typical PTC-type nuclear features. Pseudoinclusions, however, may be found. The stroma is hyalinized or rich in amyloid substance with an associated foreign body giant cell component. Foci of necrosis and dystrophic calcifications may be found.

The cytoarchitectural patterns differ a lot from case to case and may include solid/trabecular, nested, spindle cell, giant cell, small cell, oncocytic cell, clear cell, squamous

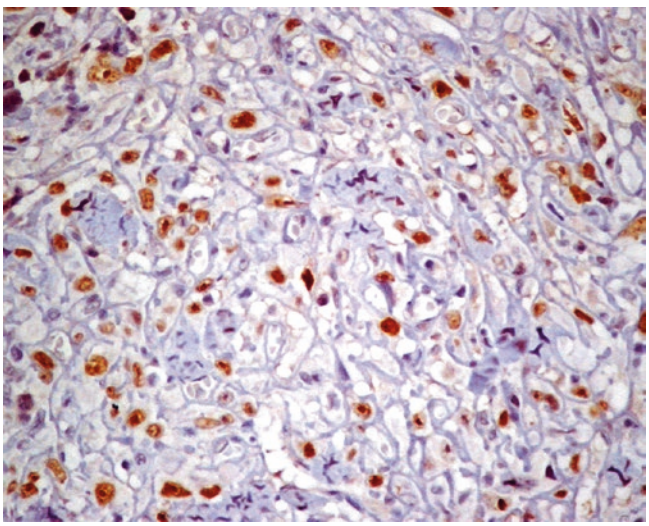

Fig. 14.62 Very high Ki-67 labeling index in an anaplastic carcinoma

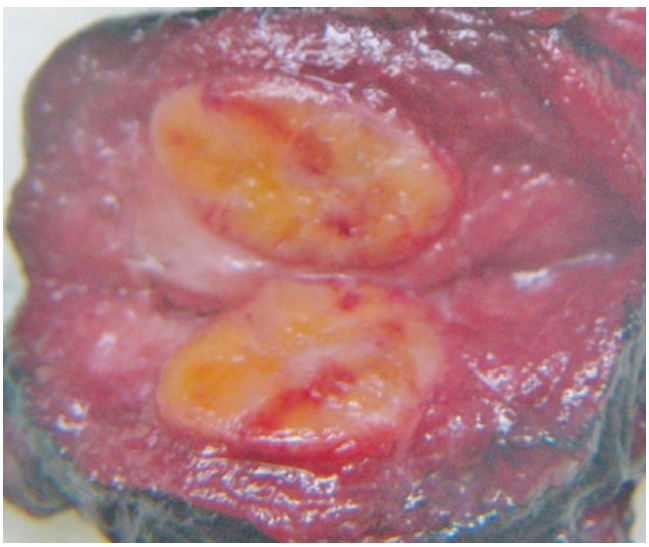

Fig. 14.63 Macroscopic aspect of medullary carcinoma

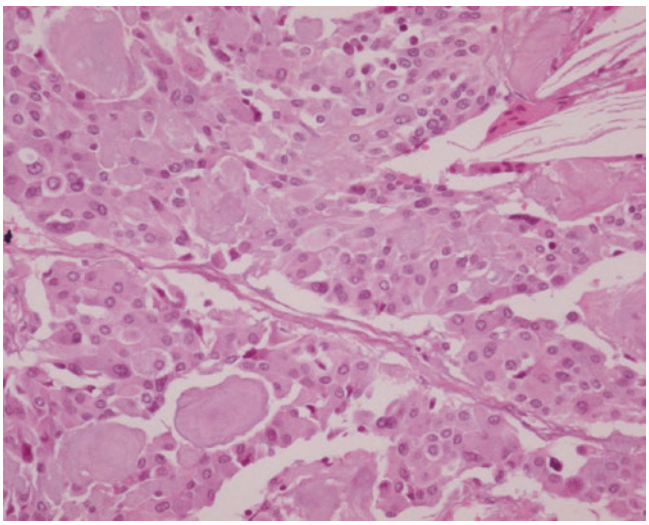

Fig. 14.64 Medullary carcinoma constituted of epithelioid cell nests and amyloid deposits surrounded by giant cells

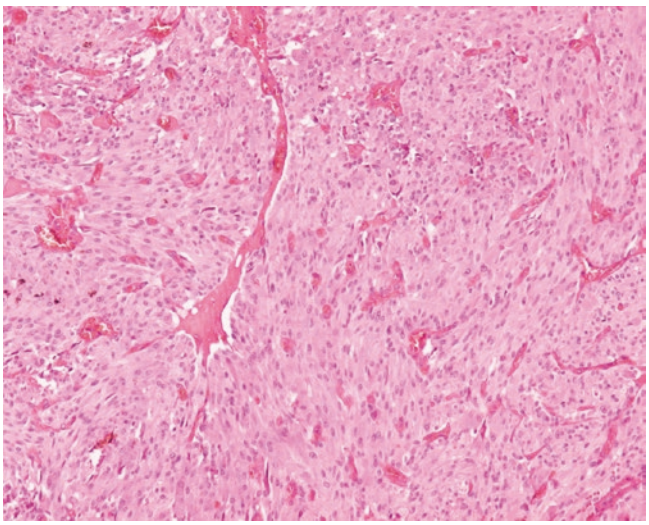

Fig. 14.65 Medullary carcinoma composed of monotonous spindle cells

cell, paraganglioma-like, pseudo-glandular, pseudo-papillary, and angiosarcoma-like features. The oncocytic (Hürthle cell or oxyphil) cell variant is difficult to distinguish from oncocytic neoplasms of follicular cell derivation, particularly in FNAB and in the frozen section setting. In FNAB, MTC is characterized by plasmacytoid to spindle isolated cells, binucleated and/or multinucleated, in a background that may have metachromatic amyloid stroma. The oncocytic variant of MTC should be suspected if the cells are amphophilic instead of eosinophilic.

MTC cells express cytokeratin 7, TTF1, calcitonin, calcitonin gene-related peptide, carcinoembryonic antigen, chromogranin, synaptophysin, and neuron-specific enolase [166]. Thyroglobulin is not expressed. The calcitonin blood levels are usually elevated in patients with MTC and can be used in follow-up as they decrease with the surgical excision of the tumor and rise up again if there is a recurrence.

MTC precursor lesion is designated C cell hyperplasia. Two types of C cell hyperplasia have been described: the reactive and the neoplastic C cell hyperplasia. The reactive C cell hyperplasia occurs in several conditions, Hashimoto thyroiditis and at the periphery of benign or malignant expansive nodules in the thyroid, other than MTC, and is thought to represent a response to mechanic stress or other stimuli to C cells. The reactive type of C cell hyperplasia is not detected by H&E and does not precede the development of MTC. The precursor lesion of MTC is the (nodular) neoplastic C cell hyperplasia that occurs in hereditary syndromes, is composed by atypical C cells that may be detected in the routine stainings, and is usually localized in the central part of the thyroid lobes. Nodular C cell hyperplasia can be difficult to distinguish from MTC measuring 1 cm or less, which is referred to as medullary microcarcinoma and is known to carry a favorable prognosis [167]. The presence of a stromal component in a small nodule composed of atypical C cell favors the diagnosis of medullary microcarcinoma.

Both reactive and neoplastic C cell hyperplasia should be distinguished from the normal clusters of C cells seen in relation to solid cell nests.

Genetics Mutations in *RET* oncogene are the critical genetic event, both in sporadic and hereditary setting. The mutations cluster in hotspots that are located at the cysteine-rich region of the extracellular domain and in the intracellular tyrosine kinase domain. Carriers of germ line *RET* mutations develop hereditary MTC as the first and most common clinical presentation. Along with hereditary MTC, patients can present with pheochromocytoma(s) and parathyroid adenoma(s). This syndromic condition is referred to as multiple endocrine neoplasia type 2 (MEN2). In clinical terms, three disease phenotypes can be recognized: MEN2A, MEN2B, and a familial form of isolated MTC. Penetrance for hereditary MTC is near complete (http://www.arup.utah.edu/database/MEN2/MEN2Welcome).

The most common *RET* germ line mutations are missense substitutions of extracellular cysteine residues, occurring at cysteine codon 634 in 80 % of cases. Mutations in cysteine 634 and, less frequently, in codons 609, 611, 618, 620, and 630 are mainly detected in MEN2A and FMTC phenotypes. Tyrosine kinase domain mutations, notably Met918Thr, are found in MEN2B phenotypes. Rare mutations found in isolated families have been reported, comprising homozygous mutations, duplications, and double mutations.

A similar spectrum of *RET* somatic mutations is observed in about 50–60 % of the sporadic MTC (http://www.sanger.ac.uk/genetics/CGP/cosmic/). Somatic Met918Thr mutation at exon 16 comprises up to 60 % of the mutation-positive

cases. Among the sporadic MTCs, cases with somatic Met918Thr mutations are associated with the worst prognosis (multifocal tumors, higher frequency of lymph node metastases and with persistent disease at advanced stage).

H- or *KRAS* mutations are present in 10–18 % of sporadic MTC negative for *RET* mutation. It was advanced that *RET* and *RAS* mutations represent alternative genetic events in sporadic MTC [168].

Treatment and prognosis MTC treatment includes total thyroidectomy and lymphadenectomy. Vandetanib, a *RET*-targeting tyrosine kinase inhibitor, has been approved for the treatment of patients with MTC that cannot be removed by surgery and is locally advanced or metastasized. A number of other *RET*-targeting tyrosine kinase inhibitors are undergoing clinical trials to evaluate their effectiveness in the treatment of MTC, and it is conceivable that the *RET* genotype may influence the response to such compounds [169].

14.5.8 Other Neuroendocrine Tumors

Definition and etiology Tumors with neuroendocrine differentiation that can be primary or metastatic.

Epidemiology Primary or metastatic neuroendocrine tumors other than MTC are rare.

Microscopy and differential diagnosis Whenever one discusses thyroid tumors with neuroendocrine differentiation, the first differential diagnosis step is to exclude the possibility of an intrathyroidal metastasis from a neuroendocrine carcinoma elsewhere or the local extension from a parathyroid carcinoma.

The expression of chromogranin in a primary thyroid tumor that does not express TTF-1, calcitonin, and carcinoembryonic antigen raises the possibility of a non-MTC neuroendocrine tumor such as paraganglioma or a true primary small cell neuroendocrine carcinoma. Paraganglioma has the same features of paragangliomas described elsewhere and expresses neuroendocrine markers, as well as S100 protein, in the absence of cytokeratin expression. Small cell neuroendocrine carcinomas are exceptionally rare and can be very difficult to distinguish from metastases of small cell neuroendocrine carcinomas primarily of the lung [170].

Extremely rare cases of aggressive cribriform-morular variant of PTC and CASTLE with neuroendocrine differentiation have been reported [133, 171].

Mixed medullary-follicular/papillary carcinomas are tumors in which the MTC coexists with a follicular or a papillary carcinoma.

14.5.9 Squamous Cell Carcinoma

Definition Primary squamous cell carcinoma (SCC) of the thyroid discloses squamous cell differentiation and arises in the thyroid without evidence of other primary origin.

Epidemiology This tumor is rare and occurs in elderly patients with long-standing nodular goiter.

Etiology and pathogenesis Primary SCC of the thyroid is thought to arise from squamous metaplasia of the follicular epithelium or from solid cell nests [172, 173].

Clinical aspects SCC manifests as a rapidly growing mass with satellite nodules and associated compressive symptoms. Patients with primary SCC can present leukocytosis and hypercalcemia [174].

Macroscopy Usually presents as a large and whitish thyroid mass displaying signs of extrathyroidal invasive growth.

Microscopy SCC of the thyroid resembles the SCCs with or without keratinization observed in other locations (Figs. 14.66 and 14.67). This tumor often shows extrathyroid extension, vascular invasion, perineural invasion, and a high mitotic index. Primary SCC shares some clinical and morphological features of ATC, an observation that led some authors to suggest that primary SCC may be considered a variant of ATC [175]. Primary SCC of the thyroid expresses cytokeratin 19, cytokeratin 5/6, and p63 [173, 176, 177]. The expression of p53 can be focal or diffuse.

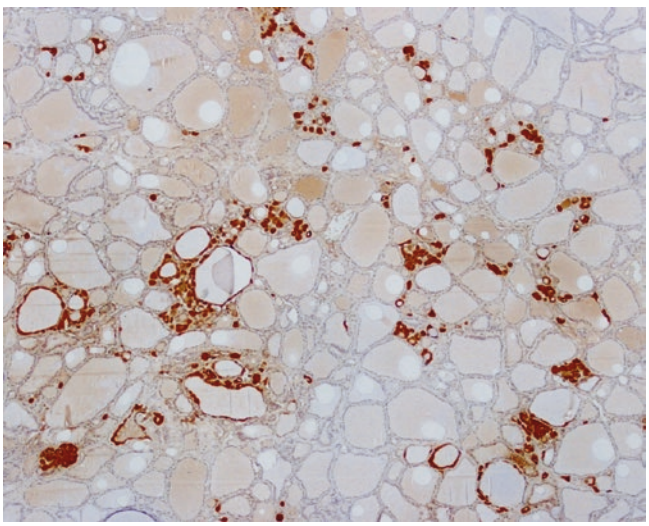

Fig. 14.66 C cell hyperplasia in the setting of multiple neuroendocrine neoplasia type 2, highlighted by immunohistochemical staining for calcitonin (calcitonin)

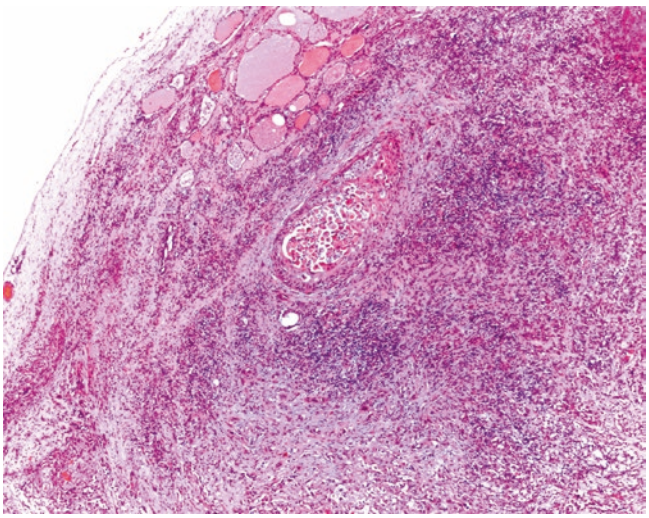

Fig. 14.67 Keratinizing squamous cell carcinoma primary of the thyroid

Thyroglobulin, calcitonin, TTF-1, and CD5 are typically not expressed [173, 176, 177].

Differential diagnosis SCC of the thyroid is a very rare tumor that must be distinguished from the more frequent secondary involvement of the thyroid by SCC with other origin, namely, those of the upper airway. Primary SCC should not also be confused with squamous metaplasia nor with primary thyroid tumors with focal squamous differentiation, such as mucoepidermoid carcinoma, carcinoma showing thymus-like differentiation (CASTLE), spindle cell tumor with thymus like elements (SETTLE), ATC, and PTC.

Genetics Due to the rarity of these tumors, the available information on molecular alterations is scarce. Nevertheless, *BRAF* mutations have been described in singular primary SCC cases [173].

Treatment and prognosis Primary SCC is a locally invasive tumor that may display regional lymph node metastases and distant metastases [178–180]. Like ATC, primary SCC can also be associated with PTC, particularly with tall cell variant [175, 180], and discloses a very poor prognosis [178–180]. Treatment is inefficient and consists in a combination of surgery followed by radio- and/or chemotherapy.

14.5.10 Mucoepidermoid Carcinoma

Definition Mucoepidermoid carcinoma (MEC) is a low-grade malignant tumor showing a combination of squamous and mucinous differentiation.

Epidemiology MEC is a rare tumor that may occur at any age, including children [181].

Etiology and pathogenesis MEC is thought to arise from either metaplastic follicular epithelium or from solid cell nests [182–184].

Clinical aspects Usually presents as a painless solitary "cold" nodule in the thyroid.

Macroscopy MECs are usually well-circumscribed, unencapsulated, more or less fibrotic tumors that may disclose extrathyroidal extension [181, 184].

Microscopy MECs arising in the thyroid are different from those arising in the salivary glands. In thyroid, MEC is characterized by confluent nests of squamous cells with pearl formation, scattered mucin-producing cells, and glandular spaces. The nests of squamous cells may show cystic spaces with necrotic debris, keratin, and mucin and are surrounded by a fibrotic stroma and, less often, by lakes of extracellular mucin. Rarely, psammoma bodies, hyaline bodies, cribriform-type pattern, and ciliated epithelium are also observed.

Nearly half of MECs coexist with PTC foci (Fig. 14.68), but the coexistence with oncocytic variant of PTC [183], classic FTC, and ATC [184] has also been reported.

The presence of squamous and mucin-producing cells in FNAB of a thyroid nodule is suggestive of MEC [185].

Low and high molecular weight cytokeratins and carcinoembryonic antigen are diffusely expressed in MEC, while thyroglobulin, TTF-1, and p63 are usually only focally expressed [181, 184]. Thyroglobulin and TTF-1 may even be entirely negative. MECs are negative for calcitonin and express P-cadherin in most of the cases [186].

Differential diagnosis The immunoprofile may help to distinguish MEC from fibrosing Hashimoto thyroiditis, primary or metastatic SSC, and follicular cell-derived tumors with focal squamous differentiation [187].

Genetics MECs are poorly characterized in molecular terms, being reported the absence of E-cadherin and *BRAF* mutations [158, 186]. *CRTC1/MAML2* fusion transcripts have been reported in a case of MEC of the thyroid [188].

Treatment and prognosis MECs are indolent tumors with a good long-term prognosis that are treated as PTC. MECs may give rise to local recurrence and to regional lymph node metastases, but distant metastases and death due to the tumor are rare [181, 184, 189]. Cases with metastases are

difficult to treat due to decreased (or absent) response to radioactive iodine.

14.5.11 Sclerosing Mucoepidermoid Carcinoma with Eosinophilia

Definition Sclerosing mucoepidermoid carcinoma with eosinophilia (SMEC) is a rare malignant tumor that arises in association with Hashimoto thyroiditis and discloses clinical and pathological features very similar to those of MEC.

Epidemiology SMEC occurs mainly in adult women but can affect patients of all ages including children.

Etiology and pathogenesis SMECs are thought to arise either from squamous metaplasia of follicular epithelium that occur in the setting of Hashimoto or lymphocytic thyroiditis [187] or from the malignant transformation of solid cell nests [190].

Clinical aspects Manifests as a solitary, slow-growing "cold" mass in the thyroid.

Macroscopy Solitary whitish nodule in a thyroid with prominent features of Hashimoto or lymphocytic thyroiditis.

Microscopy SMECs can be well circumscribed or infiltrative and are constituted by anastomosing cords of squamous cells admixed with occasional mucin-producing cells and mucin lakes. The tumor cells are surrounded by a sclerotic background infiltrated by a mixed inflammatory population of cells that is rich in lymphocytes, plasma cells, and eosinophils (despite its name, lymphocytes are much more frequent than polymorphs) [187, 191] (Figs. 14.69 and 14.70).

PTC and ATC have been described in association with SMEC [191].

In FNAB, the occurrence of both squamous and mucin-producing cells on an inflammatory background may suggest the diagnosis of SMEC.

Differential diagnosis The prominence of the squamous component and the abundance of the inflammatory cells can make the distinction from SCC and Hashimoto thyroiditis, respectively, very difficult [189, 192].

SMECs express cytokeratins and p63 and may express, or not, TTF-1 [191]. Thyroglobulin and calcitonin are not expressed in SMEC.

Treatment and prognosis Like mucoepidermoid carcinoma, SMECs are tumors with indolent clinical course that may give rise to lymph node metastases and, less often, to distant metastases to lung and bones [191, 193]. Treatment of the patients is similar to those with PTC and MEC. Recurrent or metastatic MEC or SMEC is difficult to treat due to the extremely poor response to radioactive iodine.

14.5.12 Mucinous Carcinoma

Definition Primary mucinous carcinoma of the thyroid is an exceedingly rare tumor primary of the thyroid that discloses mucinous differentiation.

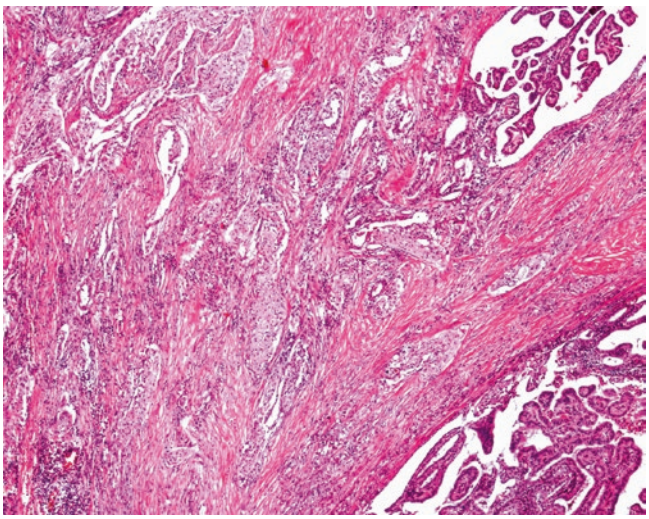

Fig. 14.68 Mucoepidermoid carcinoma (*left*) coexisting with papillary thyroid carcinoma (*right*)

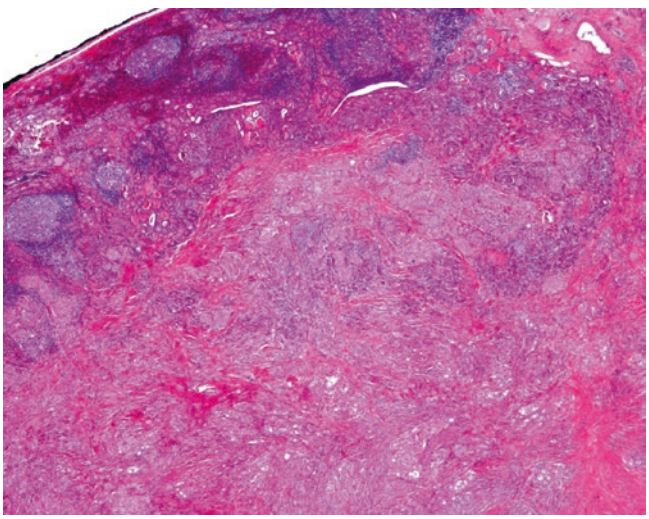

Fig. 14.69 Sclerosing mucoepidermoid carcinoma in the setting of Hashimoto thyroiditis

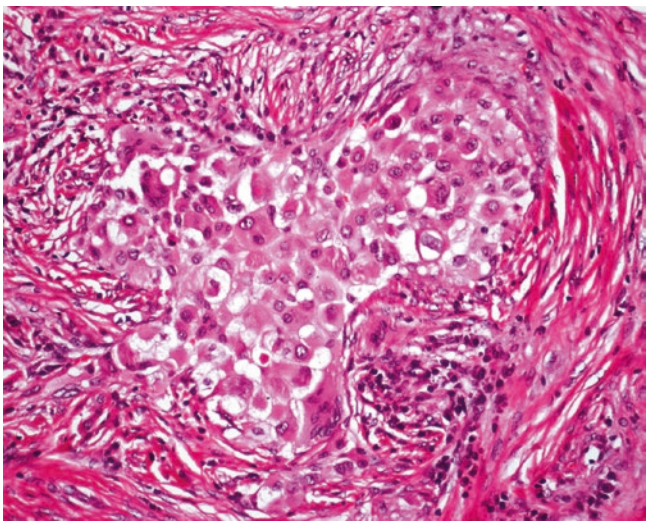

Fig. 14.70 Sclerosing mucoepidermoid carcinoma with eosinophilia composed by nests of squamoid cells with mucin vacuoles

Clinical aspects The patients with mucinous carcinoma of the thyroid present with a "cold" nodule.

Microscopy The tumor consists in a nodule that may be well or poorly circumscribed, composed by clusters of neoplastic, epithelioid cells surrounded by extensive extracellular mucin deposition [194–196]. The cells show large regular nuclei with prominent nucleoli; they may disclose focal squamous differentiation and usually express thyroglobulin, TTF-1, low molecular weight cytokeratins, and MUC2 [195, 196]. The mucin deposits stain with mucicarmine, Alcian blue, and PAS [195]. Calcitonin is not expressed.

Differential diagnosis It must not be confused with secondary involvement of the thyroid by a mucinous carcinoma elsewhere nor with other primary thyroid tumors displaying mucin production, such as FA, FTC, PTC, and MEC.

Treatment Similar to PTC and FTC.

14.5.13 Ectopic Hamatomatous Thymoma

Definition and etiology The family of thymic and branchial pouch-derived tumors encompasses tumors of the neck arising either from ectopic thymus or remnants of the third or fourth branchial pouches that retain the potential to differentiate along the thymic line, as postulated by Chan and Rosai [197]. As the majority of these tumors do not disclose thymic remnants at their periphery, other authors have suggested that this family of tumors includes also lesions related with the ultimobranchial body [198] or with the cervical sinus of His that are also elements of the branchial apparatus [199].

Ectopic hamartomatous thymoma (EHT), also known as branchial anlage mixed tumor, is a benign lesion located in the neck that displays hamartomatous and neoplastic features.

Epidemiology This rare thyroid tumor occurs predominantly in adult men in the fourth to fifth decades of life [197].

Clinical aspects and macroscopy EHT occurs as a well-circumscribed nodule in the supraclavicular, suprasternal, or presternal region, unattached to the skin, bone, or thyroid [197].

Microscopy EHT is composed by variable proportions of spindle cell areas, epithelial islands, and mature adipose tissue. The spindle cells are usually bland, arranged in fascicles [197]. The epithelial islands are composed by anastomosing cords, tubules, branching glands, and cysts. Mitotic figures are scant, and necrosis is usually absent.

The spindle cells usually express cytokeratins, p63, smooth muscle actin, and CD10, while vimentin expression is focal or absent [199]. Desmin and S100 protein are usually not expressed. The immunohistochemical profile and the presence of desmosomes and tonofilaments in the ultrastructural examination of the spindle cells favor the epithelial/myoepithelial nature of this component of EHT. The fibroblast-type cells in the stromal islands express CD34 [200].

Differential diagnosis The differential diagnosis of EHT includes schwannoma, synovial sarcoma, mixed tumor of salivary gland or of sweat gland origin, spindle cell carcinoma, and teratoma.

Prognosis There are reports of EHT that recurred after incomplete resection [199] and exceptional cases with superimposed carcinoma [201]. There are no EHT on record with documented metastases.

14.5.14 Ectopic Cervical Thymoma

Definition Ectopic cervical thymoma (ECT) is a rare tumor located in the neck that is histologically identical to mediastinal thymomas.

Epidemiology ECT occurs predominantly in middle-aged women.

Etiology and pathogenesis ECTs are thought to arise from ectopic cervical thymus as they are often associated to normal-appearing thymic remnants in their periphery [197].

Clinical aspects Presents as a deep, long-standing mass in the anterolateral region of the neck that can be related or not

with the thyroid. The occurrence of paraneoplastic manifestations that frequently accompany mediastinic thymomas has not been reported in thyroid ECT.

Macroscopy Most ECTs disclose features similar to those observed in thymomas with benign behavior. They are frequently encapsulated or circumscribed with a lobulated periphery, although some of them may exhibit infiltrative areas.

Microscopy The capsule is usually continuous with fibrous septa that divide the tumor into lobules which are composed by varying proportions of epithelial cells and small lymphocytes. These epithelial cells can form islands, fascicles, glandular structures, cleft-like spaces, and cysts [197]. Mitoses are infrequent. The epithelial cells can be highlighted by cytokeratins immunostaining, while the lymphocytes express CD3 in accordance to their T cell phenotype. The epithelial cells may express beta5t subunit of the proteasome in the absence of CD5 expression, as usually occurs in mediastinic thymomas [202].

Differential diagnosis Epithelium-rich ECT must be distinguished from ATC and SCC, particularly in ECT of the thyroid, while lymphocyte-rich ECT may be mistaken for lymphoma, particularly in a FNAB setting.

Prognosis The majority of ECTs show a benign evolution and are curable by surgical excision [197]. Rare cases developed regional lymph node metastases [203] and/or distant metastases to the lung [204].

14.5.15 Spindle Cell Tumor with Thymus-Like Differentiation

Definition Spindle cell tumor with thymus-like elements, also known by the acronym SETTLE, includes tumors previously reported as thyroid spindle cell tumor with mucous cysts and some malignant teratomas [197].

Epidemiology SETTLE is a rare malignant tumor that occurs predominantly in children and young adults.

Etiology and pathogenesis Is thought to be derived from thymus-like remnants.

Clinical aspects The patients are usually euthyroid and present with a solitary thyroid nodule associated or not with compressive symptoms.

Macroscopy SETTLE is usually lobulated tumors limited by a capsule continuous with fibrous and calcified septa, but they may also disclose an infiltrative growth pattern [197].

Microscopy SETTLE is a biphasic solid tumor with occasional cysts that encompasses a spindle cell component and a less evident epithelioid component (Fig. 14.71). The spindle cells are usually bland, and the epithelial cells form tubules, small papillae, trabeculae, or squamoid nests reminiscent of Hassall's corpuscles. Vascular invasion and necrosis are rarely found [197]. The mitotic index is low.

The spindle cell component expresses low molecular weight cytokeratins, cytokeratin 5, p63, vimentin, and, often, CD99, while the epithelioid component usually lacks vimentin and CD99 expression (Fig. 14.72). Thyroglobulin, calcitonin, TTF-1, carcinoembryonic antigen, S100 protein, CD5, and CD34 are usually negative in both components [205].

Differential diagnosis The immunohistochemical study can help to separate SETTLE from other spindle cell tumors such as ATC, PTC composed predominantly by spindle cells, and MTC. The differential diagnosis with teratoma and thymoma is based upon morphological features, but the separation from synovial sarcoma may require complementary cytogenetic studies.

Genetics The presence of *KRAS* gene mutations and absence of *TP53* mutations in a case of SETTLE. Synovial sarcoma-associated fusion genes (*SS18/SSX1* and *SS18/SSX2* or *SYT* rearrangement) were not detected in a series of 11 cases diagnosed as SETTLE.

Prognosis Metastatic disease usually occurs many years after the diagnosis being reported in over 60 % of the patients with long-term follow-up [206] into the lungs, kidney, and, rarely, lymph nodes [197, 207].

14.5.16 Carcinoma Showing Thymus-Like Differentiation

Definition Carcinoma showing thymus-like elements, also known by the acronym CASTLE, intrathyroidal epithelial thymoma or lymphoepithelioma-like carcinoma of the thyroid is a rare malignant tumor of the thyroid or perithyroid soft tissues of the neck that resembles a thymic carcinoma [197].

Epidemiology Rare tumor that occurs mainly in middle-aged patients.

Etiology and pathogenesis CASTLE is thought to originate either from ectopic thymic tissue or solid cell nests [208, 209].

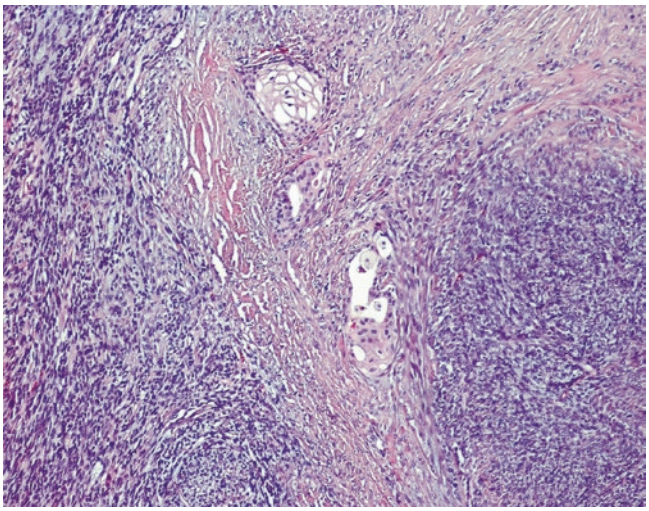

Fig. 14.71 Spindle cell dominant area and a focus of squamoid epithelial differentiation in a spindle cell tumor with thymus-like differentiation (SETTLE)

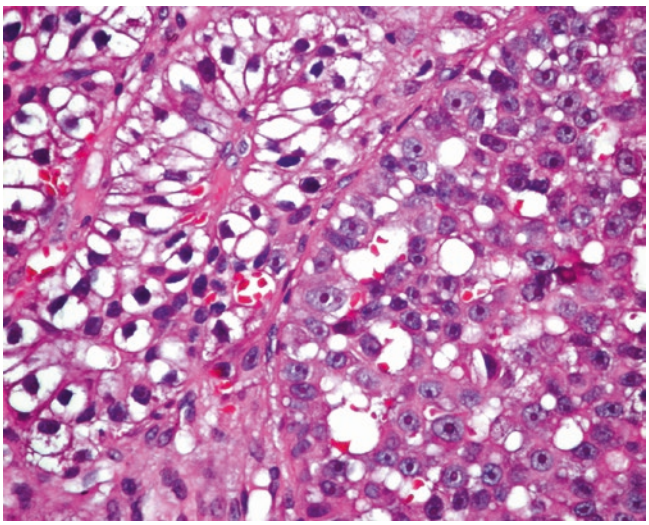

Fig. 14.73 Microscopic aspect of a carcinoma of the thyroid showing thymus-like differentiation (CASTLE) that exhibits a clear cell area

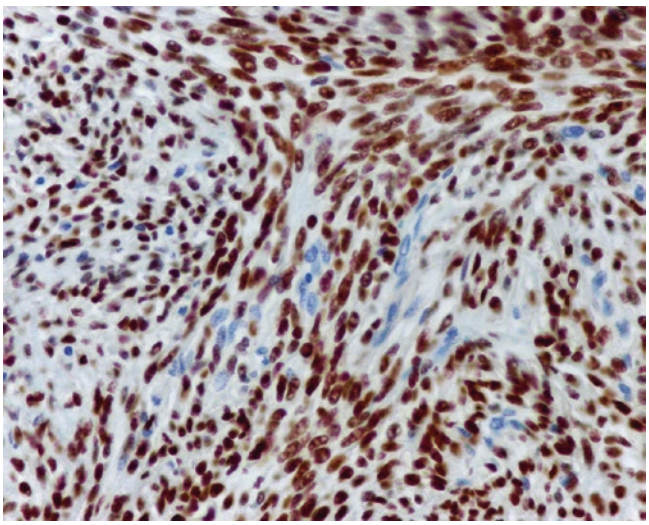

Fig. 14.72 p63 expression in the spindle cells of a spindle cell tumor with thymus-like differentiation (SETTLE)

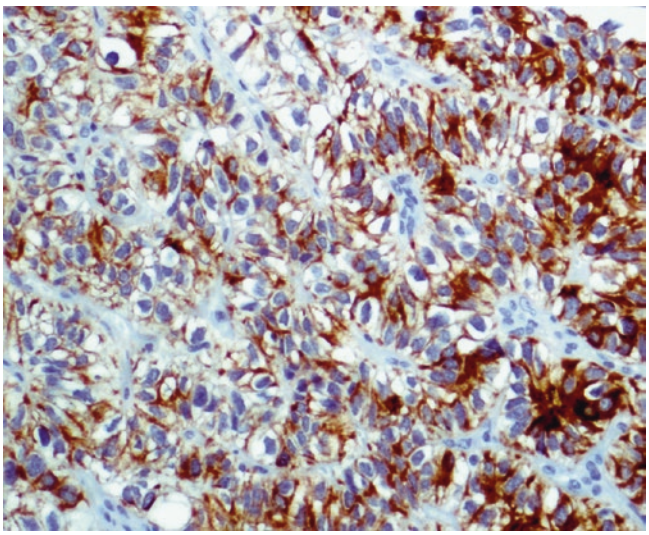

Fig. 14.74 CD5 expression in carcinoma of the thyroid showing thymus-like differentiation (CASTLE) that exhibits a clear cell area

Clinical aspects CASTLE presents with a painless neck mass accompanied or not by compressive symptoms, lymphadenopathies, and mediastinal involvement [210].

Macroscopy The tumor mass is well circumscribed and lobulated.

Microscopy CASTLE is composed by nests of epithelial cells separated by thin and thick fibrous septa infiltrated by small lymphocytes and plasma cells. The epithelial cells disclose pale to light eosinophilic vesicular nuclei with prominent nucleoli (Fig. 14.73). Mitotic index is frequently low [197].

The immunophenotype of the tumor cells is similar to that of thymic carcinoma and includes expression of cytokeratin 5/6, CD5, and CD117, in the absence of expression of thyroglobulin, calcitonin, and TTF-1 (Fig. 14.74) [211]. CASTLE also expresses p63, carcinoembryonic antigen, vimentin, and bcl-2 [208, 212].

Differential diagnosis Other rare tumors with morphological and immunohistochemical resemblance with solid cell nests such as the basaloid tumor with solid cell nests features[198] and the carcinoma of the thyroid with Ewing family tumor elements (CEFTE) [213, 214]. CASTLE must also be separated from ATC and SCC as well as from sec-

ondary carcinomas which are usually tumors with a high mitotic index.

Genetics Chromosomal imbalances similar to those found in thymomas and thymic carcinomas were reported in thyroid CASTLE with gains on chromosomal arm 1q and losses detected on 6p, 6q, and 16q [211].

Treatment and prognosis CASTLE frequently recurs and gives rise to lymph node metastases in about half of the cases [215] and/or to distant metastases to the liver, bones, mediastinum, and pleura [215, 216]. Surgical resection and adjuvant radiotherapy allow a 5-year survival rate of about 90 % [215, 216].

14.5.17 Primary Small Cell Tumors

Definition The small cell group of thyroid tumors includes rare tumors with uncertain histogenesis that are difficult to distinguish from each other.

Differential diagnosis Poorly differentiated carcinoma and lymphoma are probably the most frequent thyroid tumor histotypes displaying often a small cell phenotype. The other tumors that may present a small cell phenotype encompass MTC, squamous cell carcinoma, CASTLE, primary small cell neuroendocrine carcinoma, primary extraskeletal Ewing family tumors [217], carcinoma of the thyroid with Ewing family tumor elements (CEFTE) [214, 218], small cell secondary neoplasms, as well as some extremely rare flowers such as neuroblastoma [219] and basaloid neoplasm with solid cell nest features [198].

As in other locations, the diagnosis of thyroid primary extraskeletal Ewing family tumor has been supported by the detection of the typical *EWSR1-FLI1* rearrangement.

CEFTE is an invasive small cell tumor with favorable prognosis, associated to a PTC component, which discloses the *EWSR1-FLI1* rearrangement and an immunoprofile similar to that observed in tumors of the Ewing family [213, 214, 218]. CEFTE cells diffusely express cytokeratins, p63, e-cadherin, and CD99 in the absence of vimentin expression [213, 214, 218]. The features observed in CEFTE raise some important questions regarding histogenesis: do they derive from "dedifferentiated" PTC cells that have acquired the *EWSR1-FLI1* rearrangement and entirely lost thyroid differentiation (negativity for TTF-1 and thyroglobulin), or do they originate from thymic/branchial pouch remnants, or do they derive from main cells of solid cell nests?

Basaloid tumor with solid cell nest features is a PTC-related tumor composed by small cells that express p63, cytokeratin 5, and galectin 3 in the absence of CD5 expression [198].

14.5.18 Primary Lymphoma and Plasmacytoma

These and the following hematolymphoid diseases located in non-thyroidal sites are further discussed in Chap. 14.

Definition Lymphoma may involve the thyroid as part of a systemic disease (secondary lymphoma) or, rarely, as a primary disease originating in the thyroid (primary lymphoma).

Epidemiology Primary thyroid lymphomas account for less than 5 % of all clinically evident thyroid cancers, most of them belonging to the group of B-cell non-Hodgkin lymphomas. Diffuse large B-cell lymphoma is the most frequent primary lymphoma of the thyroid, encompassing nearly 70 % of cases, followed by marginal zone B-cell lymphoma of MALT type (mucosa-associated lymphoid tissue) which encompasses 6–27 % of cases [220]. The majority of the patients are elderly women.

Etiology and pathogenesis Nearly 40 % of diffuse large B-cell lymphomas appear to have undergone transformation from a preexistent marginal zone B-cell lymphoma of MALT type. Plasmacytoma has been reported to occur rarely in the thyroid, some in the setting of Hashimoto thyroiditis [14].

Clinical aspects Manifests as a rapidly growing mass and compressive symptoms, simulating an ATC.

Macroscopy Primary thyroid lymphomas usually occur as solid, multinodular to diffuse masses.

Microscopy The histological features of each lymphoma type in the thyroid are similar to those of corresponding lymphomas in other locations. FNAB is a useful diagnostic tool being complemented, whenever appropriate, by core needle biopsy and flow cytometry.

Histologically, plasmacytoma of the thyroid consists of sheets of mature plasma cells that replace the follicular epithelium and extend to the perithyroid tissues.

Differential diagnosis The differential diagnosis includes small cell variant of MTC, PDTC, and florid Hashimoto thyroiditis. The overall destruction of the gland architecture and the prominence of lymphoepithelial lesions that consist of thyroid follicles stuffed with neoplastic cells are, together with the coexistence of plasma cell differentiation of the neoplastic cells, the morphological clues that favor the diagnosis of marginal zone B-cell lymphoma of MALT type in opposition to Hashimoto thyroiditis. Patients with Hashimoto thyroiditis are at an increased risk of developing thyroid lymphoma. The immunohistochemical study helps to separate lymphoma from carcinoma and for the charac-

terization of the lymphoma type. Other primary lymphomas of the thyroid published as case reports include follicular lymphoma [221], mantle cell lymphoma [222], Burkitt's lymphoma [223], Hodgkin's lymphoma [224], and T cell lymphoma [225].

Treatment and prognosis The treatment for patients with primary lymphoma of the thyroid depends on the specific type of lymphoma.

14.5.19 Langerhans Cell Histiocytosis

Definition and etiology Langerhans cell histiocytosis (LCH), histiocytosis X, or eosinophilic granuloma is a monoclonal disease of histiocytes that can involve the thyroid either as an isolated event or as a part of systemic involvement.

Epidemiology LCH is more frequent in young patients, mainly children but can also occur in adults who are more frequently men [226].

Clinical aspects LCH in the thyroid can present as a diffuse goiter or as a single nodule associated to hypothyroidism in 40 % of the cases occurring in children [226]. LCH can also be found in association with thyroid tumors, namely, PTC [227].

Microscopy LCH is characterized by the presence of abundant large histiocytes with lobulated, folded nuclei, in a background of small lymphocytes and, often, abundant eosinophils (Fig. 14.75) [226].

Thyroid FNAB can be useful for the diagnosis of LCH [228].

Differential diagnosis The differential diagnosis includes other histiocytic disorders as well as PTC [229]. Immunohistochemical expression for S100 protein and CD1a, together with the observation of Birbeck granules in the ultrastructural examination of the histiocytes, supports the diagnosis of LCH [18].

Treatment and prognosis Patients with localized thyroid disease usually do not develop subsequent systemic disease which, whenever occurs, is associated to poor prognosis [229].

14.5.20 Follicular Dendritic Cell Tumor

Definition and epidemiology Follicular dendritic cell tumor is a rare neoplasm that has been exceptionally reported in the thyroid [17, 22].

Microscopy This neoplasm is usually well circumscribed and composed by cells showing morphological features of follicular dendritic cells together with variable number of scattered small lymphocytes [17, 22]. The neoplastic cells are usually positive for CD21, CD23, CD35, vimentin, and EMA, variably positive for CD69 and S100 protein and negative for cytokeratins [17, 22].

Treatment and prognosis The treatment includes surgical excision with or without adjuvant chemotherapy and radiotherapy.

14.5.21 Vascular Tumors

Definition Primary vascular tumors of the thyroid include angiosarcoma [230], epithelioid hemangioendothelioma [231, 232], and hemangioma [233].

Epidemiology Angiosarcoma of the thyroid usually manifests as cold nodule in elderly patients, most often women, with long-standing nodular goiter [94, 234]. Epithelioid hemangioendotheliomas are exceedingly rare tumors that occur in a setting similar to that reported for angiosarcomas [235]. Less than ten cases of primary hemangioma of the thyroid have been reported to date.

Etiology and pathogenesis The majority of angiosarcomas of the thyroid have been reported from European Alpine regions, linked to dietary iodine deficiency, where they encompass nearly 4 % of all clinically evident thyroid malignancies [236], but they can also occur outside those regions [237].

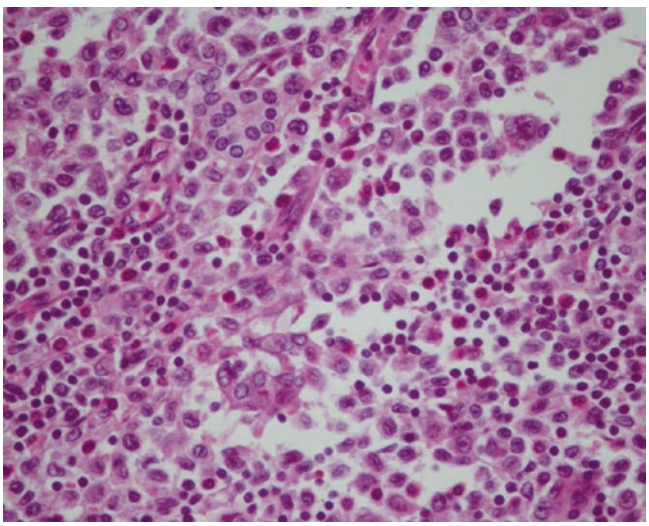

Fig. 14.75 Langerhans cell histiocytosis of the thyroid

Clinical aspects Angiosarcoma usually presents as a painful rapidly growing mass that causes pressure symptoms; some cases are diagnosed at metastatic stage.

Macroscopy Most angiosarcomas of the thyroid are large invasive tumors with cystic and solid areas with hemorrhagic and focally necrotic cut surface.

Microscopy The histological features are similar to those of the corresponding tumor in the soft tissues, being characterized by anastomosing abnormal vascular channels, some with papillary fronds, which are lined by atypical endothelial cells with frequent mitoses. Giant cells are very rare. Angiosarcomas of the thyroid are frequently epithelioid [237] with solid areas, simulating ATC or secondary neoplasms (Fig. 14.76). Epithelioid hemangioendotheliomas are characterized by an epithelioid and spindle cell proliferation in a background of chondromyxoid stroma. The tumor cells are atypical and disclose intracytoplasmatic lumina filled with red blood cells.

The immunohistochemical profile of angiosarcomas and epithelioid hemangioendotheliomas includes the expression of vascular markers such as CD31, CD34, and factor VIII and, often, the expression of low molecular weight cytokeratins (Fig. 14.77) [230, 235, 238].

The morphological features of thyroid hemangiomas are similar to those observed in hemangiomas elsewhere; most of them disclose a cavernous growth pattern, and some are considered to be secondary to hematoma formation after FNAB [239].

Differential diagnosis In the past, some authors argued that most angiosarcomas were ATCs with angiomatoid features, but the demonstration of thyroglobulin RNA by in situ hybridization in ATC [234] and the detection of cytoplasmatic structures reminiscent of Weibel-Palade bodies in angiosarcomas [238] proved the existence of thyroid angiosarcoma as a separate entity.

Treatment and prognosis Angiosarcoma and epithelioid hemangioendothelioma of the thyroid behave similarly to ATC, with invasive local disease, metastases, and a rapidly fatal outcome [235, 240].

14.5.22 Solitary Fibrous Tumor

Definition and epidemiology Less than 30 cases of primary solitary fibrous tumor (SFT) of the thyroid have been reported in the literature. This mesenchymal thyroid tumor occurs in adults with a slight predominance of men [241].

Clinical aspects Thyroid SFT presents as a painless, slowly growing mass usually in the context of a preexistent nodular goiter [242].

Macroscopy Most thyroid SFTs are solid, well-circumscribed nodules with a firm, gray-white to brown cut surface with occasional cysts that may infiltrate the thyroid parenchyma [241, 243].

Microscopy These tumors are composed by spindle-shaped cells with fibroblastic/myofibroblastic differentiation arranged in a wavy, storiform to hemangiopericytic-like pattern in a collagenous to myxoid stroma (Fig. 14.78) [243, 244]. The tumor cells disclose bland features, and the mitotic index is low [241, 243]. Interstitial inflammatory cells, mainly masts cells, can be observed. There is an adipocytic

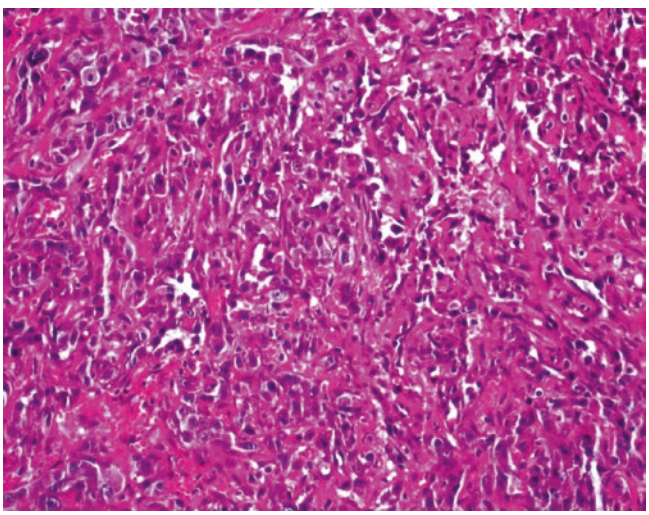

Fig. 14.76 Thyroid primary angiosarcoma with anastomosing vascular channels lined by atypical cells

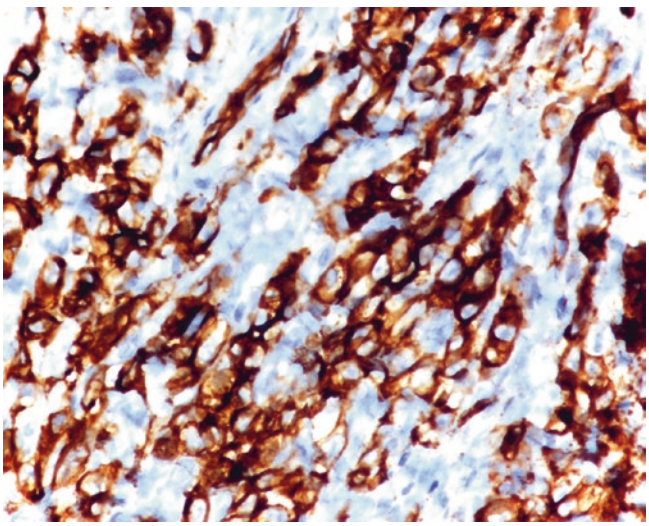

Fig. 14.77 CD31 expression in the cells of thyroid angiosarcoma

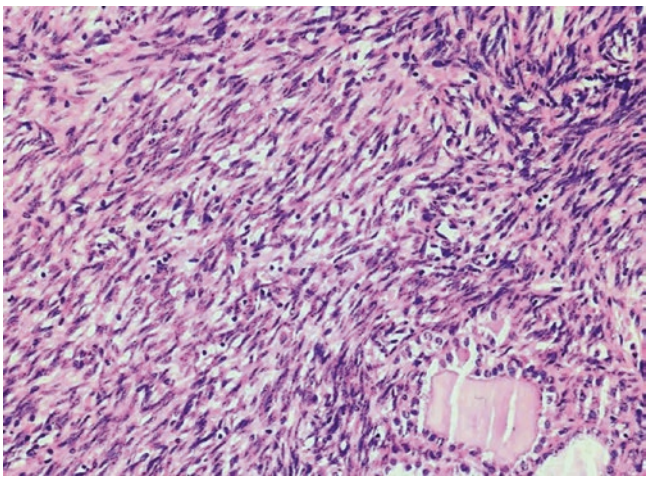

Fig. 14.78 Spindle cell growth pattern in a solitary fibrous tumor of the thyroid

variant of SFT that contains areas of mature lipomatous cells that express S100 protein [245].

Immunohistochemically, the tumor cells express vimentin, CD34, CD99, and BCL-2, whereas expression for cytokeratins, thyroglobulin, TTF1, calcitonin, and S100 protein is absent [241, 243].

Differential diagnosis The differential diagnosis of thyroid SFT includes post-FNAB lesions, spindle cell FA, spindle cell variant of PTC, spindle cell variant of MTC, SETTLE, and ATC, as well as primary and secondary mesenchymal tumors.

Treatment and prognosis The large majority of SFT of the thyroid behave in a benign fashion although there is one case of thyroid malignant SFT with pleomorphic spindle cells and abundant mitoses that recurred and gave rise to lung metastases [246].

14.5.23 Smooth Muscle Tumors

Definition and epidemiology Smooth muscle tumors of the thyroid, including leiomyoma and leiomyosarcoma, are exceedingly rare tumors derived or showing evidence of differentiation toward smooth muscle. Leiomyomas occur predominantly in middle-aged women. Leiomyosarcomas are more common in elderly patients.

Etiology and pathogenesis The smooth muscle vessels at the periphery of the gland may account for the preferential peripheral localization of these tumors [247].

Macroscopy Leiomyomas present as small encapsulated and "cold" nodules [248, 249]. Leiomyosarcomas present as large infiltrative masses that can measure up to 12 cm in largest dimension [250].

Microscopy The morphological and immunohistochemical features of thyroid leiomyomas are similar to those observed in leiomyomas located elsewhere [248]. The histological features of thyroid leiomyosarcomas include moderate to severe cellular pleomorphism, high mitotic index, coagulative necrosis, and hemorrhage [247, 250]. Tulbah et al. [251] reported a case of thyroid leiomyosarcoma that expressed Epstein-Barr virus mRNA in a child with congenital immunodeficiency.

Primary smooth muscle tumors of the thyroid express vimentin, smooth muscle actin, and muscle specific actin and desmin, in the absence of expression of cytokeratins, thyroglobulin, and calcitonin [247, 252].

Differential diagnosis The differential diagnosis of primary leiomyosarcoma of the thyroid includes ATC, SETTLE, and other primary and metastatic mesenchymal tumors.

Treatment and prognosis Patients with thyroid leiomyoma are usually cured by lobectomy, while the prognosis of thyroid leiomyosarcoma is poor regardless of the type of treatment [247, 248].

14.5.24 Other Mesenchymal Tumors

Other benign and malignant mesenchymal lesions of the thyroid published as case reports include schwannoma [253], malignant peripheral nerve sheet tumor [254], granular cell tumor [255], liposarcoma [256], osteosarcoma [257], and chondrosarcoma [258].

14.5.25 Germ Cell Tumors

Definition Primary germ cell tumors of the thyroid are very rare; most of them are classified as teratomas. A single case of yolk sac tumor primary of the thyroid was reported in a 10-year-old girl [259]. Thyroid involvement by a seminoma has been reported in a metastatic testicular neoplasm [260].

Epidemiology The peak incidence of thyroid teratomas is in the newborn [261].

Etiology and pathogenesis Primary teratomas display mature and/or immature components from ectodermic, endodermic, and mesodermic origin [262].

Clinical aspects Patients with primary teratoma of the thyroid present with a mass in the neck, accompanied or not by compressive symptoms.

Macroscopy The mass is usually expansive but may disclose an infiltrative growth pattern.

Microscopy Is similar to teratomas in gonadal location.

Differential diagnosis Primary thyroid teratoma must be distinguished from cystic lymphangioma, thyroglossal duct cyst, and branchial cleft cyst. The immature components may be confused with small cell neoplasms [262, 263].

14.5.26 Secondary Tumors

Definition and etiology Secondary tumors of the thyroid are those resulting from the lymphatic/hematogeneous spread of a primary tumor at a distant location (metastasis) or those resulting from the direct extension of tumors arising in structures contiguous to the thyroid (local invasion), namely, tumors arising in the pharynx, larynx, trachea, esophagus, cervical lymph nodes, soft tissues, and mediastinum.

Epidemiology Metastases to the thyroid are detected in up to 24 % of autopsies of patients with disseminated malignancy, while the incidence of metastases to the thyroid in the clinical setting is recorded as being much lower, accounting for less than 1 % of all thyroid malignancies [264–266].

Clinical aspects Metastases to the thyroid occur predominantly in euthyroid elderly patients [265, 266] and manifest as a clinically palpable mass, with or without concurrent neck pain, dysphonia, dysphagia, dyspnea, or hoarseness. Rarely, it may present with hyperthyroidism [267].

On ultrasound examination, the metastases appear as single or, less frequently, as multiple hypoechoic nodules that can be sampled by FNAB. FNAB constitutes the first diagnostic approach to secondary tumors achieving a very high accuracy particularly when integrated with detailed clinical information and complemented with immunohistochemical studies.

The most common primary sites giving rise to metastases in the thyroid are the kidney, lung, breast, and esophagus [264]. Primary origin is unknown in nearly 5 % of cases. In some series, lymphomas and leukemias were estimated to account for 15 % of secondary tumors in the thyroid [268]. Rare sources of primary tumors include nasopharyngeal carcinoma [269], choriocarcinoma [270], malignant phyllodes tumor [271], osteosarcoma [268], and Merkel cell carcinoma [272].

In cases in which there is local extension from a primary tumor originating in an adjacent structure, the tumor tends to be initially unilateral. Most of the tumors that extend into the thyroid are SCCs originating in the upper respiratory tract, and their secondary origin is usually clinically obvious allowing the distinction between these and the rare primary SCCs of the thyroid.

Macroscopy Metastases to the thyroid may occur as single, multiple, or diffuse lesions [268].

Microscopy Two patterns of growth can be observed in the metastases to the thyroid: an interstitial pattern of infiltration that surrounds the follicles or a nodular pattern that can mimic a primary tumor. The nodular pattern can be particularly misleading if the original source of the tumor remains occult, as often observed in renal cell carcinoma that can give rise to thyroid metastases while remaining clinically silent, or metastasize to the thyroid many years after the resection of the primary tumor [273, 274].

Metastases can occur into primary thyroid tumors in up to 42 % of cases [274, 275] being FA the most common recipient followed by follicular variant of PTC, while renal cell carcinoma is the most frequent source, followed by the lung, breast, and colon carcinoma [276]. In tumor-to-tumor metastasis, there is a dimorphic pattern characterized by an abrupt transition from the recipient thyroid neoplasm to a morphologically distinct neoplasm [276].

Secondary tumors generally retain the histological features of the primary tumor, often with less differentiation.

Differential diagnosis The differential diagnosis between poorly differentiated metastatic tumors and PDTC and ATC can be difficult, both in FNAB and histology. The distinction between renal cell carcinoma, metastases, and clear cell variant of a primary follicular neoplasm can be very difficult in morphological grounds as it relies on features such as the prominent vascularity and the glandular lumina filled with red blood cells whenever dealing with a metastasis from a kidney carcinoma [274]. Since such features are not always present, it is better to rely on immunohistochemistry. The identification of metastatic renal cell carcinoma is aided by the absence of thyroglobulin and TTF-1 expression and by the expression of CD10 in the neoplastic cells (Fig. 14.79). The absence of thyroglobulin expression in some primary thyroid tumors and the expression of thyroglobulin in entrapped follicles, that may diffuse into the adjacent structures and be absorbed by metastatic cells, constitute a pitfall. The expression of TTF-1 can also be observed in a subset of lung tumors that can be difficult to separate from primary thyroid tumors.

Treatment and prognosis The detection of a metastasis in the thyroid appears to have the same negative impact on prognosis as non-thyroidal metastases. The surgical excision of the thyroid with metastatic disease may provide relief from symptoms and, in some series of cases with solitary metastases, may prolong survival [277], while in other series, thyroidectomy did not ameliorate survival [265, 266].

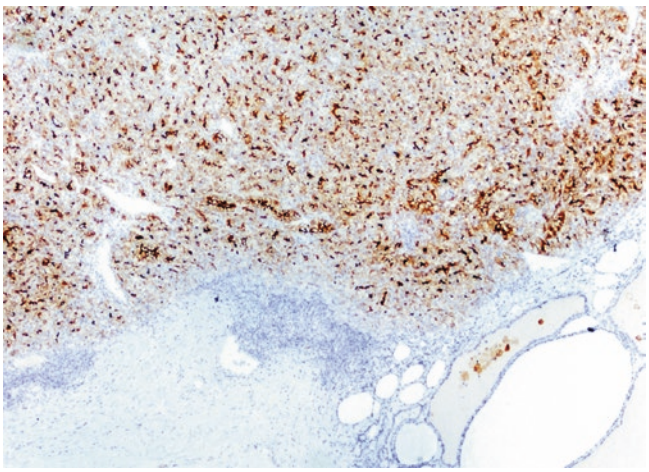

Fig. 14.79 Metastasis of renal carcinoma into a follicular adenoma (CD10)

14.6 Parathyroid Development and Normal Anatomy and Histology

At the 26th day of fetal life, the five pairs of pharyngeal pouches start being formed. The superior parathyroids develop from the fourth pharyngeal pouch, and the inferior parathyroids originate in the third and migrate in conjunction with thymic tissue.

Usually there are four parathyroid glands measuring 3–6 mm and weighing 30–40 mg each. The inferior glands are often intimately associated with thymic tissue due to their shared embryological origin. Supernumerary parathyroid glands are found at autopsy in 2–13 % of healthy individuals, most commonly with a fifth gland located in the cervical thymus.

The parathyroids have a thin fibrous capsule and are constituted by the glandular parenchyma and a variable amount of adipose tissue that increases from puberty to adulthood. Two main cell types compose the endocrine parenchyma: chief cells and oncocytic and mitochondrion-rich cells. "Water-clear cells" that correspond to chief cells with clear cytoplasm and "transitional oncocytic cells" which appear to be a sort of intermediate step between chief and oncocytic cells are also observed. Clusters of oncocytic cells that are frequently found in elderly individuals should not be misinterpreted as pathological nodules [278–280].

14.7 Hyperparathyroidism

Parathyroids are the endocrine organs mainly responsible for the control, through the production of the parathyroid hormone (PTH), of calcium level in the serum. This level is tightly regulated between 8.8 and 10.2 mg/dL and is inversely proportional to the PTH level [278–280].

14.7.1 Primary Hyperparathyroidism

Definition Primary hyperparathyroidism (PHPT) refers to an increase of serum calcium and PTH concentrations as the result of the autonomous production of PTH by a parathyroid lesion.

Epidemiology It is one of the most common endocrine diseases.

Clinical aspects Characterized by symptoms that used to be described years ago as "moans, groans, stones, and bones" [281]. These advanced findings are now rare, and most patients are asymptomatic, identified incidentally in a routine serology [278, 279]. Osteitis fibrosa cystica or von Recklinghausen disease of bone, secondary to the osteoclastic bone reabsorption stimulated by excessive PTH, is nowadays present in only about 2 % of individuals diagnosed with PHPT [280].

Etiology and pathogenesis About 90 % of cases of PHPT occur as sporadic disorders. PHPT occurring at young ages raises the suspicion of a familial syndrome. Sporadic parathyroid adenomas are the most common form of PHPT (85 %), followed by parathyroid hyperplasia (10 %), multiple parathyroid adenomas (4 %), and parathyroid carcinoma (<1 %) [280].

Hereditary disorders account for approximately 10 % of PHPT and encompass several autosomal dominant disorders, including multiple endocrine neoplasia 1 (MEN1), multiple endocrine neoplasia 2A (MEN2A), hereditary hyperparathyroidism-jaw tumor syndrome (HPT-JT), familial isolated hyperparathyroidism, neonatal severe hyperparathyroidism, familial hypocalciuric hypercalcemia, autosomal dominant mild hyperparathyroidism, and familial hypercalcemia and hypercalciuria [282].

Genetics The genetic and pathologic data of the four best known of the aforementioned conditions are summarized in Table 14.5 [281, 282].

Treatment and prognosis Surgery is the only curative procedure for PHPT.

14.7.2 Secondary Hyperparathyroidism

Definition and etiology Secondary hyperparathyroidism corresponds to a physiologic response to hypocalcemia caused by chronic renal failure, calcium deficiency, vitamin D deficiency, some disorders of vitamin D and phosphate metabolism, malabsorption, or low serum magnesium [279, 280]. All these conditions lead to an overproduction of PTH as the con-

Table 14.5 Genetic alterations in parathyroid lesions

Syndrome	Affected gene	Most frequent parathyroid disease(s)
MEN1	*MEN1*	Hyperplasia
		Adenoma
MEN2A	*RET*	Hyperplasia
HPT-JT	*HRPT2*	Adenoma
		Carcinoma
FHH	*CaSR*	Hyperplasia

MEN multiple endocrine neoplasia, *HPT-JT* hereditary hyperparathyroidism-jaw tumor, *FHH* familial hypocalciuric hypercalcemia (Table adapted from Zhang et al. [281])

sequence of an adaptive proliferation and hyperfunction of parathyroid cells.

At variance with PHPT, the parathyroid glands in almost every case of secondary hyperparathyroidism show reactive hyperplasia, which may be diffuse or nodular.

14.7.3 Tertiary Hyperparathyroidism

Definition and etiology Tertiary hyperparathyroidism corresponds to the development of apparently autonomously functioning parathyroid nodules in the hyperplastic glands of secondary hyperparathyroidism. Both parathyroid adenomas and carcinomas have been reported in tertiary hyperparathyroidism [280, 283].

14.8 Parathyroid Hyperplasia

Definition Parathyroid hyperplasia is a non-neoplastic enlargement of one or more parathyroid glands. It may lead to hyperparathyroidism.

Clinical aspects The diagnosis of hyperplasia is strongly favored if the patient has a clinical history of chronic renal failure/intestinal calcium malabsorption and/or if the surgeon does not detect an interoperatory abrupt fall in the blood levels of the patient's parathyroid hormone after the removal of an apparently single enlarged gland.

Macroscopy Parathyroids are enlarged with a nodular or multinodular cut surface. Parathyroid hyperplasia classically involves multiple parathyroid glands, typically all, but asymmetrical and even asynchronous presentation may occur [279]. In other words, if one faces four enlarged glands, the diagnosis of hyperplasia is almost unquestionable, but the same does hold true if there are only one or two enlarged glands (hyperplasia may involve just one gland, and adenoma, on the other hand, may be multiple).

Microscopy and differential diagnosis The histological features of hyperplasia are indistinguishable in primary and secondary hyperparathyroidism. The same holds true regarding the histological aspects of hyperplasia in familial and nonfamilial setting. Microscopically, the gland is hypercellular with a relative paucity of intraparenchymal fat. The predominant cell type is the chief cell which displays a faintly eosinophilic cytoplasm and a centrally placed, round, relatively monotonous nucleus without conspicuous nucleoli. Other cell types include, in variable proportions, oncocytic and transitional oncocytic cells as well as, occasionally, foci of water-clear cells. Parathyroid hyperplasia exclusively or almost exclusively composed of water-clear cells is rare. Lymphocytic infiltrate(s) may be present. The hyperplasia tends to be diffuse in the first phases but acquires often a focal nodular growth pattern that contributes to highlight the asymmetry among the variable sized glands (Fig. 14.80). Both in primary and secondary hyperparathyroidism, the progression of the hyperplastic lesions leads sometimes to dominant or "adenomatoid" nodules that may be indistinguishable from adenomas.

Treatment and prognosis Surgery is the only curative procedure, either limited parathyroidectomy or total parathyroidectomy with autotransplantation.

14.9 Parathyroid Tumors

14.9.1 Adenoma

Definition Parathyroid adenoma is a benign neoplasm of the parathyroid cells.

Etiology and pathogenesis Parathyroid adenomas occur frequently in some hereditary disorders (Table 14.5). Exposure to ionizing radiation increases the risk of developing parathyroid adenomas.

Clinical aspects It leads to hyperparathyroidism in almost every case. Nonfunctional parathyroid adenomas (like nonfunctional parathyroid carcinomas) are rare.

Macroscopy Parathyroid adenomas occur either as a well-circumscribed encapsulated thyroid nodule or as a poorly circumscribed parathyroid mass. Parathyroid adenomas are usually single, but "double" or "multiple" adenomas are also found in multiglandular disease, more commonly in older patients.

Microscopy Morphologically, the nodular growth that is typical of adenoma but also frequently occurs in parathyroid hyperplasia may be clonal or polyclonal and is constituted by

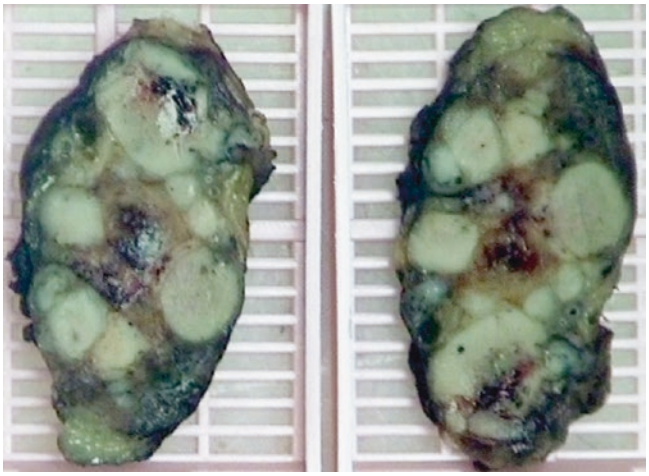

Fig. 14.80 Parathyroid hyperplasia

one or more than one type of parathyroid cells. Most adenomas are composed of chief cells. Oncocytic adenomas (>90 % of oncocytic cells) are uncommon, and "water-clear" adenomas are rare (Fig. 14.81). Lipoadenomas are also rare; initially thought to be hamartomas due to their abundant stromal fat, they are now recognized as functionally active neoplasms [280].

"Atypical adenoma" is an umbrella descriptive term that encompasses parathyroid tumors displaying some features suggesting malignancy but without reaching the diagnostic threshold of unequivocal malignancy (see parathyroid carcinoma).

Differential diagnosis The dilemma of diagnosing malignancy in difficult cases is common to other endocrine and neuroendocrine tumors in which a similar approach – creating a category of atypical, or borderline, or uncertain malignant potential – is being progressively adopted.

In the parathyroid setting, the presence of two or more of the following features is required for the diagnosis of atypical adenoma: incomplete invasion of the capsule, thick fibrous bands creating a multinodular growth pattern, prominent trabecular growth, mitotic activity greater than 1 *per* 10 high-power fields, and foci of tumor necrosis that are neither caused by previous FNAB nor by infarction [280] (Fig. 14.82).

Treatment and prognosis Van der Walt [280] refers that none of the 24 patients with parathyroid tumors classified as atypical adenoma developed tumor recurrence or metastatic disease after 8 years; this suggests that the clinical behavior of atypical adenomas does not differ from that of adenomas of the usual type. However, as stressed by DeLellis [284], close and prolonged follow-up of such

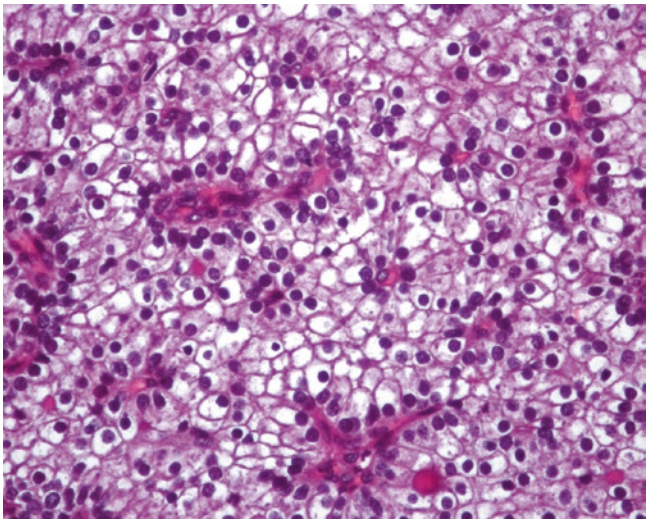

Fig. 14.81 Water-clear parathyroid adenoma

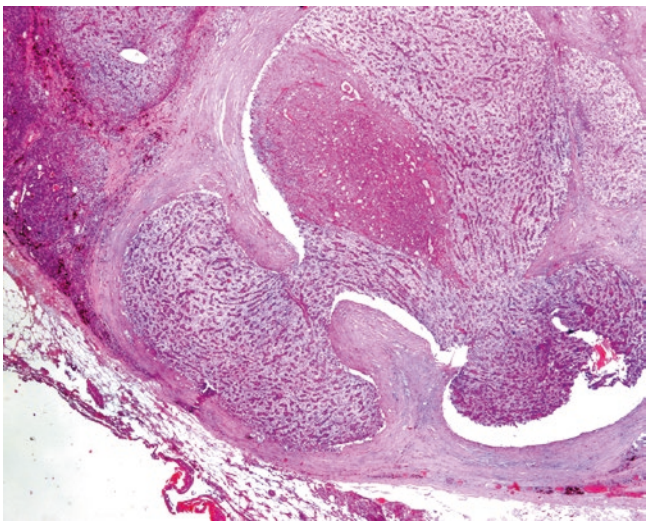

Fig. 14.82 Atypical parathyroid adenoma with signs of invasion that do not reach the peri-parathyroid soft tissue

patients is recommended due to the long natural history of many parathyroid carcinomas.

14.9.2 Carcinoma

Definition and epidemiology Parathyroid carcinoma is an extremely rare endocrine malignancy. Unlike PHPT that affects mostly women, parathyroid carcinoma occurs with equal frequency in men and women.

Macroscopy Typically, parathyroid carcinoma occurs as a solid, poorly circumscribed mass that involves the parathyroid gland and their adjacent structures. Unless the parathy-

roid tumor has overwhelming invasive features, it is very difficult to make a diagnosis of carcinoma. The situation is so problematic that the intraoperatory detection of gross invasion of soft tissues or of the adjacent organs by a parathyroid tumor remains, together with the detection of metastases, the two only reliable diagnostic criteria of malignancy (Fig. 14.83).

Microscopy The histological appearance of parathyroid carcinoma is similar to that of parathyroid adenoma. Classic histologic features suggestive of malignancy in other settings are not usable in parathyroid carcinoma. For example, the presence of abnormal mitotic figures should not lead to a diagnosis of parathyroid carcinoma; the same holds true regarding microscopic invasion of the capsule and the high mitotic activity of the tumor. It has been stressed that most parathyroid carcinomas exhibit marked nuclear pleomorphism with coarse chromatin and macronucleoli, a feature that must be distinguished from the so-called endocrine atypia (Fig. 14.84) [284]. The latter is frequently observed in hyperplasias and adenomas of the parathyroid – mainly whenever composed by oncocytic cells – as well as in other benign endocrine tumors [280, 284].

Differential diagnosis Even the nastiest nuclear morphological abnormalities like the presence of atypical mitotic figures do not serve as a diagnostic criterium of malignancy [278–280, 284]. According to some authors, the demonstration of unequivocal signs of vascular invasion appears to be the only microscopic criterium of malignancy (Fig. 14.85). The utilization of this criterium rests, however, controversial in the absence macroscopically evident invasion of the neighboring structures by the tumor.

Typically, parathyroid carcinoma expresses cytokeratins and chromogranin. It does not express TTF1, thyroglobulin, or calcitonin, a feature that allows the separation of parathyroid carcinoma from thyroid primary tumors (Fig. 14.86).

Nonfunctional parathyroid carcinomas are a rarity arising within the rarity of such malignant tumors. Besides cases which may be classified under the vague description of poorly differentiated parathyroid carcinomas, there are on record two cases of parathyroid carcinosarcoma which were accompanied by normal parathyroid hormone levels [285, 286]. Histopathology showed in both tumors a biphasic pattern characterized immunohistochemically by strong and diffuse positivity for chromogranin in the epithelioid areas and for vimentin in the sarcomatoid areas.

Genetics *MEN1* gene is a tumor suppressor gene that codifies the protein menin and is mutated in 80% of cases of MEN1 syndrome, giving rise to parathyroid hyperplasia, adenoma, and, exceptionally, carcinoma, together with other endocrine tumors in pituitary and pancreas. In the sporadic context, *MEN1* deletions are thought to represent the most prevalent somatic event, occurring in up to 54% of parathyroid lesions, including hyperplasia, adenoma, and carcinoma, a fact that weakens the utilization of this gene as a good differential diagnostic tool [287]. Menin loss of expression in parathyroid lesions does not correlate with the mutational status of *MEN1*, a gene that is difficult to study due to its long length and absence of known mutational hotspots.

The tumor suppressor gene *HRPT2* codifies the protein parafibromin and is a less frequently mutated gene in hereditary parathyroid lesions than *MEN1* but appears to be more useful for diagnostic purposes. *HRPT2* is mutated in more than half of patients with HPT-JT syndrome, a condition that

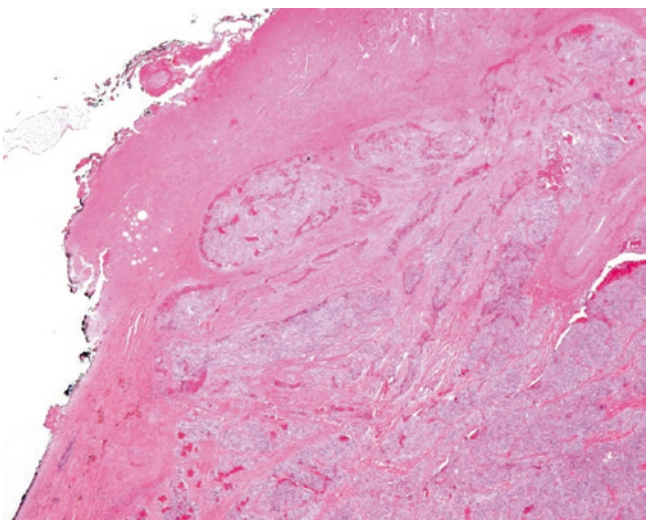

Fig. 14.83 Parathyroid carcinoma with signs of invasion that reach the peri-parathyroid soft tissue

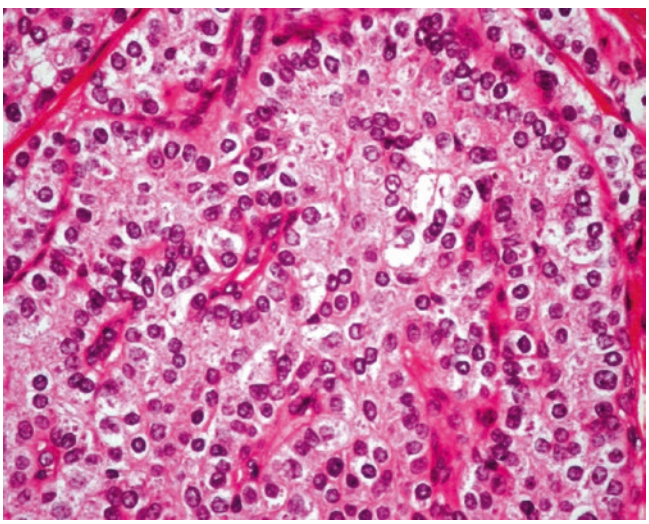

Fig. 14.84 Cytological features of parathyroid carcinoma

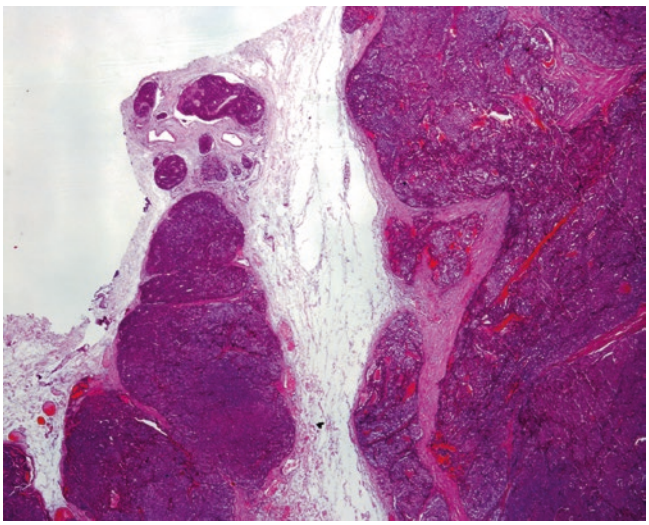

Fig. 14.85 Parathyroid carcinoma with signs of vascular invasion and invasion of the peri-parathyroid soft tissue

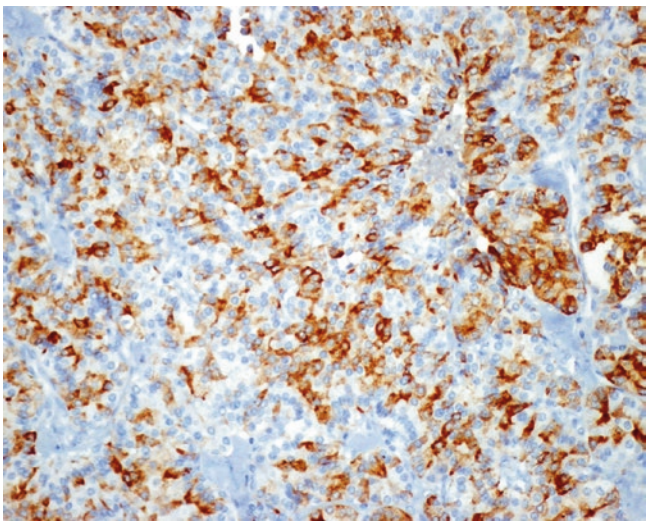

Fig. 14.86 Chromogranin expression in parathyroid carcinoma cells

is characterized by the occurrence of parathyroid adenoma and, in 15 % of cases, of parathyroid carcinoma, together with ossifying fibromas of the jaw. Nearly 20 % of apparently sporadic carcinomas are associated to germ line mutations of the *HRPT2* gene. Somatic mutations of *HRPT2* were found to be useful in the differential diagnosis of parathyroid tumors due to their detection in nearly two-thirds of the sporadic carcinomas and their rarity in sporadic adenomas [288–290]. Parafibromin expression is often but not always related to the *HRPT2* mutation status [291]. Taking together all the aforementioned data, one concludes that the molecular pathology based upon the detection of mutations of *HRPT2* gene or on the absence of nuclear expression of its product (parafibromin) by immunohistochemistry provides useful data for separating parathyroid adenoma from parathyroid carcinoma but has not solved definitively this differential diagnostic dilemma.

CCND1 gene codifies cyclinD1 and is mutated in only 5 % of patients with hereditary parathyroid lesions. Overexpression of cyclinD1 has been detected in nearly 90 % of sporadic parathyroid lesions including hyperplasia, adenoma, and carcinoma [288], a finding that reduces its differential diagnostic usefulness.

None of the abovementioned genes or proteins appears to be a good marker to distinguish hyperplasia from adenoma.

The cell cycle protein p27 has been increasingly associated to the pathogenesis of endocrine tumors and has been found to be underexpressed in parathyroid carcinoma [292]. Its differential diagnostic usefulness remains controversial. Mutations in the *CDKN1B* gene that codifies p27 are extremely rare.

It has also been suggested that the positive immunostaining for the protein gene product 9.5 (PGP9.5), a protein encoded by ubiquitin carboxyl-terminal esterase, may help, together with the absence of nuclear immunostaining for parafibromin, in establishing the diagnosis of parathyroid carcinoma. Further studies in series composed by difficult, borderline cases are necessary to confirm these findings (e.g., loss of parafibromin staining has been observed in a subset of adenomas unassociated with the HPT-JT syndrome, and positive staining has been reported in some parathyroid carcinomas) [284]. The putative value of the results that are being obtained by the massive sequencing of cases of parathyroid carcinoma remains to be proven both for diagnosis and for therapy selection.

Treatment and prognosis The reported 5- and 10-year survival rates of patients with parathyroid carcinoma vary a lot from series to series partly because of the reduced number of cases in each series. At 10 years, the survival rates vary from a minimum of 35 % to a maximum of 77 % [280]. Forty to sixty % of patients with parathyroid carcinoma develop local, single, or often multiple recurrences that are difficult to manage. Metastases to regional lymph nodes or lungs, liver, bone, pleura, and pericardium may occur, sometimes after a long disease-free interval [278, 279]. Metastatic parathyroid carcinomas are usually functional, producing hypercalcemia, and causing death as a consequence of renal disease, cardiac arrhythmia, or pancreatitis [278–280, 284].

Currently, the ideal treatment of parathyroid carcinoma is en bloc surgical resection of the tumor and adjacent grossly involved neck structures. Chemotherapy has proven largely ineffective, and radiotherapy is of limited benefit.

14.10 Other Parathyroid Lesions

Parathyroid cysts are uncommon, usually (85 a 90%) non-functional from the clinical standpoint, and may present as a cervical or retrosternal mass [280]. The diagnosis may be achieved by FNAB through the evaluation of the parathyroid hormone levels in the cystic fluid.

Lymphocytic infiltrates of the parathyroids are not uncommon and support the diagnosis of parathyroiditis in lesions in which there is evidence of a primary parathyroid immune process. There is on record a case of cytomegalovirus parathyroiditis in an immunocompromised infant [280].

Metastases to the parathyroids are exceedingly rare, and, like in the thyroid, they may be localized in preexisting parathyroid adenomas.

"Parathyromatosis" is a clinicopathological entity characterized by the presence of several nodules of hyperfunctioning parathyroid tissue occurring in the neck and mediastinum. Most frequently, parathyromatosis appears to be the result of seeding of parathyroid tissue after the fracture of the gland capsule during the surgical removal of a parathyroid neoplasm or of a hyperplastic lesion. It is crucial to distinguish this condition from metastases originating from a low-grade or, at least, not macroscopically obvious parathyroid carcinoma.

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Common Skin Tumors of the Head and Neck

15

Daniela Massi, Boštjan Luzar, and Llucia Alos

Contents

D. Massi, MD (✉)
Division of Pathology, Department of Surgery and Translational Medicine, University of Florence, Largo Brambilla 3, Florence 50134, Italy
e-mail: daniela.massi@unifi.it

B. Luzar, MD, PhD
Institute of Pathology, Medical Faculty University of Ljubljana, Korytkova 2, Ljubljana 1000, Slovenia
e-mail: bostjan.luzar@mf.uni-lj.si

L. Alos, MD, PhD
Department of Pathology, Hospital Clinic, University of Barcelona, Villarroel 170, Barcelona 08036, Spain
e-mail: lalos@clinic.ub.es

15.1 Introduction

15.1.1 Embryology

In 5–6 week-old embryos, cells of the surface ectodermal layer proliferate and form a layer of squamous epithelium, the periderm, and, beneath it, the basal layer [1]. Periderm cells undergo a process of keratinization and desquamation and are replaced by cells from the basal layer. From the basal

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layer, new cells are shifted towards the surface layers. The replacement of periderm cells continues until about 21 weeks, after which the periderm disappears in place of the newly formed stratum corneum [2]. The appearance of melanocytes in the epidermis takes place in a craniocaudal direction, in accordance with the development of the neural crest, from which melanocytes are derived. The embryonal basal cell layer differentiates also in hair germs which give rise to hair, sebaceous glands and apocrine glands, as well as eccrine gland germs, from which eccrine glands develop. The dermis derives from the mesenchyme (mesoderm located beneath the surface ectoderm) [3]. By week 11, mesenchymal cells produce collagen fibres, while elastic fibres appear in the dermis at week 22. Fat cells begin to develop in the subcutaneous tissue towards the end of the fifth month.

15.1.2 Anatomy

The skin forms a protective barrier over the body's surface and plays a key role in protecting the body against pathogens and excessive water loss. The dermis assumes the important functions of thermoregulation and sensation of touch and pressure and supports the vascular network to supply the avascular epidermis with nutrients. The thickness of skin varies according to anatomical location.

15.1.3 Histology

Three layers can be recognised in the skin: the epidermis, the dermis and the subcutaneous fat. The epidermis is the outermost part and is composed of keratinocytes arranged in four layers: the basal cell layer, the squamous cell layer (five to ten layers of polygonal cells connected to each other by intercellular bridges or desmosomes), the granular layer (diamond-shaped or flattened cells, their cytoplasm being filled with basophilic keratohyalin granules) and the horny layer (anuclear cells). A subepidermal basement membrane zone appears as a homogeneous PAS+ band at the dermo-epidermal junction. Melanocytes are recognised as clear cells between the basal cells of the epidermis, the average number being one out of ten basal cells. Langerhans cells are located in the suprabasal epidermis. Eccrine glands primarily serve in the regulation of heat and are composed of the secretory portion, the intradermal duct and the intraepidermal duct or acrosyringium. The apocrine glands represent scent glands; they are tubular glands composed of a secretory portion, the intradermal duct and the intraepidermal duct. The type of secretion occurring in apocrine glands consists of the release of portions of cytoplasm into the lumen (decapitation secretion). Sebaceous (holocrine) glands are found in association with hair structures and consist of several lobules leading into a common excretory duct; each lobule possesses a peripheral layer of cuboidal basophilic cells that do not contain lipid droplets. The hair follicle in longitudinal sections consists of three parts: the lower portion from the base of the follicle to the insertion of the arrector pili muscle; the middle portion, or isthmus, extending from the insertion of the arrector pili to the entrance of the sebaceous duct; and the infundibulum extending from the entrance of the sebaceous duct to the follicular orifice. The lower portion of the follicle is composed of five major portions: the dermal hair papilla, the hair matrix, the hair, the inner root sheath and the outer root sheath [4]. The dermis consists of collagenous and elastic fibres embedded into ground substance which contains glycosaminoglycans or acid mucopolysaccharides. The largest portion of the dermis is referred to as the reticular dermis (composed of thick collagen bundles), while papillary dermis is characterised by a finely woven meshwork of collagen fibres in the subepidermal papillae and subpapillary layer. Cutaneous blood vessels consist of a subcutaneous plexus of small arteries from which arterioles ascend into the dermis and are interconnected. The skin is supplied with sensory nerves and autonomic nerves, which permeate the entire dermis with branching nerve fibres.

15.2 Cutaneous Epithelial Neoplasms

15.2.1 Benign Tumors of the Epidermis

15.2.1.1 Seborrheic Keratosis

Definition Seborrheic keratosis (SK) is a benign proliferation of keratinocytes.

Epidemiology SK is an extremely common lesion occurring in adults and elderly patients, arising mostly on the face and trunk.

Clinical aspects SK appears as solitary or multiple plaques with a greasy appearance, measuring from a few mm to a few cm. The surface can be verruciform or smooth, the consistency generally friable. SK can be brown-black in colour, being clinically mistaken for melanoma. A sudden onset of multiple SK may occur in association with the development of visceral tumours including gastrointestinal tract carcinomas (Leser–Trélat sign).

Microscopy SK is an exophytic or endophytic proliferation of cytologically bland basaloid and squamoid cells, associated with orthokeratosis and numerous keratin-filled invaginations and pseudohorn cysts (Fig. 15.1). Nests of keratinocytes (squamous eddies) are observed in the irritated/clonal form. Many other histological variants have been described, including acanthotic, papillomatous, adenoid (reticulated), lichenoid and inflammatory.

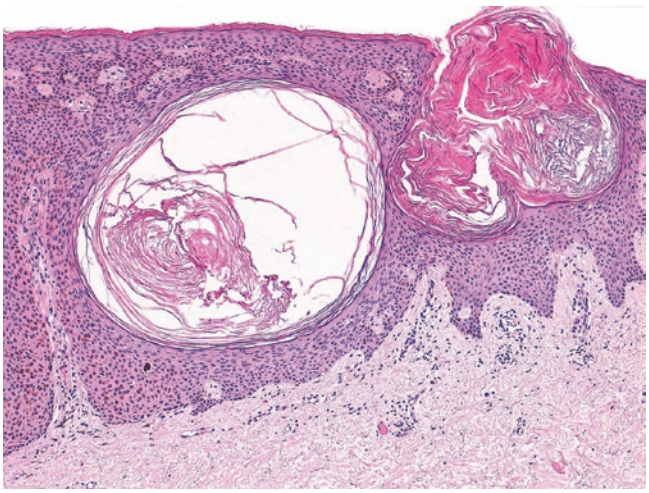

Fig. 15.1 Seborrheic keratosis. Proliferation of pigmented basaloid and squamoid cells associated with keratin-filled invaginations and horn cysts

Differential diagnosis Verruca vulgaris (VV), epidermal nevus, hidroacanthoma simplex (intraepidermal poroma) and hypertrophic actinic keratosis (AK) should be considered in the differential diagnosis.

Treatment and prognosis SK are benign tumors.

15.2.1.2 Verruca Vulgaris

Definition Verruca Vulgaris (VV) (common warts) are benign squamous papillomatous lesions caused by human papilloma virus (HPV) infection.

Epidemiology VV are common in children and young adults but can occur at any age.

Etiology and pathogenesis Numerous HPV types, including HPV 1, 2, 3, 4, 7 and 10, have been implicated in their pathogenesis.

Clinical aspects They appear as keratotic papules of variable size or more filiform exophytic lesions. They occur on any skin surface, but they are frequently localised to the extremities and the face, and they may be single or grouped lesions. Verruca plana commonly occurs as multiple recurrent flat papules, mostly on the face, and is generally associated with HPV types 3 and 10.

Microscopy VV show marked acanthosis and hyperkeratosis, and columns of parakeratosis overlie the papillomatous proliferation (Fig. 15.2). Intracorneal haemorrhage is typically present. Perinuclear halos are seen in superficial keratinocytes (koilocytes). Hypergranulosis is associated with coarse clumps of keratohyalin granules.

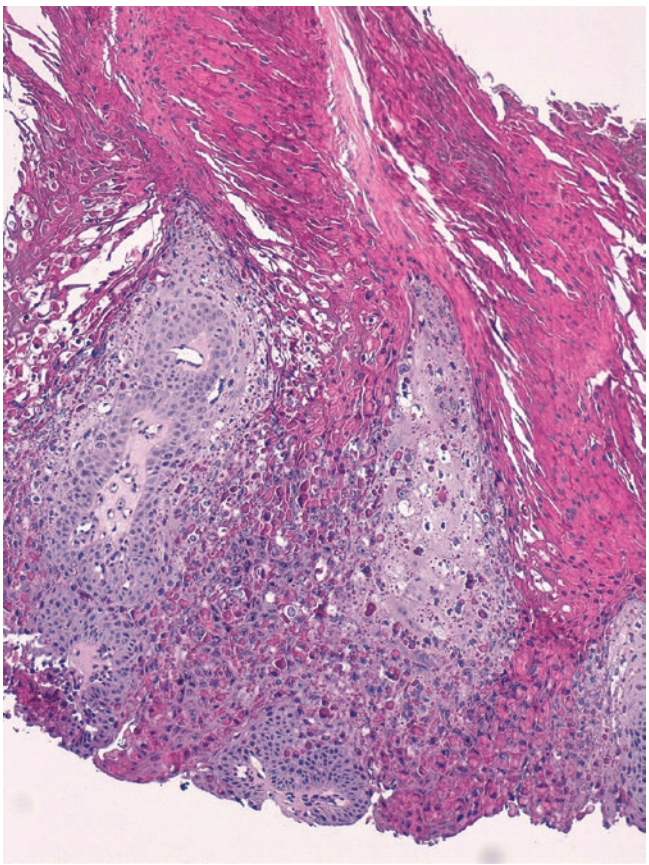

Fig. 15.2 Verruca vulgaris. Papillomatosis and large keratohyalin granules are observed

Differential diagnosis The differential diagnosis includes SK, AK, verrucous carcinoma (VC) and epidermal nevus.

Treatment and prognosis Most VV are only a cosmetic problem. Prognosis is excellent, with the exception of immunosuppressed patients where cutaneous carcinomas may develop on HPV-related lesions.

15.2.1.3 Warty Dyskeratoma

Definition Warty dyskeratoma (WD) is a cup-shaped benign epidermal proliferation characterised by acantholysis and dyskeratosis.

Epidemiology WD affects middle-aged to older patients.

Clinical aspects WD presents as a solitary umbilicated papule or nodule on the head or neck region.

Microscopy Under the microscope, it shows a cup-shaped keratinocytic hyperplasia associated with a hair follicle infundibulum (Fig. 15.3). Typically, there is hyperkeratosis, parakeratosis, suprabasilar acantholysis and dyskeratosis (corp ronds and grains) [5].

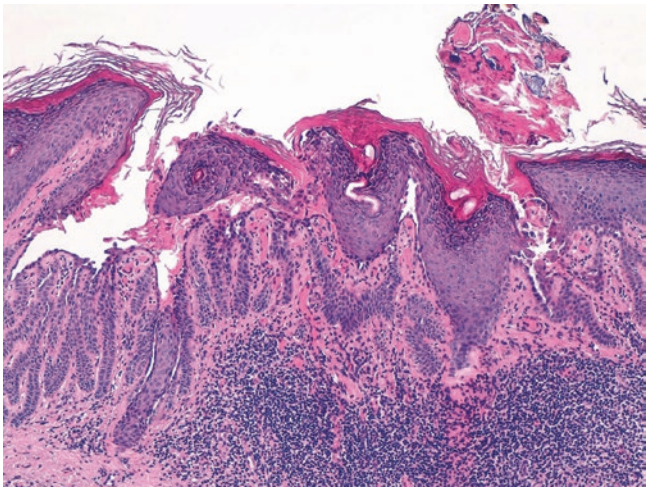

Fig. 15.3 Warty dyskeratoma. Cup-shaped invagination filled with keratin associated with acantholysis and dyskeratosis

Differential diagnosis Clinicopathologic correlation is necessary to rule out acantholytic dermatoses such as Darier or Grover disease. Acantholytic acanthoma does not show dyskeratosis; acantholytic dyskeratotic acanthoma unlike warty dyskeratoma has a flat profile. Acantholytic AK is not cup shaped, typically spares hair follicles and is composed of atypical keratinocytes.

Treatment and prognosis Excisional biopsy is suggested for a benign lesion that does not have any malignant potential.

15.2.1.4 Keratoacanthoma

Definition Keratoacanthoma (KA) is an exoendophytic squamoproliferative lesion which can spontaneously regress. Some authors regard KA as a well-differentiated variant of squamous cell carcinoma (SCC), but this hypothesis has not been universally accepted.

Epidemiology Male adults or elderly patients are more frequently affected. The head and neck is the most common location. Recently, occurrence of cutaneous squamoproliferative lesions, including KA, pathology-proven SCC and verrucous keratosis, in association with *BRAF* inhibitors, has been increasingly recognised [6].

Etiology and pathogenesis The lesion is pathogenetically related to excessive sunlight exposure and viruses, particularly in immunosuppressed patients, in whom HPV has been detected.

Clinical aspects KA is typically described as a dome-shaped cutaneous nodule with a central keratinous plug. It may reach large size, up to 10 cm (giant KA). KA tend to grow rapidly over 1–2 months with spontaneous involution after 3–6 months.

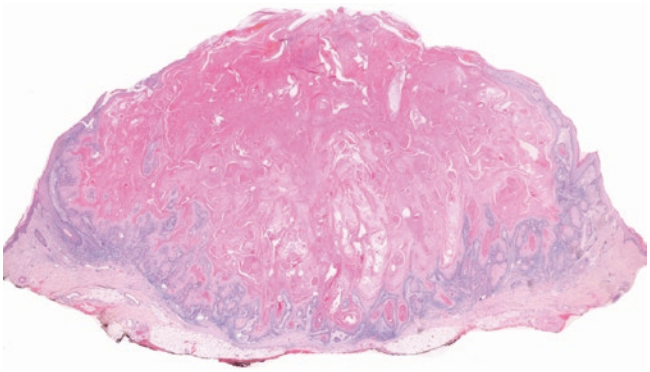

Fig. 15.4 Keratoacanthoma. Exoendophytic lesion showing an invaginating mass of keratinizing squamous epithelium

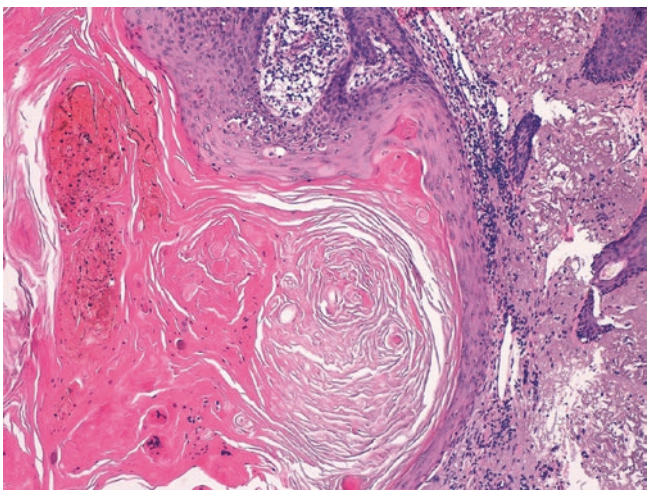

Fig. 15.5 Keratoacanthoma. Regressing phase showing a large keratin plug

Microscopy At low magnification, fully developed KA appears as a well-differentiated squamous proliferation with a central keratin-filled crater (Figs. 15.4 and 15.5). Lipping of the edges overlap the central keratin-filled crater, giving it a symmetrical appearance. Keratinocytes show abundant glassy eosinophilic cytoplasm and large nuclei. Perineural invasion has been rarely described in face lesions. There is prominent inflammatory infiltrate, usually with numerous eosinophils and neutrophils within the lesion.

Differential diagnosis A long-lasting debate exists on the distinction between SCC and KA, since some authors regard KA as a variant of SCC [7, 8]. SCC arising from KA have also been described [9]. Those who believe that these are distinct entities underline that well-differentiated SCC of the skin lacks a central keratin-filled crater and generally shows more cytological atypia, atypical mitoses and infiltrative pattern of growth. VC can be recognised from its endophytic–exophytic architecture, deep, bulbous rete ridges and lack of central crater.

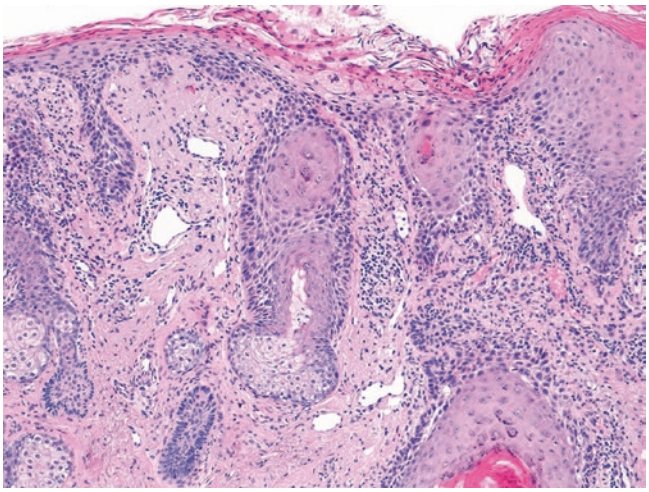

Fig. 15.6 Actinic keratosis. There is moderate atypia of epidermal and adnexal keratinocytes

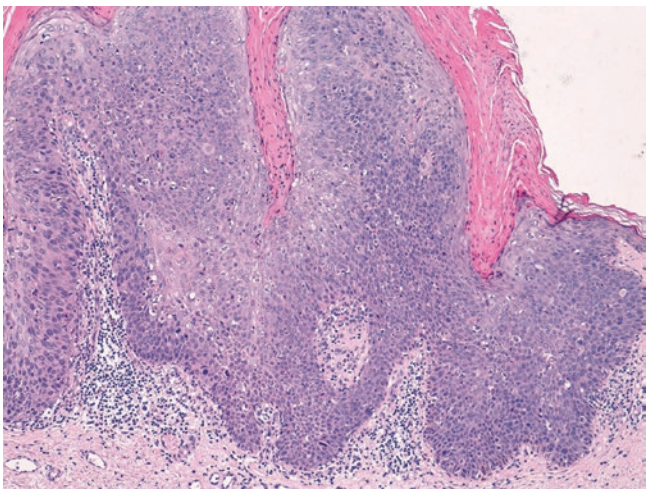

Fig. 15.7 Bowen's disease. There is remarkable full-thickness atypia of intraepidermal keratinocytes within an acanthotic epidermis

Treatment and prognosis Surgical excision is the main choice but probably not necessary in most cases due to spontaneous regression. In contrast, complete surgical excision is recommended for giant, subungual and KA arising in immunosuppressed patients since in these cases lesions may not regress and rarely metastasise.

15.2.1.5 Actinic Keratosis

Definition Actinic keratosis (AK) (solar keratosis) is a proliferation of atypical keratinocytes confined to the epidermis, with limited risk of progression to invasive SCC.

Epidemiology AK is a very common lesion with higher incidence in areas with heavy sun exposure, such as face, head and neck, dorsal hands and forearms. Older Caucasian adults are typically affected, males more commonly than females.

Etiology and pathogenesis Ultraviolet B radiation induces mutations in DNA, which lead to abnormal proliferation of intraepidermal keratinocytes [10]. P53 mutations are the most common genetic alteration identified.

Clinical aspects AK presents as scaly macules or slightly elevated papules or plaques, ranging from erythematous to grey-brown with adherent yellow-brown scale. Some are hyperkeratotic or verrucous, and a keratin horn may be produced.

Microscopy AK shows an intraepidermal proliferation of atypical keratinocytes, typically confined to the lower third of the epidermis, and overlying parakeratosis (Fig. 15.6). Unlike Bowen's disease (BD) (Fig. 15.7), atypical keratinocytes usually do not involve hair follicles or eccrine ducts. An increased number of mitotic figures is observed. Several variants have been described, including hypertrophic, atrophic, acantholytic, pigmented, lichenoid and bowenoid.

Differential diagnosis In small superficial biopsies, the differential diagnosis includes invasive SCC, BD and basal cell carcinoma (BCC, basosquamous and superficial types). Pigmented AK should be differentiated from in situ melanoma on sun-exposed skin (lentigo maligna).

Treatment and prognosis Treatment consists in conservative excision or topical therapies. For multiple or extensive lesions, photodynamic therapy can be used. Prognosis is excellent, as only approximately 6–10 % may progress to invasive SCC, if left untreated.

15.2.2 Malignant Tumors of the Epidermis

15.2.2.1 Basal Cell Carcinoma

Definition Basal cell carcinoma (BCC) is the most common malignant neoplasm of humans and the most frequent cutaneous carcinoma of the head and neck region. BCC is a low-grade malignancy of basaloid-appearing keratinocyte-derived follicular stem cells, and some authors regard BCC as a trichoblastic carcinoma, although this is not unanimously accepted.

Epidemiology BCC arises most commonly in the head and neck region (up to 80 % of cases), typically in older adults. There is a greater incidence in fair-skinned individuals. Few cases are observed in young adults and children, and in these cases, a clinical association with basal nevus syndrome, Bazex syndrome, xeroderma pigmentosum or an organoid nevus should be considered.

Etiology and pathogenesis Pathogenesis is multifactorial, but the vast majority of BCC is strongly related to prolonged sun exposure. Previous radiation, immunosuppression (organ transplantation) and burn scars increase the risk of BCC development. Genetic factors also play a role in the susceptibility of some individuals to BCC. Mutations in the *PTCH1* gene on chromosome 9q, responsible for the nevoid basal cell carcinoma syndrome, have also been encountered in 30–40 % of sporadic BCC. Mutations in *TP53* have been reported in 33–50 % of BCC [11].

Clinical aspects BCC may show diverse clinical appearances, such as a papule, a plaque or a skin-coloured nodular lesion; size ranges from few mm to several cm. Lesions often appear as a pearly papule or nodule with telangiectasia. Larger lesions often erode or ulcerate with bleeding. A minority of cases is pigmented and may clinically simulate melanocytic lesions.

Microscopy Histopathologically, BCC are characterised by a proliferation of basaloid cells arranged in islands (Fig. 15.8), nests and/or infiltrating cords, generally with outer peripheral palisading and stromal retraction artefacts (clefting) between tumor aggregates and stroma. The stroma surrounding islands of tumor cells is newly formed and different from the adjacent dermis. Cells are uniform in size and show a hyperchromatic nuclei with inconspicuous nucleoli and scant cytoplasm. Numerous mitotic and apoptotic figures are observed. Mucinous cystic degeneration may be present. There is considerable variability in the morphology of BCC, and a number of histopathological subtypes can develop in the head and neck region. Histopathological subtypes, based upon growth pattern and risk for recurrence, include low-risk types (superficial basal cell carcinoma (Fig. 15.9) and nodular basal cell carcinoma) and high-risk types [micronodular basal cell carcinoma, basosquamous carcinoma and infiltrative basal cell carcinoma, the later further subclassified into infiltrative (NOS) and sclerosing/morphoeic (Fig. 15.10)]. In the basosquamous/metatypical variant, nests and strands of tumor cells maturing into larger and paler cells with more abundant cytoplasm, with more marked keratinization than typical BCC, palisading may be focally lost.

Immunohistochemistry Occasional cases of BCC may be diagnostically challenging, particularly tumors that may simulate benign cutaneous adnexal tumors, and in these cases, immunohistochemical stains may be helpful. BCL-2, p53 and Ki-67 are more expressed in BCC in comparison with trichoepithelioma (TE) and trichoblastoma (TB). In addition, CK20 shows scattered CK20+ Merkel cells in benign follicular tumors, while in BCC, they are rare or absent. BCC tumor cells are generally BER-EP4+, while TE and TB are almost always negative. In BCC, CD10 is positive in tumor cells, while in TE and TB, CD10 is positive in the stroma. CD34 is negative in BCC and positive in the

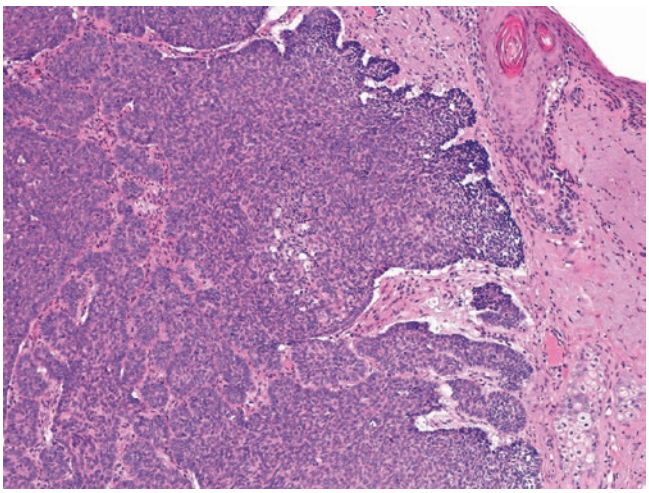

Fig. 15.8 Basal cell carcinoma, nodular type. Island of basaloid cells with peripheral palisading

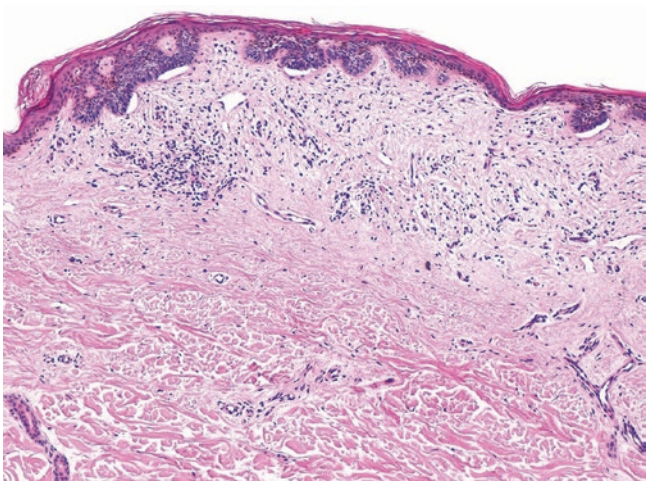

Fig. 15.9 Basal cell carcinoma, superficial type. Multiple small islands of pigmented basaloid cells with peripheral palisading and retractions artefacts

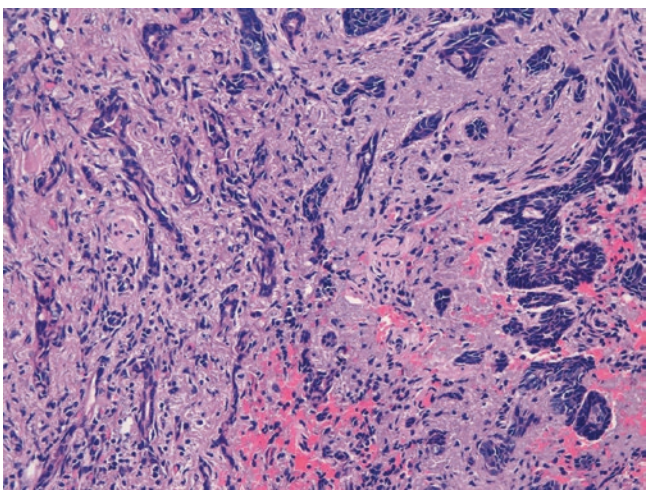

Fig. 15.10 Basal cell carcinoma, infiltrating type. Infiltrating elongated cords and strands of basaloid cells within the stroma

stroma of TE and TB. Finally, AR is positive in 60–78 % of BCC, while it is negative in benign follicular tumors.

Differential diagnosis Superficial biopsies of sclerosing/morpheaform BCC may pose differential diagnosis with desmoplastic trichoepithelioma (DT) [12] or microcystic adnexal carcinoma (MAC). DT is CK20+, CK15+ and p75+, while BCC is CK20-, CK15- and p75- (most cases) [13, 14]. Recently, PHLDA1 (pleckstrin homology-like domain, family A, member 1) has been reported as useful in distinguishing DT from morpheaform BCC, with positive staining in the former and negative in the latter [15]. MAC is CEA+, CK15+, while BCC is CEA-, CK15-. TE may resemble keratotic BCC, although TE shows a much more organoid pattern. In comparison to TE, stroma in BCC generally shows more frequently myxoid features. BCC with advanced follicular differentiation may be mistaken for TB, a tumor that generally lacks the high number of mitoses and apoptotic figures of BCC, and may show retractions within the fibrocytic stroma and not between tumor and the fibromyxoid stroma, as in BCC. Sebaceous carcinoma (SC) can show areas of basaloid differentiation, but clear and multivacuolated cells with nuclear indentations should be present. Furthermore, SC lacks peripheral palisading, myxoid/mucinous stroma or stromal retraction artefacts.

Treatment and prognosis Most BCC are slow-growing, relatively non-aggressive tumors that are cured by complete excision or electrodessication and curettage. Recently, Food and Drug Administration (FDA) approval of the new agent vismodegib, a Hedgehog pathway-targeting agent, has provided another option for patients who have exhausted surgical or radiation options for advanced BCC. For low-risk tumors, the prognosis is usually excellent and tumors are cured by local excision. BCC that develop in the head and neck area (and particularly the high-risk mask area of the face) are more likely to recur than those tumors located on the extremities and trunk. More aggressive histologic subtypes also have a higher rate of recurrence and increased risk of metastasis. Overall risk of metastasis is estimated at 0.05 %.

15.2.2.2 Squamous Cell Carcinoma

Definition Cutaneous squamous cell carcinoma (SCC) (also known as epidermoid carcinoma) is a malignant tumor of epidermal keratinocytes.

Epidemiology Elderly male patients are the most affected, especially on sun-exposed skin (forehead, face, ears, scalp, neck, dorsum of the hands and the vermilion part of the lower lip).

Etiology and pathogenesis Most SCC are related to UV-B radiation. However, some cases may be pathogenetically related to previous radiation therapy, chronic inflammation, chronic wounds, long-lasting ulcers, burn scars, immunosuppression and HPV infection.

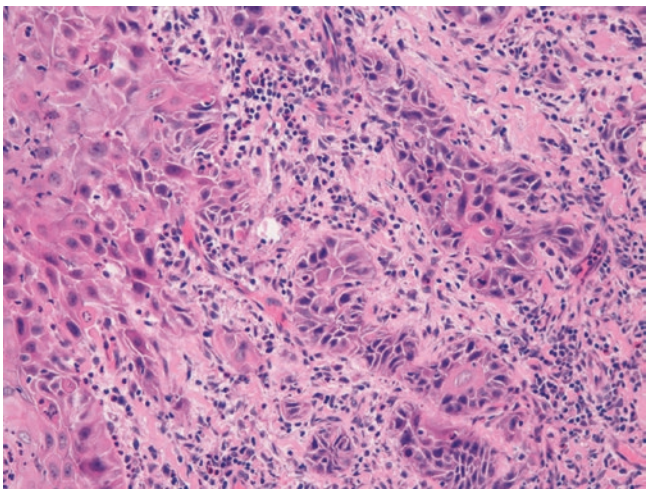

Fig. 15.11 Squamous cell carcinoma. Nests of atypical squamous cells infiltrating the dermis surrounded by inflammatory cells

Clinical aspects Clinically, they appear as papules, plaques or nodules of variable size; the lesions may be ulcerated or haemorrhagic [16]. The vast majority of SCC is associated with a pre-existing AK. Bowen disease (BD) is a form of SCC in situ that is recognised as a scaly patch or plaque lesion on the head and neck region or other sites.

Microscopy Conventional SCC consists in a proliferation of atypical keratinocytes arranged in nests, sheets and infiltrative cords (Fig. 15.11). Keratin pearls and squamous eddies may be observed. Typically, the amount of keratinization decreases and cytologic atypia increases with higher grades of differentiation. Tumor cells show an abundant eosinophilic cytoplasm, large nucleus with vesicular chromatin and prominent nucleoli. At high power, intercellular bridges and dyskeratotic cells are observed. Mitotic figures are usually numerous, and atypical forms may be found in moderately to poorly differentiated cases. In poorly differentiated tumors, necrosis is typically prominent and lymphatic, and perineural invasion is detected in approximately 50 % of cases. Prominent dermal elastosis is seen in tumors arising in sun-exposed areas. A number of histological variants of SCC have been described, and those that may occur in the skin of the head and neck region include spindle cell SCC, adenoid (acantholytic or pseudoglandular) SCC, lymphoepithelioma-like SCC, carcinosarcoma (or metaplastic carcinoma), follicular SCC (face, elderly patients), pseudovascular SCC, adenosquamous carcinoma, clear cell SCC, inflammatory SCC, basaloid SCC, desmoplastic SCC and pigmented SCC.

BD is characterised by full-thickness intraepidermal keratinocytic atypia with numerous mitotic figures and apoptotic cells. Keratinocytes show marked pleomorphism,

hyperchromatic nuclei and prominent nucleoli. Basilar keratinocytes are often spared, while follicular involvement is typically seen. Overlying parakeratosis is often present.

Immunohistochemistry Immunohistochemistry is necessary for the diagnosis of poorly differentiated tumors and spindle cell/sarcomatoid SCC. In this context, high molecular weight cytokeratins are the most important markers. P63 is also a very sensitive marker and can be used to confirm diagnosis. Negative staining for melanocytic markers, smooth muscle markers, CD10, CD68 and CD99 (generally positive in atypical fibroxanthoma) should be demonstrated. In the differential diagnosis between SCC with basaloid features and BCC with squamous features, it has recently been suggested that a panel of three markers (BER-EP4, CK17 and CK14) are expected to be diffusely positive in BCC with squamous features, while no basaloid SCC displayed diffuse staining for all of them [17]. EMA is expressed more frequently in poorly differentiated tumors, while it tends to be negative in well-differentiated SCC. Thus, in the differential diagnosis between BCC and SCC, the recommended panel includes BER-EP4 and EMA. BCC is generally BER-EP4+/EMA−, while SCC is BER-EP4−/EMA+. Finally, androgen receptors are almost always positive in BCC and negative in SCC, while cytokeratins are not helpful in discriminating the two types of keratinocytic tumors.

Differential diagnosis Pseudoepitheliomatous hyperplasia generally does not demonstrate the degree of atypicality observed in SCC. Poorly differentiated and spindle cell SCC should be distinguished mainly from atypical fibroxanthoma, leiomyosarcoma and spindle cell amelanotic melanoma, and a wide panel of immunohistochemical markers is generally necessary. Among adnexal carcinomas, a major simulator of SCC is porocarcinoma from which it should be distinguished on the basis of recognition of ductal differentiation and immunohistochemistry.

Treatment and prognosis Treatment options for cutaneous SCC include Mohs micrographic surgery, surgical excision, electrodessication and curettage and cryosurgery. Non-surgical modalities include radiation therapy, photodynamic therapy and topical treatments with 5-fluorouracil (5-FU) and imiquimod. While most tumors are adequately treated with these measures, a small percentage of tumors are associated with higher rates of recurrence and metastasis. Poorly differentiated, deeply invasive tumors of more aggressive subtypes (morpheaphorm/sclerosing, infiltrative, micronodular basosquamous) and/or high-risk anatomical sites (central face, eyelids, periorbital, nose, lip, ear) may be associated with an adverse outcome.

15.3 Adnexal Tumors of the Head and Neck

Cutaneous adnexal tumors are a difficult subject, not least because of their rarity, histopathological diversity and complex classification, together to our limited understanding of the precise derivation of many of them [18]. Although they are usually encountered as single, sporadic tumors, they may occasionally be multiple and hereditary; in that case, they may herald complex genetic syndromes that comprise visceral cancers [reviewed in 19]. Embryologically, the eccrine apparatus develops separately from the follicular–apocrine sebaceous unit; thus, tumors of follicular, apocrine or sebaceous lineage may, on occasion, have elements of the other within the tumor. In most instances, the histologic diagnosis can be rendered with the routine histologic sections; however, immunohistochemistry can help to narrow the differential in diagnosing neoplasms of cutaneous appendages in some settings [20, 21]. Several adnexal tumors of the skin may arise in the head and neck region, and we will address only those entities that can be observed with some regularity.

15.3.1 Benign Sweat Gland Tumors

15.3.1.1 Eccrine and Apocrine Hidrocystoma

Definition Eccrine and apocrine hidrocystomas (or cystadenomas) are cystic lesions preferentially located on the face, mostly in the periorbital region. Apocrine hidrocystoma are thought to arise from the apocrine secretory coil, while eccrine hidrocystomas are not regarded as true neoplasms but rather retention cysts of the eccrine cyst duct.

Epidemiology Relatively common in adults, females are slightly more affected than males. They arise commonly on the face, predominately in the periorbital region, but may occur on scalp, neck and other sites.

Clinical aspects Clinically, they appear as solitary or multiple, translucent to bluish, dome-shaped papules.

Microscopy Eccrine hidrocystomas are unilocular cysts lined by cuboidal epithelium; no decapitation secretion is seen (Fig. 15.12). Apocrine hidrocystomas are usually multiloculated cysts lined by columnar eosinophilic epithelium with luminal decapitation secretion and elongated myoepithelial cells beneath. Projections may protrude into the cyst lumen. In some cases, it can be difficult to differentiate with certainty between eccrine or apocrine origin, and sometimes both components can be found.

Differential diagnosis The differential diagnosis includes apocrine (papillary) cystadenoma, which is a true benign tumor.

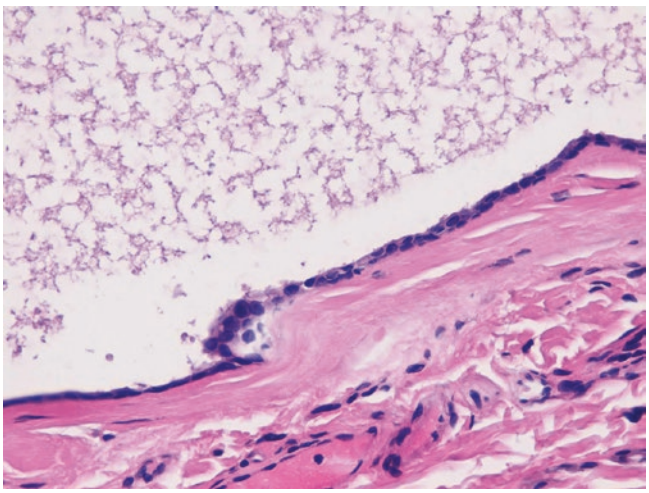

Fig. 15.12 Eccrine hydrocystoma. A unilocular cyst is lined by two layers of cuboidal epithelium

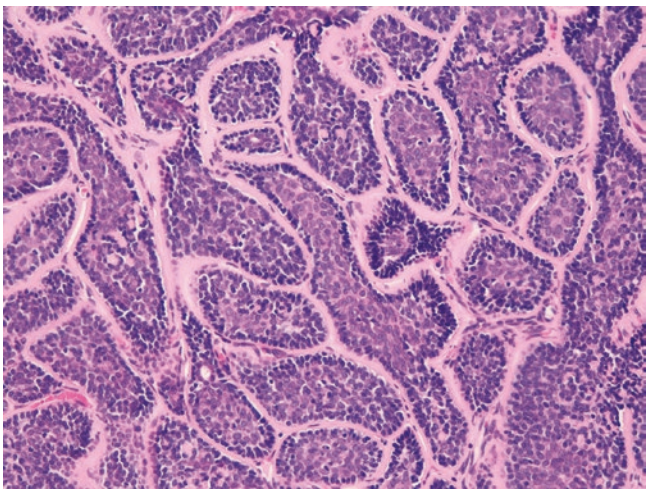

Fig. 15.13 Cylindroma. Irregularly shaped islands and cords of basaloid cells surrounded by conspicuous eosinophilic hyaline bands

Treatment and prognosis Treatment is not necessary; simple excision may be offered for cosmetic purposes.

15.3.1.2 Cylindroma

Definition and synonyms Cylindroma is a benign adnexal tumor with distinctive 'jigsaw puzzle' pattern. In its multiple form on the head region, it is also known as 'turban tumor'.

Epidemiology Cylindromas may be solitary or multiple, sporadic or familial as part of Brooke–Spiegler syndrome, in association with multiple eccrine spiradenomas and trichoepitheliomas.

Etiology and pathogenesis Lesions developing in the context of Brooke–Spiegler syndrome are associated with germ line, and somatic mutations in the *CYLD* gene on chromosome 16q are found in both sporadic and familial cases.

Clinical aspects Most cylindroma develop in early adulthood, with female predominance; 90 % of them are present on the scalp or face.

Microscopy Cylindroma is a poorly circumscribed dermal or subcutaneous proliferation in which islands of tumor cells are separated by deposits of eosinophilic PAS+, diastase-resistant basement membrane material (jigsaw puzzle piece pattern) (Fig. 15.13). Globules of PAS+ diastase-resistant hyaline basement membrane material are often present in the interior of tumor nodules. Two cell types are present, a uniform basaloid population with dark-staining nuclei and the other showing a polygonal shape with more abundant amphophilic cytoplasm. Sometimes both cylindroma and eccrine spiradenoma may be seen in the same lesion suggesting a close relationship between the two.

Differential diagnosis Spiradenoma shows significant histologic overlap with cylindroma, and hybrid lesions are not rare. Cylindrocarcinoma shows an infiltrative growth with loss of jigsaw pattern, marked cytologic atypia and numerous mitotic figures, including atypical forms.

Treatment and prognosis Cylindroma is a benign tumor that may recur if incompletely excised. Rare transformation to malignant cylindroma (cylindrocarcinoma) may rarely occur, usually in large, long-standing tumors.

15.3.1.3 Spiradenoma

Definition Spiradenoma is a benign adnexal tumor showing significant morphological overlap with cylindroma. It may have either eccrine or apocrine differentiation.

Epidemiology Spiradenoma arises most commonly in adults, but it may occur at any age. Most spiradenomas appear on the face but can also affect other sites.

Clinical aspects Clinically, it usually appears as a nodular, painful, cutaneous nodule, less than 1 cm in size. It may have bluish colour.

Microscopy Histopathologically, spiradenoma appears as a solid tumor composed of aggregations of tumor cells in the dermis, sometimes in the subcutaneous fat (Fig. 15.14). Two cell types are present, similar to cylindroma, but there is no jigsaw puzzle pattern, and instead intralesional dilated vascular channels and intratumoral lymphocytes are observed. Tumor lobules may sometimes be surrounded by thickened basement membrane, like cylindroma.

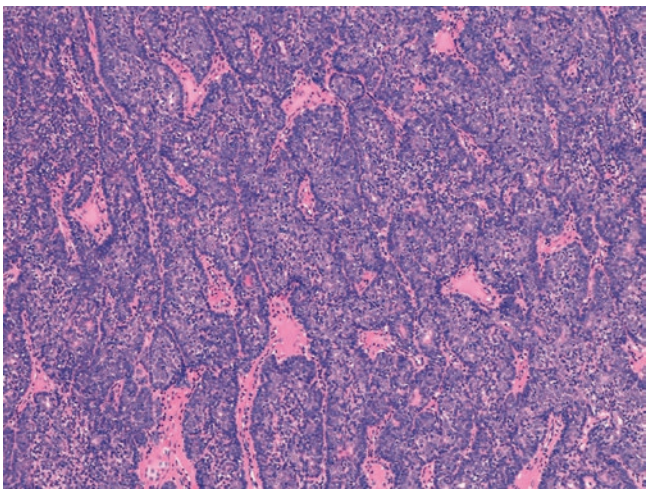

Fig. 15.14 Spiradenoma. Tumor aggregates are composed of small dark basaloid cells intermingled with larger cells with pale nuclei

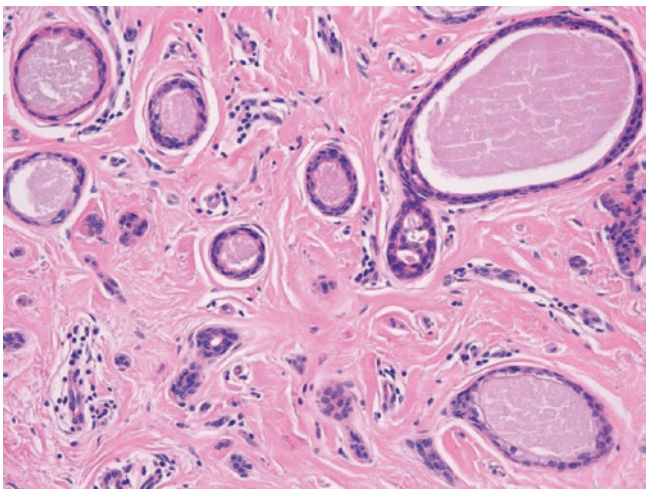

Fig. 15.15 Syringoma. Small ductal structures, partially dilated, lined by cuboidal epithelium and embedded in a fibrous stroma

Differential diagnosis Cylindroma and spiradenoma significantly overlap, and combined tumors may show areas that are indistinguishable. Differential diagnosis includes spiradenocarcinoma, which has a more infiltrative growth, prominent atypia, numerous mitotic figures and necrosis.

Treatment and prognosis Spiradenoma is a benign tumor, but local recurrence may occur. Malignant transformation is very rare.

15.3.1.4 Syringoma

Definition Syringoma is a benign eccrine ductal proliferation involving the superficial dermis.

Epidemiology Syringoma is a relatively common tumor which arises more commonly in females in adolescence or early adulthood in the head and neck region, with predilection for the cheek and lower eyelid.

Etiology and pathogenesis Most cases are sporadic, though some eruptive and disseminated forms may be familial. The clear cell variant is associated with diabetes mellitus [22].

Clinical aspects The lesions appear as multiple, asymptomatic, firm skin-coloured to translucent papules, 1–3 mm in diameter. Rare solitary cases have been described.

Microscopy Histopathologically, syringoma shows nests, cords and ductal structures with tadpole or comma-like configuration in the superficial dermis (Fig. 15.15). Dilated ducts may have eosinophilic contents. Horn cysts can rarely be present. The stroma is dense and fibrosclerotic. In the clear cell variant, epithelial cell lining ducts have clear cytoplasm due to abundant glycogen (Fig. 15.16).

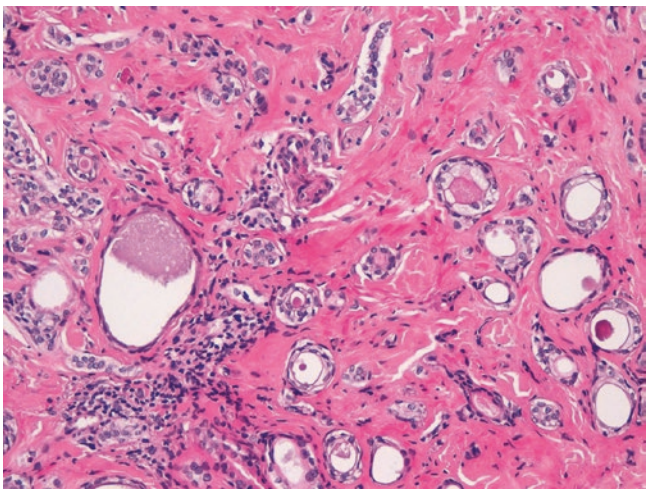

Fig. 15.16 Clear cell syringoma. The ductal structures are lined by clear cells

Immunohistochemistry Tumor cells are positive with CEA, EMA and cytokeratins.

Differential diagnosis Desmoplastic trichoepithelioma presents as a single lesion rather than multiple. It often shows multiple basaloid islands with signs of follicular differentiation, as well as superficial keratinizing horn cysts, and lacks true ductal differentiation. Syringoma should be also differentiated from Microcystic adnexal carcinoma, which often presents as a single larger lesion, and histopathologically as a more infiltrative tumor with common perineural invasion that extends into deep dermis and often subcutaneous tissue. Morpheaform BCC also enters in the differential diagnosis in superficial biopsies, clinically recognised as a single indurated lesion in which ductal differentiation, only very rarely present in BCC, and retraction of tumor cells from stroma are often seen.

Treatment and prognosis Excision is not necessary, unless for cosmetical purposes.

15.3.1.5 Hidradenoma

Definition Hidradenoma is a benign adnexal tumor showing either apocrine or eccrine differentiation. There are several synonyms for this tumor, including clear cell hidradenoma, nodular hidradenoma, solid–cystic hidradenoma, cystic hidradenoma, eccrine acrospiroma, eccrine sweat gland adenoma, clear cell myoepithelioma, poroid hidradenoma and apocrine hidradenoma.

Epidemiology Hidradenomas are sporadic tumors that generally develop in adults, with no sex predilection.

Clinical aspects Clinically, they appear as solitary dermal nodules, on the scalp and other locations.

Microscopy Hidradenoma appears as a well-circumscribed dermal tumor composed of solid and/or cystic areas (Fig. 15.17). Solid areas are composed of varying proportion of different cell types: glycogen-containing clear cells, poroid cells, squamoid cells and rare mucinous cells. Ducts are observed in solid areas. Cystic areas are lined by cuboidal cells, sometimes with evidence of apocrine differentiation. The stroma is often sclerotic.

Immunohistochemistry Glandular structures stain for CK7, CAM 5.2, EMA and CEA (lumina). A t(11;19) translocation that results in fusion of the mucoepidermoid carcinoma translocated 1 (*MECT1*) gene on 19p13 with the mastermind-like 2 (*MAML2*) gene on 11q21 [23].

Differential diagnosis Hidradenoma should be distinguished from metastatic clear renal cell carcinoma (which shows a prominent vascular pattern, CD10 and EMA positivity) and hidradenocarcinoma (more atypical cytology, infiltrative features and mitotic activity) [24].

Treatment and prognosis Excision is generally curative. Malignant transformation into hidradenocarcinoma is very rare.

15.3.1.6 Tubular Apocrine Adenoma

Definition Tubular apocrine adenoma is a rare benign adnexal neoplasm showing apocrine differentiation.

Epidemiology It occurs in a wide age distribution and females are more affected.

Clinical aspects Tubular apocrine adenoma may present as a solitary nodule on the scalp and face.

Microscopy Tubular apocrine adenoma is characterised by a dermal proliferation of variable-sized tubular structures, some of which cystically dilated or with papillary projections (Fig. 15.18). There is an outer layer of myoepithelial cells and a luminal (inner) layer with columnar cells showing decapitation secretion.

Immunohistochemistry Luminal cells are EMA+/CEA+ while myoepithelial cells are S100 protein+/SMA+.

Differential diagnosis Syringocystadenoma papilliferum (SP) shows connection with the epidermis and dense plasma cell-rich inflammatory infiltrate in the stroma; papillary eccrine adenoma lacks true apocrine differentiation; apocrine carcinoma (most often arising in the scalp) has more infiltrative features; the architecture can be papillary, tubular or solid. Pagetoid epidermal spread is present in a minority

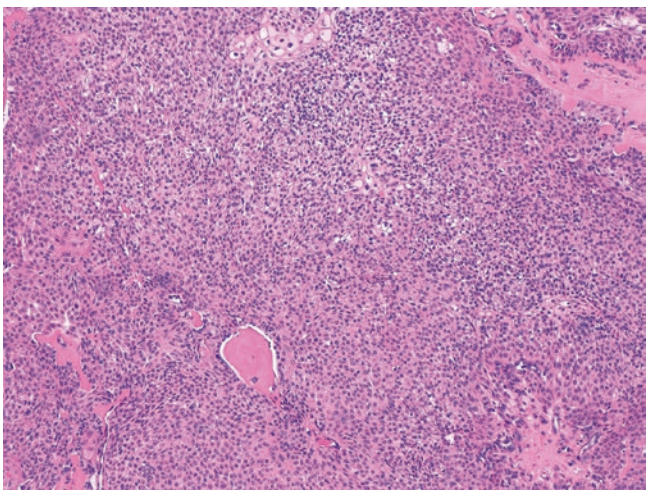

Fig. 15.17 Hidradenoma. Nodular and partly cystic proliferation composed of clear cells and cells with eosinophilic cytoplasm

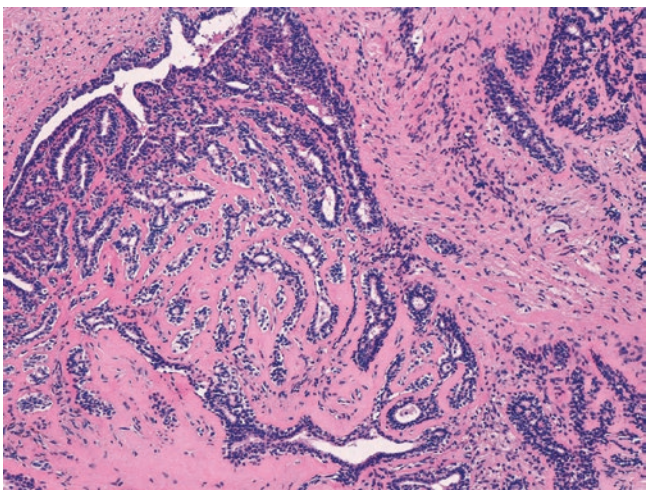

Fig. 15.18 Apocrine tubular adenoma. Well-differentiated tubular structures showing decapitation secretion embedded in a desmoplastic stroma

of cases. Tumor cells are large oval cells, sometimes with plentiful eosinophilic granular cytoplasm and pleomorphism, and numerous mitotic figures are present.

Treatment and prognosis Complete excision is curative. Prognosis is excellent.

15.3.1.7 Syringocystadenoma Papilliferum

Definition Syringocystadenoma papilliferum (SP) is a benign adnexal tumor that may occur in association with nevus sebaceous.

Epidemiology The tumor usually develops in childhood and adolescence; some cases may arise at birth. Association with nevus sebaceous occurs in 5–19 % of cases. The scalp is the most common location, followed by the face.

Clinical aspects Clinically, it presents as a warty papule, or as a solitary grey to dark brown plaque with papillomatous features.

Microscopy SP is characterised by epithelial invaginations that communicate with the overlying epidermis (Fig. 15.19). Invaginations form papillary structures that protrude into cystic spaces and are lined by squamous epithelium at the surface and by columnar epithelium with myoepithelial cells in the deeper portions. A heavy plasma cell infiltrate is evident in the stroma (Fig. 15.20).

Differential diagnosis SP in the head and neck region should be distinguished from tubular apocrine adenoma; they are similar in the dermal component, but the latter lacks the overlying epidermal attachments and the plasma cell-rich stroma. Syringocystadenocarcinoma papilliferum is very rare and recognised by the presence of increased nuclear atypia, multilayering of glandular epithelium and increased mitotic activity and may show considerable diversity. A pre-existing contiguous SP is generally found.

Treatment and prognosis Simple excision is curative, and prognosis is benign. Rare cases may have an associated BCC or other benign adnexal tumors (e.g. trichilemmoma, apocrine hidrocystoma). Rare cases may transform to syringocystadenocarcinoma papilliferum.

15.3.1.8 Cutaneous Mixed Tumor

Definition Cutaneous mixed tumor (CMT) is a benign adnexal tumor composed of epithelial elements in a chondromyxoid matrix. It may show either apocrine or eccrine differentiation [25].

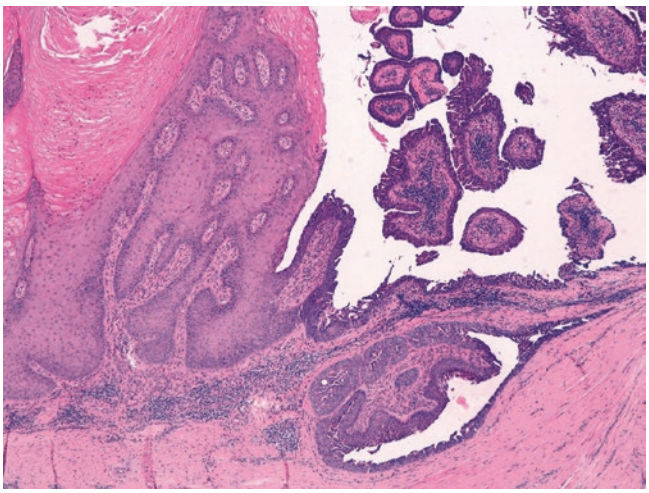

Fig. 15.19 Syringocystadenoma papilliferum. Duct-like structures protrude as papillary projections from the epidermis

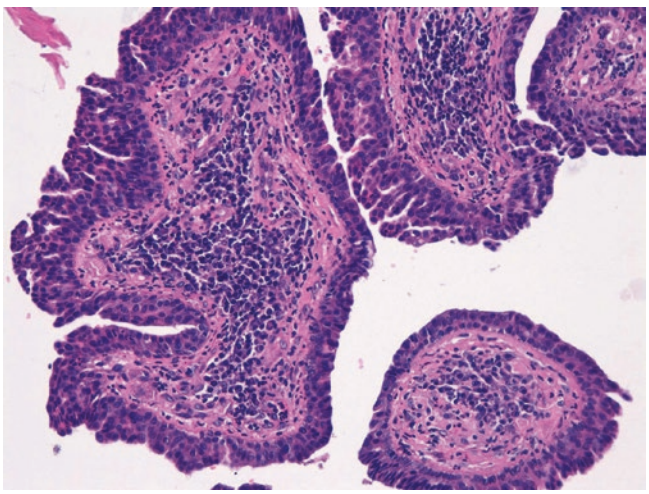

Fig. 15.20 Syringocystadenoma papilliferum. The stroma contains abundant plasma cells

Epidemiology CMT is a rare tumor that occurs in adult to elderly patients, with male predominance. Head and neck is the most common site.

Clinical aspects It appears as a skin-coloured, asymptomatic, slowly growing nodule of 1.0–3.0 cm in size.

Microscopy The tumor is composed by both epithelial and mesenchymal elements (Fig. 15.21). The epithelial component consists of double-layered ducts and solid islands of plasmacytoid, squamous or spindled cells. The epithelial elements are immersed in a chondromyxoid or hyalinised stroma (Fig. 15.22). The stroma stains strongly for alcian blue with hyaluronidase resistance.

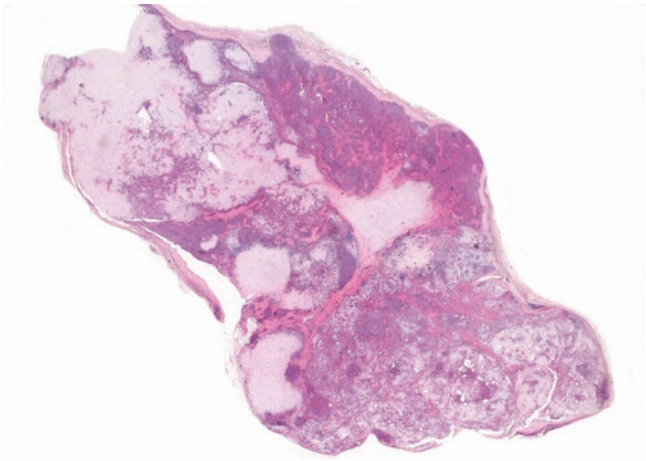

Fig. 15.21 Apocrine mixed tumour. Biphasic tumour showing ductal structures embedded in a chondro-myxoid stroma

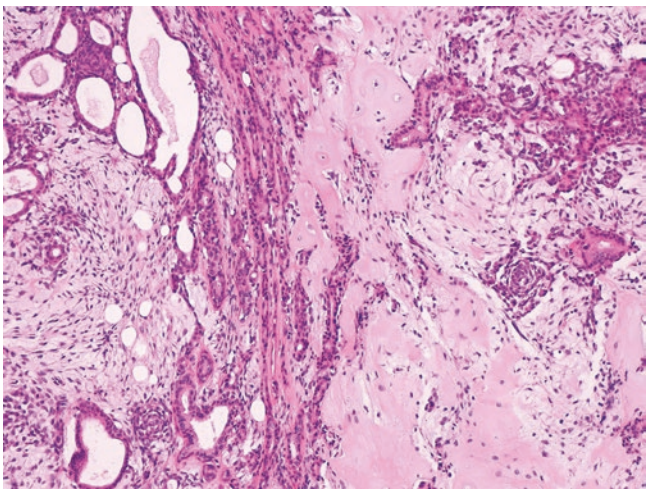

Fig. 15.22 Apocrine mixed tumour. The epitelial component shows signs of decapitation secretion. There is a prominent myxoid and chondroid matrix

Immunohistochemistry Ductal inner cells express cytokeratin, EMA and CEA; outer cells express S100 protein, smooth muscle markers.

Differential diagnosis Differential diagnosis includes myoepithelioma, which rarely occurs in the skin and is composed of a pure myoepithelial population (lacks epithelial elements with ductal differentiation of chondroid syringoma). CMT should also be distinguished from malignant mixed tumor (malignant chondroid syringoma) on the basis of cytological features and necrosis.

Treatment and prognosis Surgical excision is recommended; prognosis is excellent.

15.3.2 Malignant Sweat Gland Tumors

The distinction of primary adnexal carcinoma from metastatic adenocarcinoma in other sites (including breast and lung) can be challenging. A panel of immunohistochemical markers has been proposed, including CK 5/6, CK15, p63, D2-40, which have been reported to be preferentially expressed in the primary tumors [26–29]. The sensitivity and specificity in diagnosing primary cutaneous adnexal neoplasms were 91 % and 73 % for CK5/6 and 91 % and 100 % for p63, respectively [26, 27]. The sensitivity and specificity of gross cystic disease fluid protein-15 are both 99 % for metastatic breast carcinoma; however, this marker can be seen in both apocrine and eccrine carcinoma of the skin.

15.3.2.1 Porocarcinoma

Definition Porocarcinoma is a malignant adnexal tumor with ductal differentiation that shows both intraepidermal (in situ) and dermal components.

Etiology and pathogenesis It may arise from malignant transformation of long-standing poroma.

Epidemiology It is a rare tumor occurring in elderly patients, often located in the head and neck region and extremities. Both sexes are equally affected.

Clinical aspects Clinically, it appears as a cutaneous ulcerated nodule or a verrucous plaque.

Microscopy In situ and invasive porocarcinomas have been described. In situ porocarcinoma (10 % of all porocarcinomas) is a lesion confined to the epidermis, while invasive porocarcinoma is a dermal neoplasm with a solid–cystic architecture and infiltrative borders. Tumor cells show eosinophilic or clear cytoplasm and a variable grade of nuclear atypia; squamoid differentiation is common (Fig. 15.23). Duct formation and intracytoplasmic lumina are important features. Comedo-type necrosis is often seen. Lymphovascular invasion, intraepidermal pagetoid spread and connection with the epidermis (with intact basal layers) are helpful diagnostic clues.

Immunohistochemistry Ductal structures are EMA+ and CEA+. Tumor cells are usually diffusely positive for pancytokeratins, high molecular weight cytokeratins and p63.

Differential diagnosis Poroma may be difficult to distinguish from porocarcinoma (Figs. 15.24 and 15.25) and displays a bland cytology. Hidradenocarcinoma may have clear cell change, but squamoid features are less common than in

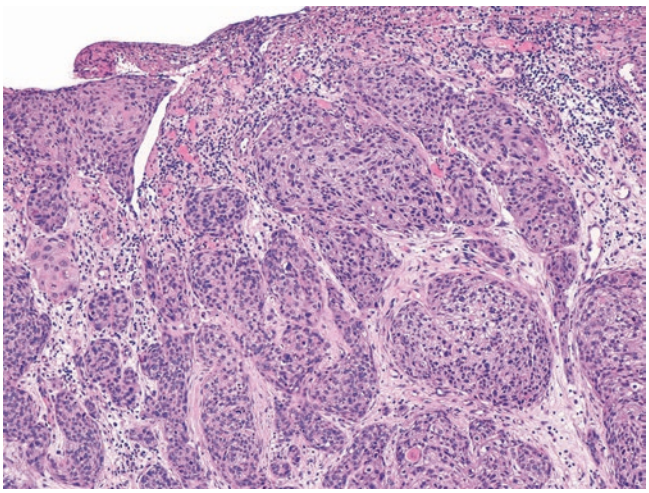

Fig. 15.23 Porocarcinoma. Ulcerated tumor showing broad aggregates of neoplastic cells with clear cytoplasm, squamous differentiation and focal ductal structures

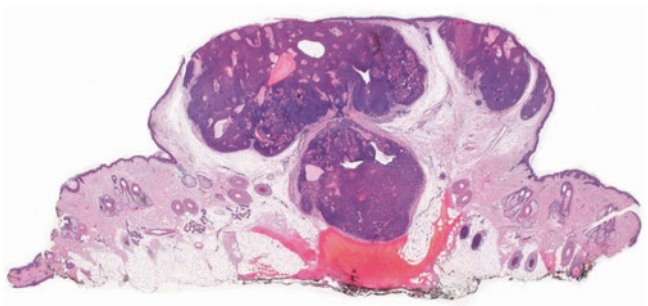

Fig. 15.24 Poroma. Multilobular proliferation of basaloid cells associated with ducts

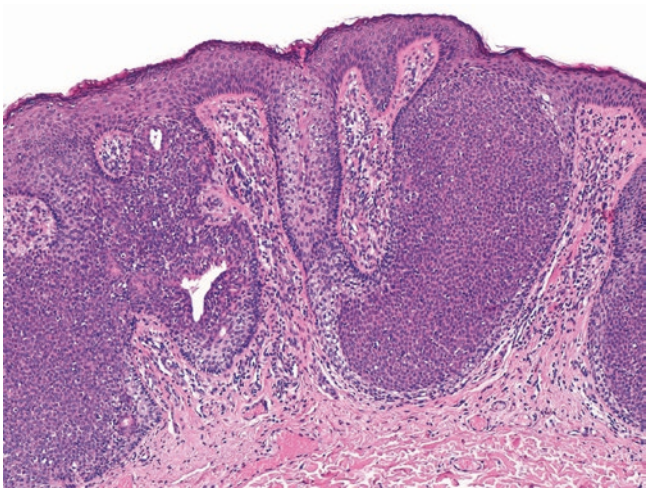

Fig. 15.25 Intraepidermal poroma. The tumor shows well-circumscribed islands of basaloid cells confined to the epidermis

poroid neoplasms. The most important differential diagnosis is SCC. Duct formation (highlighted by EMA and CEA positivity) helps in the correct identification of porocarcinoma.

Treatment and prognosis Complete surgical excision is recommended since the tumor may pursue an aggressive clinical course and a significant rate of metastasis (up to 40–50 %, usually to lymph nodes).

15.3.2.2 Malignant Hidradenoma

Definition Malignant hidradenoma (or malignant acrospiroma) is a malignant adnexal tumor arising from hidradenoma.

Epidemiology It arises more frequently in the head and neck region.

Clinical aspects Clinically, it appears as a cutaneous nodule.

Microscopy Histopathologically, it is characterised by dermal tumor aggregates composed of the same cell types seen in hidradenoma with tubular or ductal structures, in solid or cystic patterns. Necrosis may be evident, infiltrative growth, nuclear pleomorphism, and numerous mitoses are often present.

Immunohistochemistry Malignant hidradenomas are generally positive with p63 and cytokeratin 5/6. CEA and EMA highlight glandular/ductal foci.

Differential diagnosis Hidradenoma lacks high-grade atypia, necrosis, infiltrative features and atypical mitoses. The differential diagnosis includes tumors with clear cell features, including metastatic renal cell carcinoma and clear cell SCC.

Treatment and prognosis Wide local excision is necessary. Clinical course can be very aggressive, with local recurrences and distant metastases.

15.3.2.3 Eccrine Carcinoma

Definition Eccrine carcinoma is a malignant adnexal tumor with eccrine differentiation and ductal architecture.

Epidemiology Eccrine carcinoma is a very rare tumor usually present in middle-aged patients, more common in females. The scalp and face are common affected sites.

Clinical aspects It presents as a cutaneous plaque or nodule.

Microscopy Eccrine carcinoma is a dermal or hypodermal tumor with infiltrative growth composed of mildly atypical basaloid cells arranged in ductal structures with lumen formation. Occasional clear cells due to glycogen depositions may be observed. Perineural invasion is common.

Immunohistochemistry Lumina are highlighted by EMA and/or CEA. Tumor cells positive for high and low molecular weight cytokeratins may show focal immunoreactivity for S100 protein.

Differential diagnosis MAC has a similar growth pattern and is closely related to eccrine carcinoma. Keratinous cysts are generally present and help in its recognition.

Treatment and prognosis Wide excision is the optional treatment for a locally aggressive tumor that may develop rare lymph node metastasis.

15.3.2.4 Mucinous Carcinoma

Definition Primary cutaneous mucinous carcinoma (PCMC) is a rare malignant cutaneous tumor characterised by epithelial islands floating in mucin pools.

Epidemiology PCMC is very infrequently observed. Men are affected more than women, and it occurs in adults and elderly. It commonly arises on the face, with higher incidence on eyelids.

Clinical aspects Slow-growing, asymptomatic, solitary, reddish skin nodule

Microscopy The tumor is composed of nests and cords of epithelial cells suspended in large pools of PAS+, diastase-resistant, basophilic mucin in the dermis with occasional extension into subcutaneous tissues. Small glandular or tubular structures rarely occur.

Immunohistochemistry Tumor cells are generally CK5/6+/p63+/CK7+/CK20–, although p63-negative PCMC has been described. ER, PR and GCDFP-15 are often positive, but are not useful in the differential diagnosis with metastatic breast carcinoma.

Differential diagnosis PCMC should be differentiated from metastatic mucinous carcinoma mostly from breast and colon cancer, and extensive clinical work-up is necessary [30]. Positivity for CK5/6 and p63 (suggesting the presence of a myoepithelial component) favours primary over metastatic carcinoma. CK20 and CDX-2 staining favour metastasis from gastrointestinal tract carcinoma. Breast carcinoma shows significant morphological and immunohistochemical overlap.

Treatment and prognosis Wide excision and dissection of regional lymph nodes has been proposed. Up to 36 % of cases develop local recurrence and up to 15 % experience lymph nodes or distant metastases.

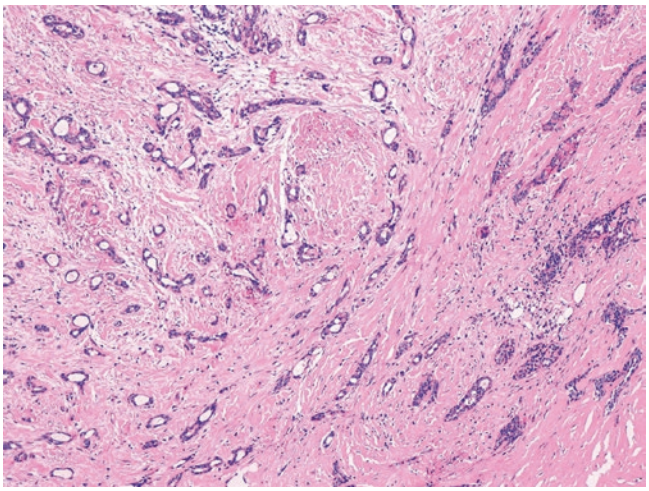

Fig. 15.26 Microcystic adnexal carcinoma. The tumor shows an infiltrating growth and is composed of strands of basaloid and squamous epithelium showing ductal differentiation

15.3.2.5 Microcystic Adnexal Carcinoma

Definition and synonyms Microcystic adnexal carcinoma (MAC) (known as sclerosing sweat duct carcinoma or syringoid carcinoma) is a low-grade malignant adnexal tumor with follicular and ductal differentiation.

Epidemiology MAC usually occurs in adults, more commonly in women, and usually affects the face and upper lip.

Etiology and pathogenesis Some cases may be related to solar damage or previous radiation therapy.

Clinical aspects Indurated, plaque-like or nodular lesion, typically measuring >1 cm in diameter

Microscopy There are numerous infundibular cysts as well as islands and strands of basaloid and squamous epithelium with a variable duct formation extending into the deep dermis, often subcutis (Fig. 15.26). In the deeper portions, strands of tumor cells are embedded in a dense fibrous stroma. Perineural invasion is a typical feature. The lining epithelium may look deceptively benign.

Immunohistochemistry Tumor cells are AE1/AE3+/CK7+/CK15+ [31]. P63 has a unique staining pattern of positive cells around the periphery of tumor nests with minimal staining in the tumor islands in contrast with the diffuse staining of p63 throughout BCC and desmoplastic trichoepithelioma [32]. Luminal cells are often positive for EMA and CEA. MAC has been reported as BER-EP4 negative [33]. However, Hoang et al. recently found that 38 % of MAC exhibited positivity for BER-EP4 [31].

Differential diagnosis Desmoplastic trichoepithelioma appears almost identical to the superficial portion of MAC. It

typically shows more basaloid-appearing nests and islands. It lacks deep subcutaneous and perineural invasion. Syringoma, usually superficial and noninfiltrative, has ductal structures that often show tadpole-like projections, lacks evidence of follicular differentiation (typical of MAC) and has no perineural invasion. Desmoplastic BCC shows infiltrative small nests and cords of atypical basaloid cells and shows multiple apoptotic and mitotic figures, which are absent in MAC. Focal mucinous stroma and tumor-stromal retraction artefacts may be present.

Treatment and prognosis Complete excision with clear margins is indicated. MAC is associated with a high incidence of local recurrence (up to 50%), but almost never metastasises distantly.

15.3.2.6 Adenoid Cystic Carcinoma

Definition Adenoid cystic carcinoma (ACC) is a rare malignant adnexal tumor showing prominent cribriform pattern and perineural invasion.

Epidemiology ACC typically occurs in adults.

Clinical aspects Clinically, it appears as a slow-growing nodule.

Microscopy It is a dermal tumor composed of lobules, islands and cords of basaloid cells with numerous cystic spaces and ductular structures, in a typical cribriform pattern, similar to that seen in adenoid cystic carcinoma of the salivary glands and breast. Perineural invasion is very common.

Immunohistochemistry ACC stains positive with EMA, CEA-M and cytokeratins. S100 protein, p63 and GFAP, and muscle markers including SMA and calponin often stain peripheral cells, consistent with myoepithelial cells.

Differential diagnosis Metastatic adenoid cystic carcinoma shows essentially identical histologic features.

Treatment and prognosis Upon excision, up to 70% of cases have been reported to recur (due to perineural invasion). Only a few cases have metastasised, usually to lymph nodes and lungs [34].

15.3.3 Benign Pilosebaceous Tumors

15.3.3.1 Tumor of the Follicular Infundibulum

Definition Tumor of the follicular infundibulum (TFI) is a benign adnexal follicular tumor characterised by a superficial plate-like proliferation with epidermal attachments.

Epidemiology It is a rare tumor occurring on the face and head and neck region of adult patients, more commonly in women [35].

Etiology and pathogenesis Some cases may be associated with nevus sebaceous. It may represent a mani festation of Cowden syndrome, in association with trichilemmomas.

Clinical aspects TFI clinically presents as a small, slow-growing skin papule.

Microscopy Microscopically, it is characterised by a superficial, plate-like proliferation of cytologically bland keratinocytes, with multiple anastomosing connections to the epidermis.

Differential diagnosis TFI may mimic superficial-type BCC, although in TFI tumor cells it has a pale to clear cytoplasm. TFI also lacks of cytologic atypia, and numerous mitoses and apoptosis are not observed.

Treatment and prognosis Surgical excision is curative.

15.3.3.2 Trichilemmoma

Definition Trichilemmoma (TL) is a benign follicular tumor with external root sheath differentiation.

Epidemiology TL is a relatively common tumor, affecting more often adults, although patients with Cowden syndrome present multiple trichilemmomas at an earlier age. Most occur on the face, especially nose and upper lip.

Etiology and pathogenesis Some authors have considered TL to be related to HPV infection. Some cases are associated with Cowden syndrome, characterised by multiple trichilemmomas, hamartomas and visceral tumors (including breast and thyroid carcinomas).

Clinical aspects TL appears as a small papule that may mimic BCC or VV.

Microscopy Histologically, the tumor is connected to the epidermis and shows lobules of squamoid cells that often show marked clear cell change due to abundant glycogen (PAS+, PASD−) (Fig. 15.27). There is peripheral palisading, with tumor cells resting on a thickened basement membrane. Desmoplastic trichilemmoma is a variant with prominent desmoplastic stroma (Fig. 15.28).

Immunohistochemistry Immunostaining with calretinin may be helpful in TL and other adnexal proliferations showing differentiation towards the innermost cell layer of the

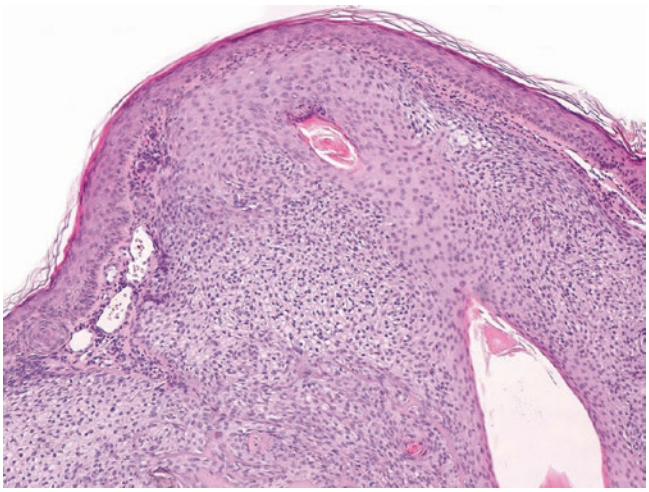

Fig. 15.27 Trichilemmoma. Lobules of clear cells in a tumor showing continuity with the follicular epithelium

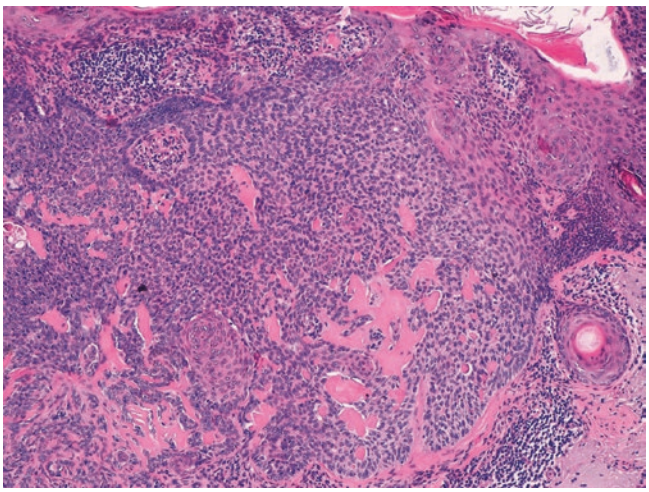

Fig. 15.28 Desmoplastic trichilemmoma. A thickened basement membrane surrounds the tumor

outer root sheath, as well as eccrine gland and sebaceous tumors while neoplasms with predominantly apocrine differentiation are negative [36].

Differential diagnosis TL should be distinguished from its malignant counterpart, tricholemmal carcinoma (TC), on the basis of cytologic atypia, mitotic activity, infiltrative features and presence of necrosis.

Treatment and prognosis TLs are benign tumors; prognosis is excellent.

15.3.3.3 Trichofolliculoma

Definition Trichofolliculoma (TF) is considered a hamartoma with prominent follicular differentiation.

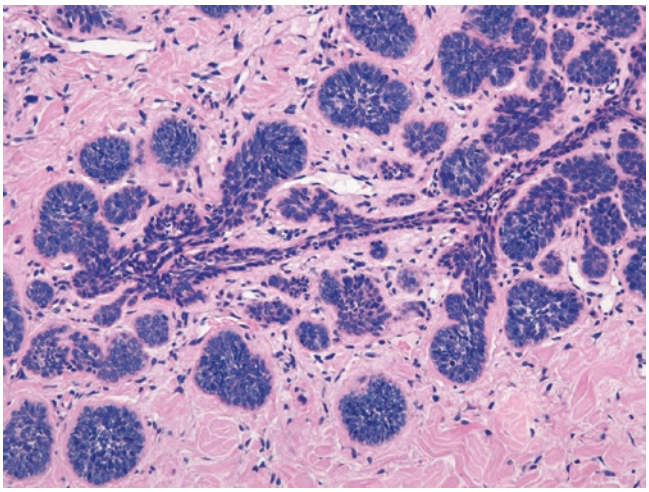

Fig. 15.29 Trichofolliculoma. Small follicles radiate at the periphery branching off the central follicle

Epidemiology TF is a rare tumor that usually presents in adults. The face is the most common location, especially around the nose, scalp and neck.

Clinical aspects It appears as a dome-shaped solitary papule with central dilated pore, from which a tuft of white hairs may protrude.

Microscopy Histologically, there is one or more central dilated hair follicle from which numerous smaller hair follicles of various degrees of maturity radiate (Fig. 15.29).

Differential diagnosis Dilated pore of Winer shows a cystically dilated follicle that communicates with overlying epidermis but lacks numerous primitive true follicular structures of trichofolliculoma. TE lacks central cystic space lined by keratinizing squamous epithelium.

Treatment and prognosis Simple excision is curative, but usually not necessary due to benign behaviour.

15.3.3.4 Trichoblastoma

Definition Trichoblastoma (TB) is a benign adnexal tumor showing primitive follicular differentiation.

Epidemiology It usually occurs in adults in the head and neck area, especially the scalp.

Clinical aspects Clinically it appears as a skin nodule, usually single but may rarely be multiple.

Microscopy TB shows a dermal proliferation of irregular cords or nests of basaloid cells, with no epidermal connections (Fig. 15.30). There is a typical fibrotic stroma with increased numbers of fibroblasts associated (Fig. 15.31).

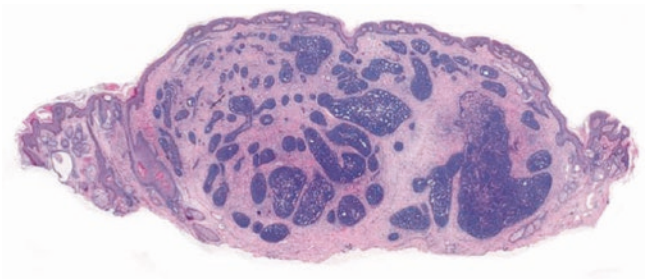

Fig. 15.30 Trichoblastoma. At low power, a large basaloid tumor showing no epidermal connection is observed

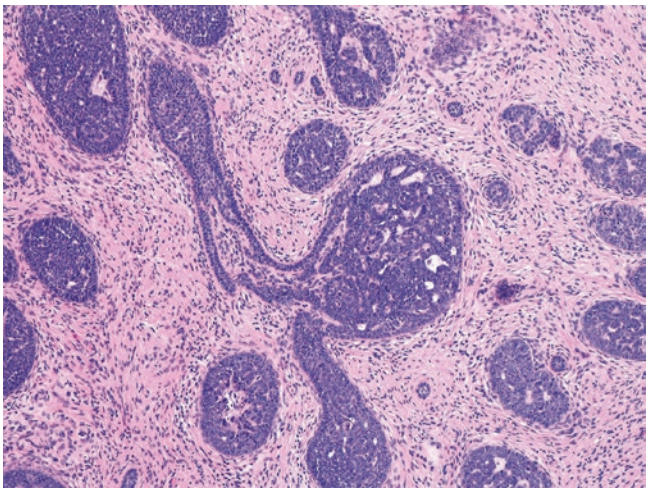

Fig. 15.31 Trichoblastoma. Basaloid cells are reminiscent of basal cell carcinoma but there are no retraction artefacts between the tumor and the stroma. The surrounding stroma is highly cellular and fibrocystic

Immunohistochemistry Immunohistochemistry may be useful in some cases (particularly in small, partial biopsies).

Expression of BCL-2, p53 and Ki-67 is generally elevated in BCC, while it should be low in TB. CK20 highlights Merkel cells in TB (they are generally absent in BCC).

Genetics In a minority of sporadic TB, mutations in the *CTNNB1* gene (*β-catenin* gene) and the *PTCH1* have been reported. Syndromic cases (Brooke–Spiegler syndrome) are associated with germ line mutations in the *CYLD* gene.

Differential diagnosis BCC usually shows multiple attachments to the overlying epidermis (focal or absent in TB), prominent peripheral palisading and mucinous stroma and tumor–stromal retraction artefacts (while stromal–stromal retraction seen in TB). Numerous mitotic and apoptotic figures are seen in BCC. TE shows overlapping histologic features, but is smaller, more superficial than TB and usually shows more prominent folliculocysts, calcifications and granulomatous inflammation.

Treatment and prognosis Complete excision is curative.

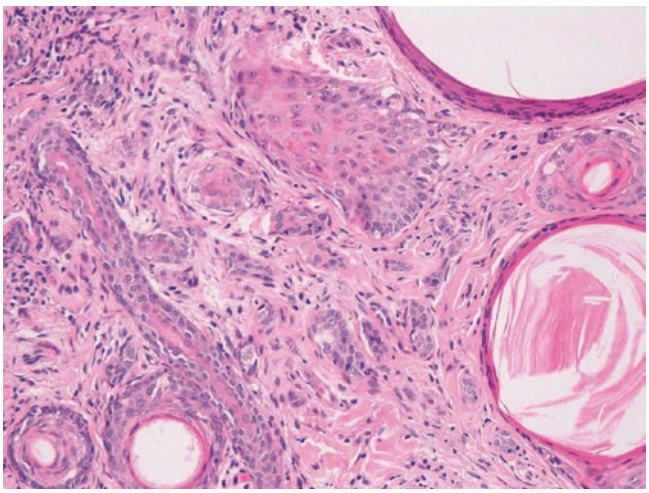

Fig. 15.32 Desmoplastic trichoepithelioma. There are cords and small nests of basaloid cell and horny cysts embedded in a desmoplastic stroma

15.3.3.5 Trichoepithelioma

Definition Trichoepithelioma (TE) is a benign follicular tumor with a mixture of primitive follicular structures and keratinizing infundibular microcysts.

Epidemiology TE is a relatively common tumor, diagnosed in adulthood. Genetic forms present in adolescence. Most commonly, it occurs on the face, but may involve other head and neck sites.

Etiology and pathogenesis Some cases are inherited as part of a genetic syndrome [37], including Brooke–Spiegler syndrome, inherited in an autosomal recessive fashion and characterised by the occurrence of spiradenomas, trichoepitheliomas and cylindromas. The tumors are generally benign but may become malignant. Affected individuals are also at increased risk of developing extracutaneous tumors, including tumors of the salivary glands.

Clinical aspects TE appears as solitary or multiple papules with a superficial indentation or dell.

Microscopy Superficially, there are keratinous cysts. There may be calcification and granulomatous inflammation. The tumor consists of lobules of basaloid cells with peripheral palisading and poorly formed hair follicles with papillary mesenchymal bodies. Tumor lobules are surrounded by a fibrocytic stroma. DT shows slender cords or small nests within a more fibrosclerotic stroma (Fig. 15.32).

Immunohistochemistry BCL-2, p53 and Ki-67 are highly expressed in BCC (low to negative in TE). Podoplanin is diffusely positive in TE (95.5 % of cases), while only 22.2 %

of BCC express this marker [38]. CK20 highlights Merkel cells in TE (absent in BCC).

Differential diagnosis BCC usually shows connections to the overlying epidermis, peripheral palisading, mucinous stroma, tumor–stroma retraction artefacts, numerous mitotic and apoptotic figures. TB is a larger, more nodular tumor that often extends into deep dermis/superficial subcutis, infundibulocystic differentiation more common in TE and calcifications and granulomatous inflammation also more typical of TE.

Treatment and prognosis Surgical excision is curative. Prognosis is excellent.

15.3.3.6 Pilomatrixoma

Definition Pilomatrixoma (pilomatricoma or calcifying epithelioma of Malherbe) is a benign follicular tumor derived from the matrical portion of a hair follicle.

Epidemiology It is relatively common in children; 30–50 % of cases are diagnosed before 30 years, but it may occur in any age, with no gender predilection. Head and neck and upper limbs are the most common locations.

Etiology and pathogenesis Mutation in the *CTNNB1* gene has been reported in both pilomatrixoma and pilomatrical carcinomas.

Clinical aspects Patients present with solitary, rarely multiple, slowly growing cystic or firm nodules 0.5–3 cm in diameter. Lesions are commonly skin coloured but may acquire a bluish to reddish discolouration.

Microscopy Pilomatrixoma is a dermal to subcutaneous tumor composed of two cell types: basaloid cells and shadow cells (Fig. 15.33). Basaloid cells are more peripherally located and have scant cytoplasm, indistinct cellular borders, slightly open chromatin and small nucleoli. Basaloid cells merge gradually or abruptly with keratinizing eosinophilic cells called shadow cells. Shadow cells have abundant eosinophilic cytoplasm and faint outlines of the nuclear contour in the centre where the nucleus used to be. Mitoses are easily identified. Calcifications are seen in about 75 % of tumors; ossification is seen in 15–20 % of cases. Foreign-body giant cell reaction surrounding tumor is common. Melanin may be present within tumor cells or melanophages. Focal areas of extramedullary haematopoiesis can rarely be present.

Immunohistochemistry Nuclear and cytoplasmic β-catenin staining is observed in matrical cells with loss of nuclear expression in shadow cells.

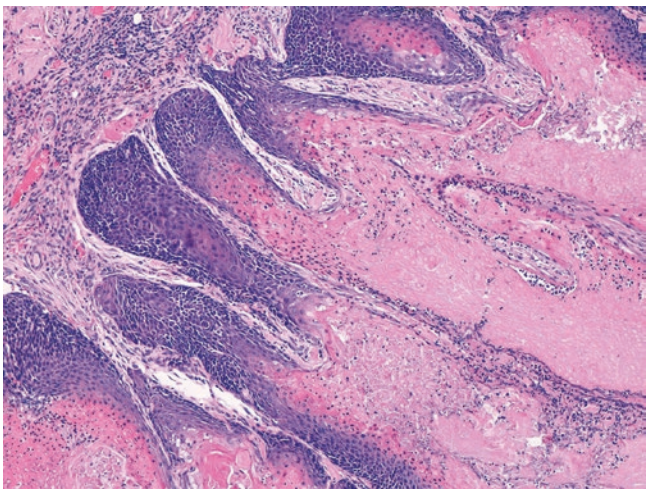

Fig. 15.33 Pilomatrixoma. Basophilic cells at the periphery and shadow cells are seen

Genetics The Wnt/β-catenin signalling pathway and BCL-2 oncoprotein are both implicated in the pathogenesis of pilomatrixoma. Activating mutations in the *CTNNB1* gene that encodes for β-catenin have been reported in sporadic pilomatrixoma.

Differential diagnosis Pilomatricoma should be distinguished from pilomatrical carcinoma, an often ulcerated tumor, asymmetrical, with infiltrative growth, areas of en masse necrosis, numerous mitoses and more cytological atypia.

Treatment and prognosis Simple excision is curative, with excellent prognosis. Malignant transformation to pilomatrical carcinoma is very rare.

15.3.3.7 Proliferating Pilar Tumor

Definition Proliferating pilar tumor (proliferating trichilemmal cyst or proliferating trichilemmal tumor) is a multicystic squamous neoplasm composed of mature keratinocytes lining keratin-filled spaces.

Epidemiology It is a rare tumor which occurs in older adults. It is much more common in females than males, typically occurs on scalp (90 % of cases).

Etiology and pathogenesis Most cases arise in pre-existing trichilemmal cyst.

Clinical aspects Clinically, it appears as a large cutaneous nodule.

Microscopy Microscopically, it is a multicystic, multilobulated nodular mass composed of dermal tumor with anastomosing cystic spaces with squamous lining (Figs. 15.34 and 15.35). Cysts are filled with abundant, dense, eosinophilic

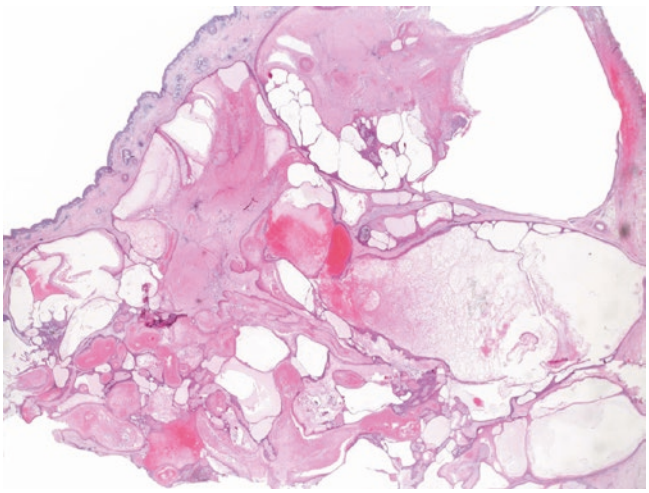

Fig. 15.34 Proliferating trichilemmal cyst. At low power, the tumor appears as a large lobular proliferation of squamous cells with prominent cystic areas

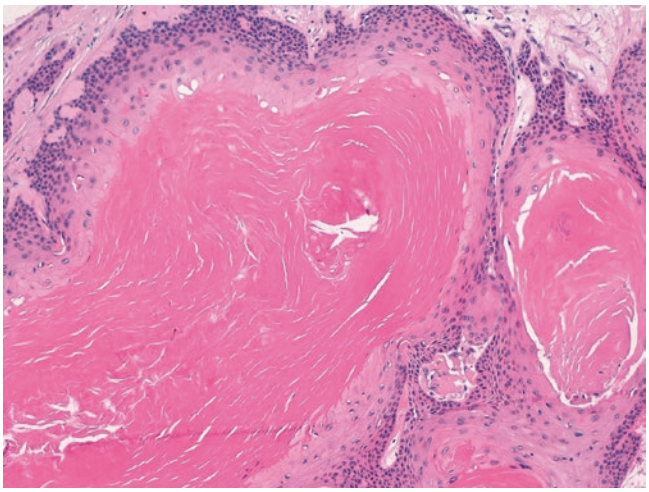

Fig. 15.35 Proliferating trichilemmal cyst. At higher magnification, areas of trichilemmal keratinization surrounded by squamous epithelium

keratin (similar to trichilemmal cysts). Occasional mitotic figures are present, but no high-grade atypia or increased mitotic activity should be present.

Differential diagnosis SCC may arise from proliferating pilar tumors. In this case, increased cytological atypia and mitotic figures should be seen.

Treatment and prognosis Proliferating pilar tumors should be treated with complete surgical excision in order to prevent recurrence and possible malignant transformation.

15.3.3.8 Sebaceous Adenoma

Definition Sebaceous adenoma (SA) is a benign adnexal tumor with sebaceous differentiation, composed mainly of mature sebocytes (more than 50 % of the tumor) and more than one layer of basaloid germinative cells at periphery.

Epidemiology SA occurs mostly as solitary lesions in adult patients on sun-damaged skin of the head and neck area. In case of multiple lesions, the possibility of a Muir–Torre syndrome (MTS) should be investigated. MTS is characterised by the association of sebaceous tumors (sebaceous adenoma, sebaceoma, sebaceous carcinoma) or multiple keratoacanthomas with internal malignancies. Loss of immunohistochemical staining with MSH2 and MLH1 (or possibly MLH6) in tumor tissues and/or presence of microsatellite instability is supportive of hereditary germ line mutation.

Clinical aspects Often on the head and neck, but any site possible, SA appears as a pale or yellow papule often covered by scale or crust.

Microscopy In SA, there are lobular aggregations of mature sebocytes, characterised by abundant, finely vacuolated cytoplasm and central ovoid nucleus that outnumber basaloid undifferentiated germinative cells (Fig. 15.36). SA often shows connection to the overlying epidermis.

Immunohistochemistry In fresh, frozen sections, Oil Red O and Sudan Black can be used to detect intracytoplasmic lipids of sebaceous lesions. Specific immunohistochemical markers for formalin-fixed and paraffin-embedded tissues are limited. Recently, antibodies to adipophilin were used to identify sebaceous differentiation [39]. Mature sebocytes are EMA+/adipophilin+/CK7+, while germinative cells are EMA−/adipophilin−/CK7−/AR+/SOX-9+.

Genetics Sebaceous tumors, including SA, have inactivating mutations in *LEF1* (lymphocyte-enhancing factor 1), a tumor suppressor gene involved in the Wnt pathway.

Differential diagnosis SA should be differentiated from sebaceous hyperplasia in which sebaceous lobules are arranged around a central placed follicular infundibulum connected to the epidermis. Sebaceoma is composed of more than 50 % germinative basaloid cells and less than 50 % mature sebocytes. Sebocytes and germinative/basaloid cells often arranged haphazardly may or may not connect to epidermis. Sebaceous carcinoma is often a larger, infiltrative tumor in which basaloid/germinative cells generally predominate and shows prominent cytologic atypia and numerous mitoses.

Treatment and prognosis SA is a benign tumor.

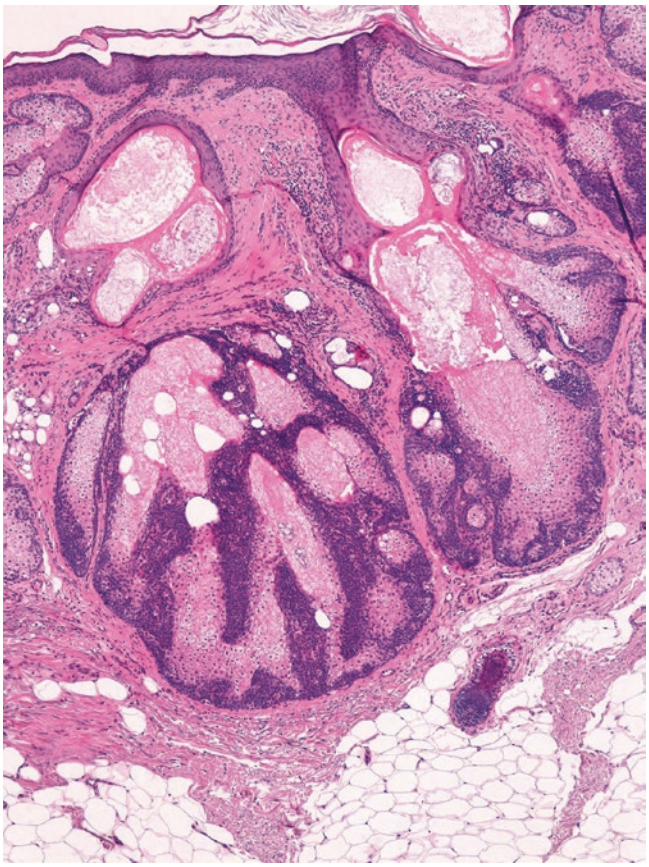

Fig. 15.36 Sebaceous adenoma. Lobules of mature sebocytes surrounded at the periphery by small basaloid immature sebocytes

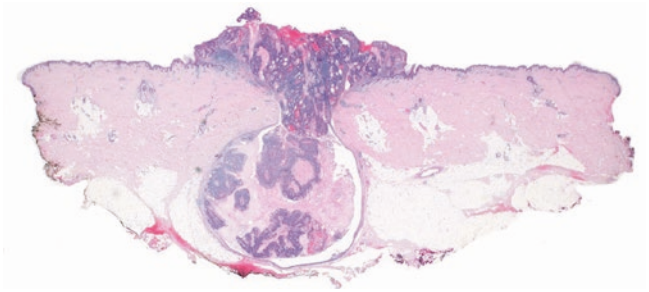

Fig. 15.37 Sebaceoma. The tumor basaloid cells with random admixture with mature sebaceous cells. The deeper portion shows cystic dilation containing debris of holocrine degeneration

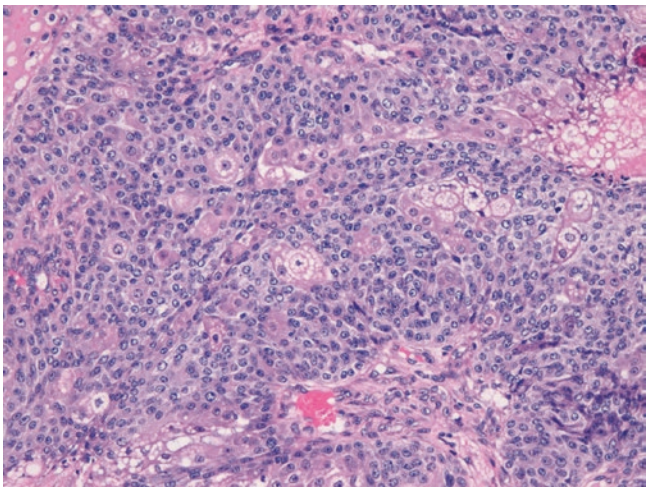

Fig. 15.38 Sebaceoma. At higher magnification, sebocytes of different degrees of maturity do not display cytological atypia

15.3.3.9 Sebaceoma

Definition Sebaceoma is a benign proliferation of mature sebaceous cells associated with a predominant basaloid germinative cell population.

Epidemiology It is a rare tumor, typically occurring in adults on the face, but may also present on trunk.

Some cases are part of Muir–Torre syndrome.

Clinical aspects Clinically, it appears as a slow-growing, skin-coloured to yellowish papule or nodule. It is a solitary lesion but may be multiple, especially in Muir–Torre syndrome.

Microscopy Histologically, there is a greater proportion of basaloid germinative cells admixed with mature sebocytes, and there may be mitoses and mild atypia (Figs. 15.37 and 15.38). Cyst formation is common. Rippled pattern sebaceoma is a rare variant showing palisading of basaloid nuclei.

Immunohistochemistry EMA is usually positive in mature sebocytes.

Differential diagnosis The differential diagnosis includes BCC with sebaceous differentiation, sebaceous carcinoma and sebaceous adenoma.

Treatment and prognosis Complete conservative excision is curative.

15.3.4 Malignant Pilosebaceous Tumors

15.3.4.1 Trichilemmal Carcinoma

Definition Trichilemmal carcinoma is a low-grade malignant adnexal tumor characterised by clear-staining squamous cells with tricholemmal/outer root sheath differentiation.

Epidemiology The tumor occurs in elderly patients, with no gender predilection. It most often presents in head and neck region.

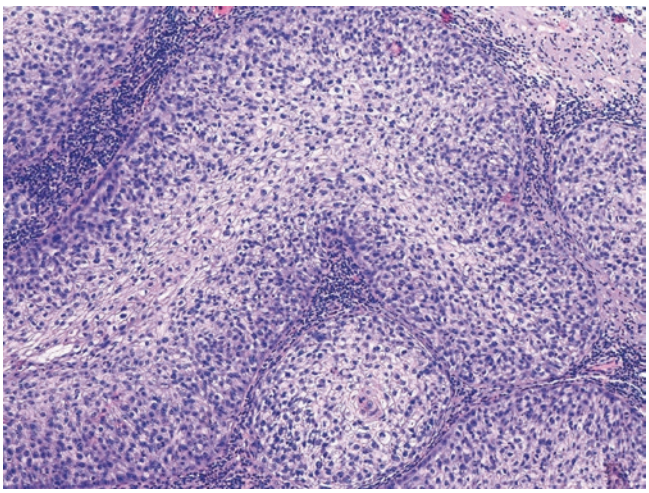

Fig. 15.39 Trichilemmal carcinoma. Multilobulated malignant infiltrating tumor showing signs of outer root differentiation

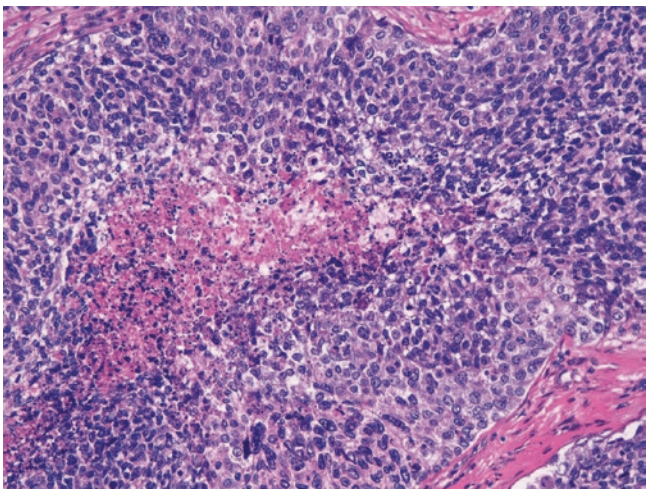

Fig. 15.40 Sebaceous carcinoma. Malignant necrotising tumor displaying signs of sebaceous differentiation, as vacuolated or foamy cytoplasm

Etiology and pathogenesis Trichilemmal carcinoma has been associated to ultraviolet light exposure, previous radiation or immunosuppression.

Clinical aspects Clinically, it appears as a papule or nodule. It may be ulcerated.

Microscopy Trichilemmal carcinoma is characterised by lobules or atypical clear cells (Fig. 15.39) which may show connections to the epidermis and/or hair follicles [40]. Neoplastic cells show an abundant clear cytoplasm, a hyperchromatic nucleus and prominent nucleoli. Some cells may show prominent keratohyalin granules. Peripheral palisading at the periphery of the lobules and thickened basement membrane are often seen. Mitotic figures are usually abundant; necrosis may be present.

Differential diagnosis Trichilemmoma is usually smaller and more superficial; it lacks significant cytologic atypia and mitotic activity. Conventional clear cell SCC does not show tricholemmal differentiation, peripheral palisading or thickened basement membrane. Sebaceous carcinoma cells typically show abundant multivacuolated cytoplasm with nuclear indentations, unlike the uniformly clear-staining cytoplasm of trichilemmal carcinoma.

Treatment and prognosis Complete excision should be performed. Local recurrences have been described but no confirmed metastatic cases.

15.3.4.2 Sebaceous Carcinoma

Definition Sebaceous carcinoma (SC) is a malignant adnexal tumor demonstrating sebaceous differentiation.

Epidemiology Most tumors occur in middle-aged to elderly individuals, with female preponderance. Eyelids are by far the most common site (75 % of cases). The remainder of cases occurs in other head and neck sites, followed by the trunk and extremities.

Etiology and pathogenesis Some cases are related to solar UV exposure. SC may be encountered in the context of Muir–Torre syndrome in patients who have multiple sebaceous tumors and/or multiple keratoacanthoma and internal organ malignancies.

Clinical aspects Clinically, they are nodular, firm lesions, often ulcerated, usually 1–4 cm in diameter.

Microscopy The tumor extends in the dermis and consists of lobules of variably atypical clear cells containing abundant cytoplasmic lipid, often producing multiple vacuoles and nuclear indentation (Fig. 15.40). Nuclei are enlarged and vesicular or hyperchromatic staining, with prominent nucleoli. Depending upon the degree of differentiation, the identification of sebaceous differentiation can be difficult. Moderately and poorly differentiated tumors show few to rare clear cells and may be composed predominantly of basaloid or squamoid cells. Prominent cytologic atypia and pleomorphism and mitotic figures, including atypical forms, are usually seen. Areas of necrosis, with comedo pattern, are common. Lymphovascular invasion is frequently seen. Pagetoid involvement of epidermis may be seen in up to 30 % of cases.

Immunohistochemistry Adipophilin was positive in a membranous pattern in all sebaceous tumors, including poorly differentiated sebaceous carcinoma [39]. In compari-

son to SA, sebaceoma and sebaceous hyperplasia, SC shows significant higher levels of p53 and Ki-67 and lower levels of BCL-2 and p21 [41]. EMA is positive in most well-differentiated SC but does not highlight ductal structures, unlike other adnexal carcinomas (like porocarcinoma and hidradenocarcinoma). Androgen receptors (AR) are positive (nuclear staining) in most cases, including poorly differentiated carcinomas. SCC and most other primary cutaneous carcinomas are negative for AR. However, AR is often positive in BCC (>60 % of cases) and some metastatic carcinomas to the skin. High molecular weight cytokeratins (CK5/6 and 34βE12) and p63 are typically strongly and diffusely positive. D2-40 is positive in a subset of cases, especially in more basaloid sebaceous carcinomas. Other markers that may be positive include CAM5.2, BER-EP4, CK7 (~50 % of cases) and CD10 (~50 %).

Differential diagnosis SCC with clear cell features can be difficult to distinguish from SC. Some cases of BCC are predominantly clear cell, but typically show at least focal areas of more conventional BCC with peripheral palisading and mucinous stroma. With respect to the clear cell BCC and SCC, the cytoplasmic vacuolation is due to glycogen; therefore, a PAS stain with and without diastase will demonstrate the abundant globules of PAS+ material. Other cutaneous adnexal carcinomas, like porocarcinoma and hidradenocarcinoma with clear cell features, may enter the differential diagnosis. Porocarcinoma shows multiple epidermal attachments, whereas hidradenocarcinoma is dermal-based tumor typically lacking epidermal connections. Both tumors show at least focal ductal differentiation (rare in SC).

Treatment and prognosis Complete excision is necessary to ensure local removal. Sentinel lymph node biopsy may be useful for staging purposes. Aggressive tumors are associated with high incidence of metastasis (>30 % of cases) and generally poor prognosis unless discovered early.

15.4 Primary Neuroendocrine Carcinoma of the Skin

Definition Primary neuroendocrine carcinoma of the skin (PNCS) (previously known as Merkel cell carcinoma, primary small cell carcinoma of skin or trabecular carcinoma) is thought to represent a malignant transformation of a primitive pluripotential (stem) cell that can differentiate along multiple different lines, rather than a specific neuroendocrine cell.

Epidemiology PNCS is a rare tumor typically observed on sun-damaged skin (usually head and neck or extremities) of Caucasian middle-aged to elderly patients, with a predilection for male gender.

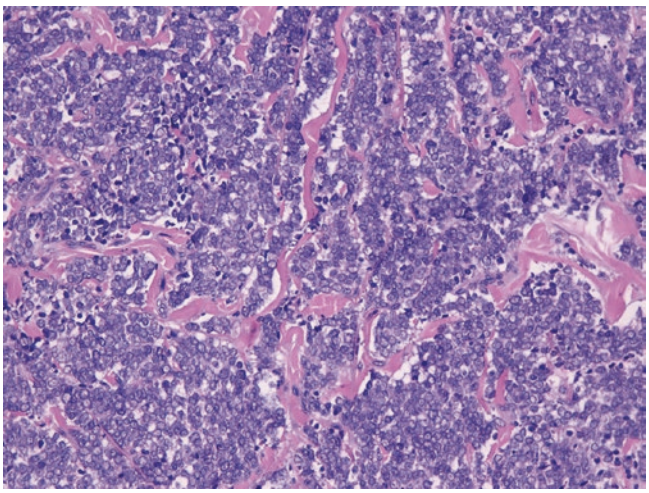

Fig. 15.41 Merkel cell carcinoma. Aggregates of small, hyperchromatic cells

Etiology and pathogenesis Recent studies have shown that a strong link to infection with polyomavirus and Merkel cell polyoma virus infection is found in up to 90 % of cases.

Clinical aspects It appears as a rapidly enlarging erythematous to violaceous nodule or plaque; it may show extensive ulceration and/or become hemorrhagic.

Microscopy PNCS is generally a dermal tumor composed of infiltrative cords, trabeculae and sheetlike areas of hyperchromatic cells with scant amphophilic cytoplasm, oval to roundish nuclei, evenly dispersed chromatin and indistinct nucleoli (Fig. 15.41). Nuclear clearing is a distinctive feature often seen. Numerous apoptotic bodies and mitotic figures are observed. Nuclear crush artefact and streaming may be seen, similar to small cell carcinoma. Although a grenz zone is usually present, epidermal (pagetoid) involvement is seen in 10–20 % of cases. Angiolymphatic invasion is identified in approximately 20 % of cases. Associated dermal desmoplasia may be present. Areas of squamoid, adnexal (Figs. 15.42, 15.43 and 15.44) or, more rarely, melanocytic differentiation may be present. The association between PNCS and SCC has been well described.

Immunohistochemistry PNCS is positive for cytokeratins and CK20 shows a typical perinuclear dot-like staining. EMA immunostaining is observed in 75 % of cases. CD56 is the most sensitive neuroendocrine marker, while chromogranin and neurofilaments positivity is seen only in 33 % of cases. CD99 can be detected in 55 % of cases [42].

Genetics Trisomy 6 has been demonstrated in up to 50 % of cases.

Differential diagnosis The differential diagnosis of PNCS is broad, and correct identification relies on clinical

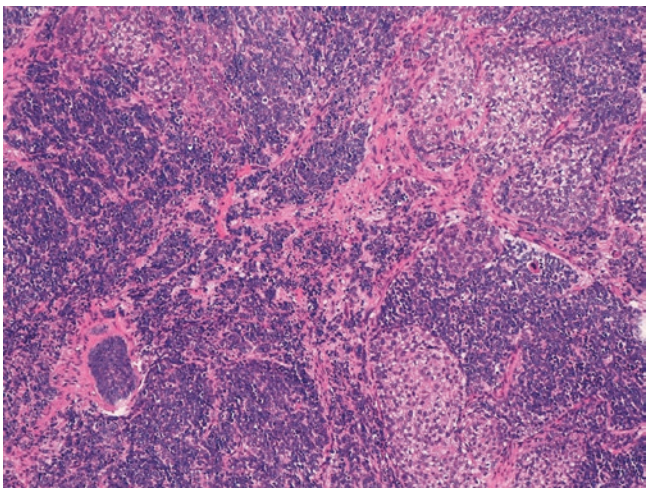

Fig. 15.42 Merkel cell carcinoma, divergent differentiation. In this case, there are also aggregates of squamoid, clear cells morphologically distinct from the classical neuroendocrine component

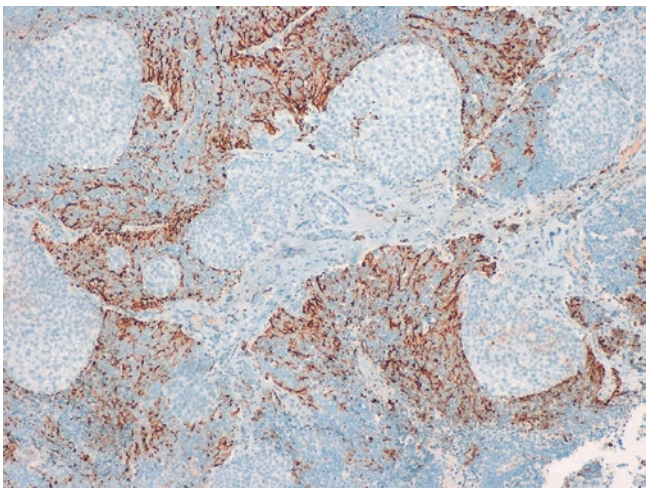

Fig. 15.43 Merkel cell carcinoma, divergent differentiation. Immunohistochemical stain with anti-cytokeratin 20

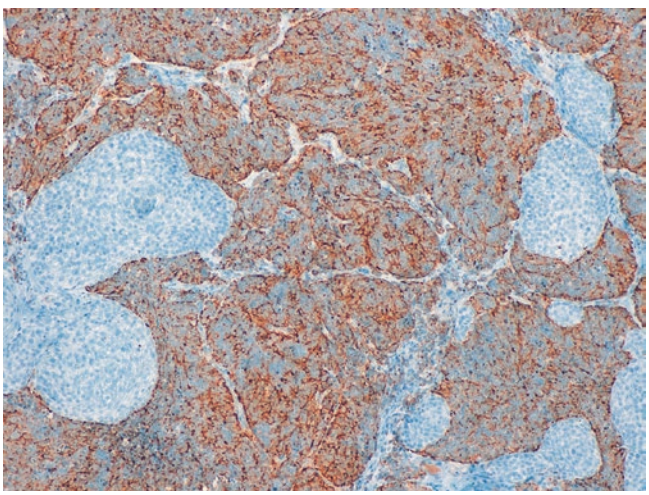

Fig. 15.44 Merkel cell carcinoma, divergent differentiation. Immunohistochemical stain with anti-chromogranin-A

history and immunohistochemical studies. It includes BCC (almost always positive for EpCAM/BER-EP4, but negative for CK20, chromogranin-A and synaptophysin), small cell SCC, small cell melanoma (S100 protein, HMB45, MART-1/melan-A typically positive; negative for cytokeratins, CK20 and neuroendocrine markers) and lymphoma. Metastatic neuroendocrine carcinoma from visceral sites (especially lungs, generally TTF-1+, while PNCS is TTF-1–) and small round blue cell tumors (neuroblastoma, Ewing/primitive neuroectodermal tumor (PNET), rhabdomyosarcoma) should be also ruled out on the basis of clinical history and immunohistochemistry.

Treatment and prognosis Treatment is based on complete surgical excision. Sentinel lymph node biopsy may be considered for selected patients, although SLN positivity does not seem to be very reliable for regional lymph node involvement, especially in the head and neck region, as many patients progress to distant metastases. Radiotherapy is used to minimise regional disease, while chemotherapy may be offered for distant metastatic disease. PNCS are aggressive tumors with 25–30 % of local recurrences, 52–59 % of regional diseases and 34–36 % of distant metastases. Worse prognosis is associated with advanced age, head and neck location, large size and immunosuppression.

15.5 Conventional Cutaneous Melanocytic Lesions

15.5.1 Lentigo Simplex and Labial Melanotic Macule

Definition Lentigo simplex or simple lentigo and labial melanotic macules are homogeneously pigmented and sharply demarcated macules characterised by hyperpigmentation of the epidermal/epithelial basal layer.

Epidemiology Both lentigo simplex and labial melanotic macule can be solitary or multiple. Lentigo simplex frequently develops in childhood, but it is more conspicuous in the adult life. It is frequently found in the face [43]. Melanotic macule usually affects older patients and arises in the lower vermilion border of the lip, rarely in the oral mucosa [44]. It is more common in women. A higher incidence of this lesion in patients with human immunodeficiency virus infection has been described.

Etiology and pathogenesis Rare cases of multiple lentigines in the face, lips and oral mucosa are a hallmark systemic diseases. Most of them are inherited, with an autosomal dominant pattern, such as Peutz–Jeghers syndrome, centrofacial lentiginosis, LEOPARD and Carney complex [45], as well as acquired conditions, such as Laugier–Hunziker

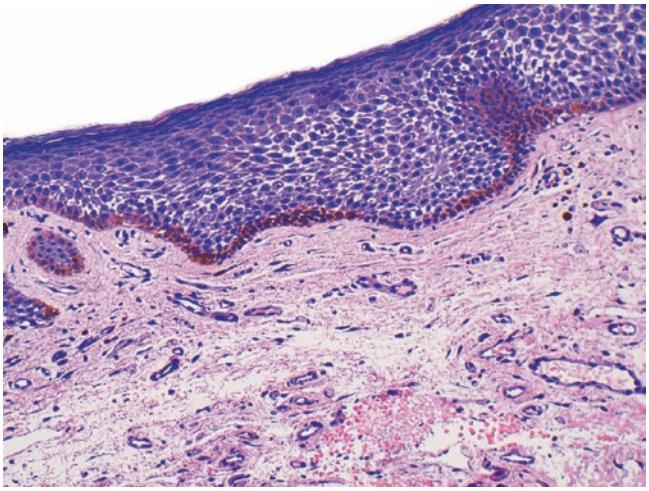

Fig. 15.45 Melanotic macule. There is a hyperpigmentation of the basal layer of the epithelium, which is acanthotic and flat

syndrome, characterised by multiple macular pigmentation of the lips and oral cavity.

Clinical aspects Lentigo simplex or simple lentigo presents as a small, homogeneously pigmented and well-demarcated macule measuring few millimetres [43]. Labial melanotic macule has a similar appearance, affects the lip and can measure up to 15 mm in diameter [44].

Microscopy In lentigo simplex, there is hyperpigmentation of the epidermal basal layer, often with a slight increase of melanocyte number. The epidermis is frequently acanthotic, with elongation of the rete ridges. Sometimes, a slight inflammatory component with presence of melanophages is seen in the superficial dermis. Melanotic macules have similar histological characteristics to lentigo simplex, but the epithelium is usually slightly acanthotic and flat (Fig. 15.45).

Genetics Lentigo simplex fails to show *BRAF*V600E mutations, in contrast with melanocytic nevi and actinic lentigo [46].

Differential diagnosis Differential diagnoses must be performed, mainly with actinic lentigo and lentiginous melanocytic nevi.

Treatment and prognosis Lesion excision is recommended only for cosmetic purposes because neither lentigo simplex nor labial melanotic macule has malignant potential.

15.5.2 Actinic Lentigo

Definition Actinic lentigo, also called senile lentigo, is a pigmented macule in sun-damaged skin, characterised by hyperpigmentation of basal layer of the epidermis [43].

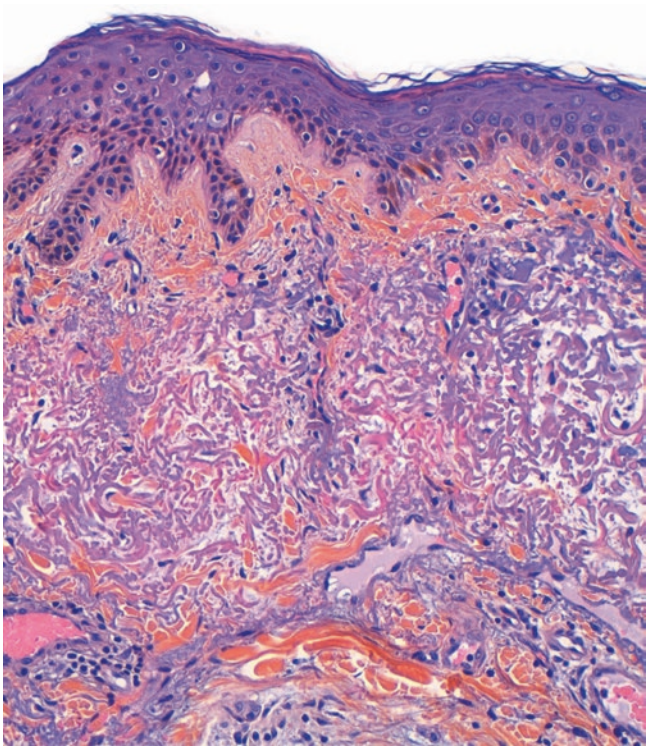

Fig. 15.46 Actinic lentigo. The epidermis shows an elongation of the rete ridges and a hyperpigmentation of the basal layer. There is also a slight increase of the melanocyte number. Note the marked actinic elastosis in dermis

Epidemiology It is frequent in the face of the middle aged or elderly [47].

Etiology and pathogenesis Its development is attributed to high sun exposure. One study using microarrays analysis has shown upregulation of genes related to chronic inflammation, fatty-acid metabolism and melanocytes, suggesting that actinic lentigo is induced by the mutagenic effect of repeated ultraviolet light exposures in the past [48].

Clinical aspects The lesion usually presents as an irregularly pigmented macule. It is often multiple, measuring from 1 to 10 mm or more in diameter.

Microscopy Histologically, there is an elongation of the rete ridges and a hyperpigmentation of the basal layer (Fig. 15.46), usually with a slight increase of the melanocyte number.

Treatment and prognosis The lesion excision is usually performed for cosmetic reasons. Actinic lentigo can enlarge slowly over many years, and some cases can evolve into a reticulate seborrheic keratosis, with a verrucous surface. Some cases may develop a lichenoid keratosis with a marked inflammatory dermal infiltrate and regression of the lesion. Evolution to a lentigo maligna has also been documented.

15.5.3 Melanocytic Nevi

Definition Melanocytic nevi or banal nevi are benign proliferations of melanocytes which are also called nevocytes when they form a nevus.

Epidemiology Most of them are acquired and appear in childhood and adolescence, although some are congenital and are present at birth. They can increase in number during second or third decades but later usually involute. There is not a gender predilection. Melanocytic nevi usually affect Caucasians, especially individuals with pale skin and light-coloured eyes. Ancient nevus is frequently found in the face of the elderly.

Etiology and pathogenesis Sun exposure in childhood predisposes to the development of nevi [49].

Clinical aspects The clinical aspect of melanocytic nevi can change according to the evolution of the lesion. Initially, in the junctional stage, the nevus is a flat, pigmented lesion, well demarcated. Later, when corresponding to a compound nevus, the lesion becomes more elevated and acquires a dark brown colour, sometimes with black dots. Finally, in the purely dermal nevus stage, the lesion will become a dome-shaped nodule, a papillomatous lesion or a pedunculated skin tag.

Microscopy The junctional nevus is composed of discrete nests of melanocytes at the dermo-epidermal junction, usually located on the rete ridges. The cells are cuboidal or oval in shape, containing a variable amount of melanin pigment. Nuclei may have a prominent nucleoli in the initial lesion. Mitoses are rare or absent. In dermis, there can be a lymphohistiocytic infiltrate and some melanophages.

The lentiginous melanocytic nevus subtype has a lentiginous pattern of growth, characterised by single melanocytes which display in the dermo-epidermal junction along with elongated rete ridges.

The compound melanocytic nevus has both junctional nests and an intradermal component of nevus cells. The cells in the upper dermis are cuboidal, larger than those located in the lower parts, and can have melanin pigment in the cytoplasm. The nevus cells are arranged in orderly nests and cords. The overlying epidermis may be flat or can show some hyperplastic, papillomatous changes.

In intradermal nevus, nevus cells are located in the dermis and arranged in nests and cords (Fig. 15.47). Cells are cuboidal and usually small. It is frequent to find nuclear pseudoinclusions, which are intranuclear cytoplasmic invaginations. Multinucleated cells may be seen (Fig. 15.48). Some cases may have neuroid differentiation (neurotized melanocytic nevus), with spindle shape and structures resembling Meissner's tactile body. Ancient nevus usually shows fibrotic changes (Fig. 15.49). Cytological pleomorphic and hyperchromatic nuclei may be seen (Fig. 15.50) [50]. The related hair follicle in dermal melanocytic nevi may sometimes become dilated, cystic and inflamed. Meyerson's nevus

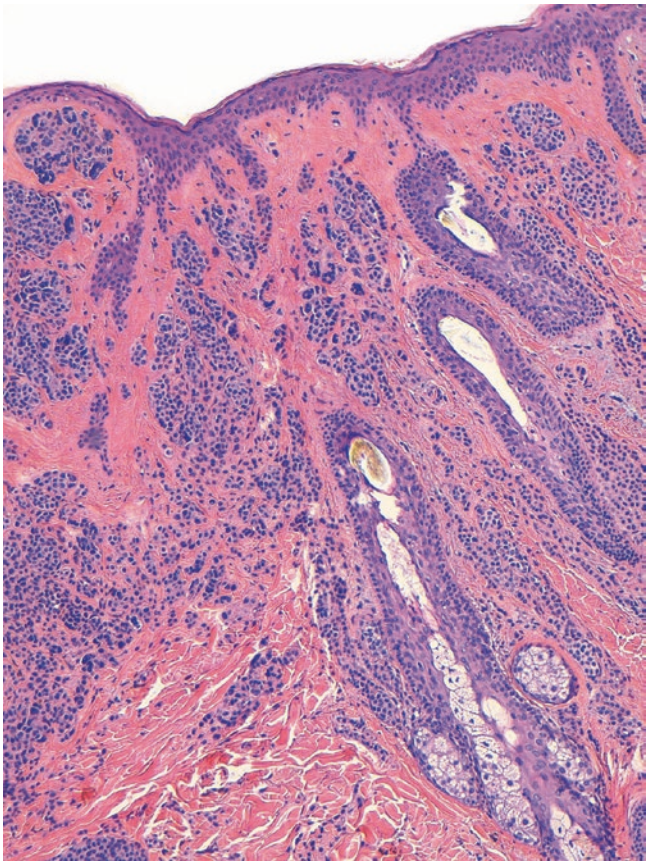

Fig. 15.47 Intradermal melanocytic nevus. Nevus cells are located in dermis and are arranged in nests and cords

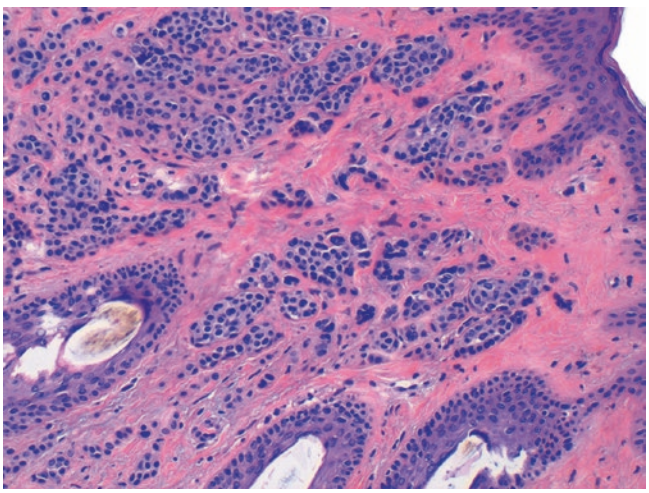

Fig. 15.48 Intradermal melanocytic nevus at a higher magnification. Nevus cells are small, cuboidal. Some multinucleated cells can be seen. Some cells have nuclear pseudoinclusions, which are intranuclear cytoplasmic invaginations

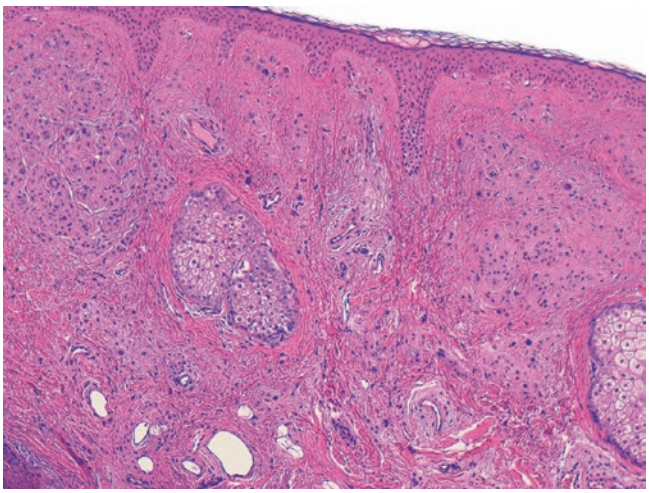

Fig. 15.49 Ancient nevus. There is a markedly sclerotic stroma

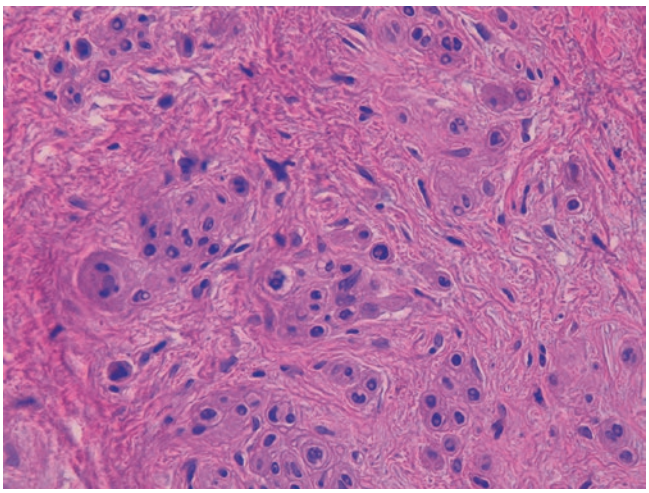

Fig. 15.50 Ancient nevus. Nevus cells show hyperchromatic, pleomorphic nuclei

is a melanocytic nevus with marked spongiosis of the epidermis due to a concurrent skin inflammation and eczema. In halo nevus or Sutton nevus, marked inflammation due to an immune response with predominant T-lymphocytes and consequent regression features are characteristic (Fig. 15.51) [51]. Recurrent nevus usually occurs after the shave of the lesion, a trauma or chronic inflammation (Fig. 15.52). Histologically, this may be worrisome and has been called pseudomelanoma changes. In these cases, there is a lentiginous and junctional epidermal component, but the lesion is well circumscribed and does not extend beyond the dermal scar.

Atypical histological features can be seen in about 10 % of nevi arising in the scalp, especially in adolescents [52, 53]. They can show asymmetry and poor lateral circumscription, and junctional components may show large confluent nests, as occurs in nevus of special sites (of the vulva, flexural sites and mammary skin). Cytological atypia is usually mild.

In the auricular area, nevi may also show histological atypical characteristics, such as poor circumscription, shouldering and bridging. Some cases may show melanocytic atypia and pagetoid spread in the centre of the lesion [54, 55].

Immunohistochemistry Nevi cells are positive for melanocytic markers, such as S100 protein, MART-1 (melan-A), microphthalmia transcription factor (MITF-1) and SOX10.

Genetics The melanocytic cells of the nevi are genetically altered, suffer clonal expansion and ultimately arrest their growth [56]. Loss of heterozygosity, microsatellite instability and other markers of malignancy have also been found in melanocytic nevi, suggesting that they can be melanoma precursors [57]. The *BRAF*V600E mutation that is frequent in melanomas has also been detected in 17 % of lentiginous junctional nevi, 55 % of compound nevi and 78 % of intradermal nevi [58].

Studies based on comparative genomic hybridisation (CGH) have evidenced that nevi usually do not show chromosomal abnormalities, in contrast with melanomas [59].

Differential diagnosis The differential diagnosis between melanocytic nevi and melanoma is based on histological criteria (Table 15.1) [60]. It is usually straightforward. However, some histological criteria that are usually used to diagnose a melanoma, such as the asymmetry of the lesion, irregular confluent nests, focal cellular atypia and even mitosis in the junctional or superficial dermic component, can be found in benign compound nevi. Conversely, some melanomas fail to show overt histological criteria of malignancy. Indeed, some melanocytic tumors cannot be categorised as melanocytic nevus or based on histological criteria, and the term 'melanocytic tumor of uncertain malignant potential (MELTUMP)' has been proposed for these unclassifiable tumors [61]. Immunohistochemistry and molecular analysis can assist in the diagnosis of these tumors. The most useful immunohistochemical stains are HMB45 and cell-cycle proteins. HMB45 expression is often positive in the junctional component of the nevi, but it is lost in the deep areas. In contrast, in melanoma the tumor cells are often positive throughout the lesion. In banal nevi, a small number of Ki-67 and cyclin D1+ positive cells may be seen in the more superficial dermal component of the lesion. In contrast, in melanoma, they are usually much more numerous and often present throughout the thickness of the lesion. p53 protein is rarely expressed in banal dermal nevi, but it is frequently present in melanoma.

Based on the results of CGH (see above), a commercially four-colour FISH assay has been developed, targeting genes on chromosomes 6p, 6q, 11q and centromere 6, to assist in the differential diagnosis between melanocytic nevi and melanomas. Only a scarce number of melanocytic nevi can show

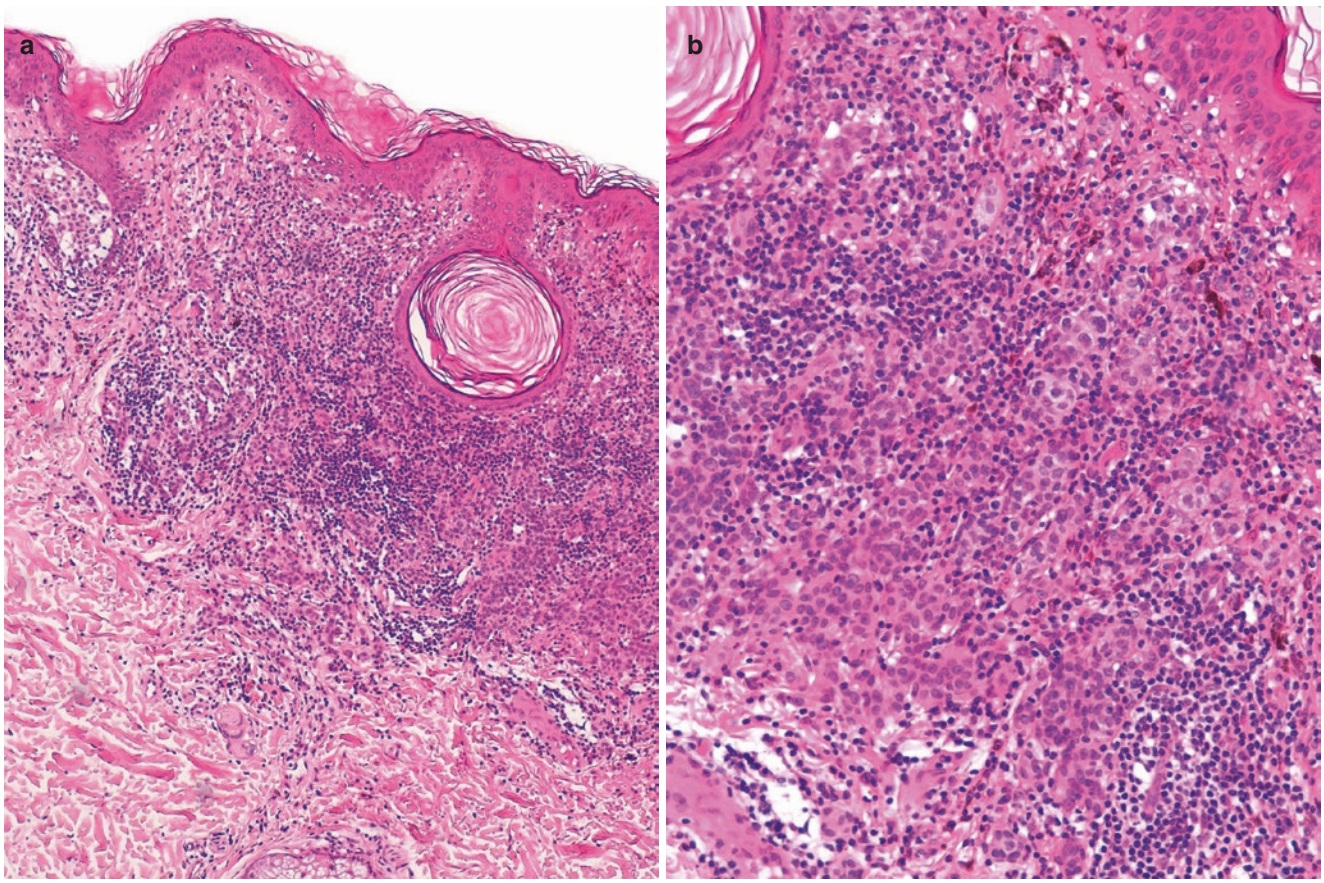

Fig. 15.51 Halo nevus. (**a**) This particular case is a compound melanocytic nevus with a marked lymphocytic inflammatory reaction. Regressive features are seen at the *left* of the picture. (**b**) The lesion at a higher magnification. The melanocytic cells do not show atypical features, and are admixed with abundant lymphocytes

chromosomal changes, giving a high specificity (97 %) and sensitivity (85 %) to detect melanomas [62].

Treatment and prognosis The melanocytic nevi have potential malignisation, but this is very low (about 1/100,000) and there is no indication for prophylactic excision of these benign lesions. However, melanocytic nevi that undergo clinical changes must be followed and excised if there is a suspicion of malignisation. The melanoma subtypes that usually develop from melanocytic nevi are superficial spreading and nodular types.

15.5.4 Giant Congenital Nevi

Definition Congenital nevi are melanocytic nevi that appear at birth. Giant congenital nevi are those tumors that measure 20 cm in diameter or more.

Epidemiology Congenital nevi affect approximately 1 % of newborn infants [63]. Some children present early-onset nevi in the first 2 years of life, with the clinical and histological characteristics of congenital nevi. Giant congenital nevi can cover a limb or an extensive area of the trunk. In the head and neck region, the most frequent site is the scalp.

Etiology and pathogenesis Congenital nevi may be associated with several syndromes, including Carney syndrome, epidermal nevus, neurocutaneous melanosis, neurofibromatosis type I, the premature ageing syndrome and the occult spinal dysraphism [64]. Giant congenital nevi may be associated with leptomeningeal melanocytosis (neurocutaneous melanosis).

Clinical aspects When they appear, congenital nevi usually measure less than 1 cm in diameter, but later can enlarge up to 4 cm or more. Initially, the lesion is pale brown and slowly becomes darker, thicker and hairy. They can have a verrucous surface, with small nodules.

Microscopy Congenital nevi can be junctional, compound or intradermal in type. In neonates, they are usually junctional, and histologically, there is a melanocytic hyperplasia in the epidermis and adnexal structures. Two types of cells can be seen in congenital nevi in first years of life. The cells in the underlying

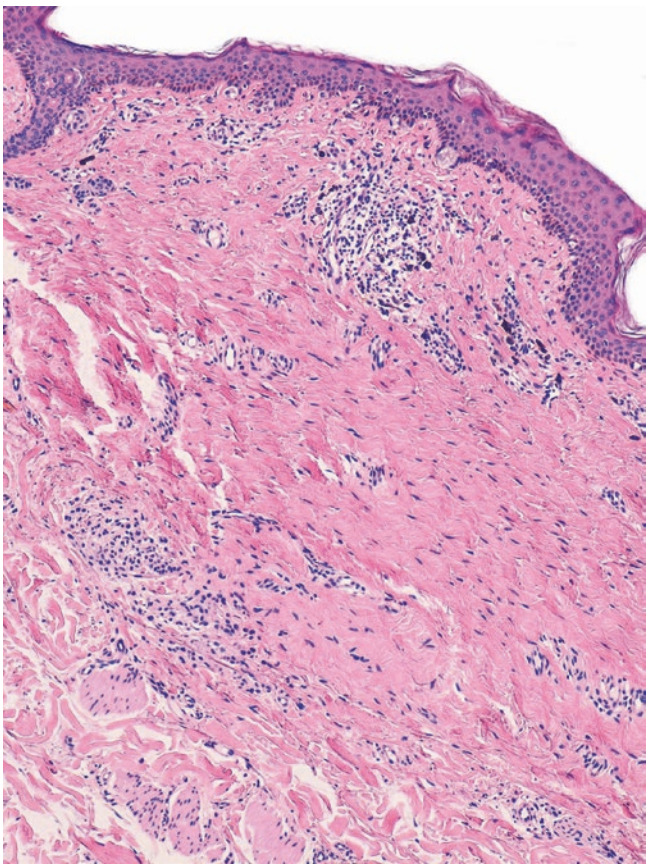

Fig. 15.52 Recurrent nevus. There is a lentiginous junctional melanocytic component over a scar. The persistent intradermal melanocytic nevus is seen at the *bottom*

Table 15.1 Histological criteria that favour the diagnosis of melanoma

Architectural criteria
Asymmetry
Poor circumscription
Epidermal consumption
Predominance of epidermal single melanocytes over nests
Pagetoid spread
Confluent and haphazardly arranged melanocytic nests
Variability of size and morphology of melanocytic nests
Irregular and asymmetrical melanin distribution
Vascular invasion
Cytological criteria
Nuclear pleomorphism
Nucleolar variability
Dermal mitoses
Atypical mitoses
Lack of cellular maturation in depth

Modified from Tan and Ackerman[60]

epidermis are larger than those in the reticular dermis, which are usually small. Characteristically, the dermal cells have a tendency to extend in deep parts, between collagen bundles around adnexae, nerves and vessels. Lipomatous and cartilaginous differentiation can be found in giant congenital nevus. Proliferative nodules can develop in congenital nevi, as a cellular proliferation composed of epithelioid cells, usually measuring less than 0.5 cm. in diameter. However, some larger proliferative nodules can develop. The proliferative nodule may have features of deep-penetrating nevus, Spitz nevus and balloon cell (melanocytic cells with wide, clear cytoplasm) nevus.

Immunohistochemistry The immunophenotype of giant congenital nevi does not differ from conventional melanocytic nevi. Proliferating antigen Ki67 is usually higher in proliferative nodules [65].

Genetics Giant congenital nevi frequently harbour *NRAS* mutations, but *BRAF* mutations are very rare [65, 66].

Differential diagnosis Differential diagnosis with melanoma can be difficult in the proliferative nodules. Features of malignancy in these lesions include lack of circumscription, atypical mitoses, cellular atypia and necrosis.

Treatment and prognosis Excision of giant congenital nevi is recommended for cosmetic reasons and the risk of malignancy. In adults, congenital nevi can be stable in appearance, in the absence of malignancy or trauma. Involution of giant congenital nevi has been reported [67].

The risk of malignancy is about 5 % in giant congenital nevi, higher than small congenital nevi [68].

15.5.5 Blue Nevus

Definition Blue nevus is a pigmented dermal tumor composed of dendritic-type melanocytes.

Epidemiology It is a frequent, usually acquired lesion. However, congenital cases do exist and a giant congenital form has been reported. It usually affects adults between the second and fourth decades, with a predilection for females. In the head and neck region, the most frequently involved site is the scalp, followed by the face.

Etiology and pathogenesis As with other dermal melanocytic proliferations, blue nevus is due to an arrested melanocytic migration. The epithelioid variant can be associated to Carney syndrome [69].

Clinical aspects The common or classic blue nevus is a small slate-blue to blue-black macule or papule. The epithelioid variant has a similar clinical appearance, but it has a tendency to be multiple. The cellular variant is usually larger and can present as a nodule. The plaque-like blue nevus is an

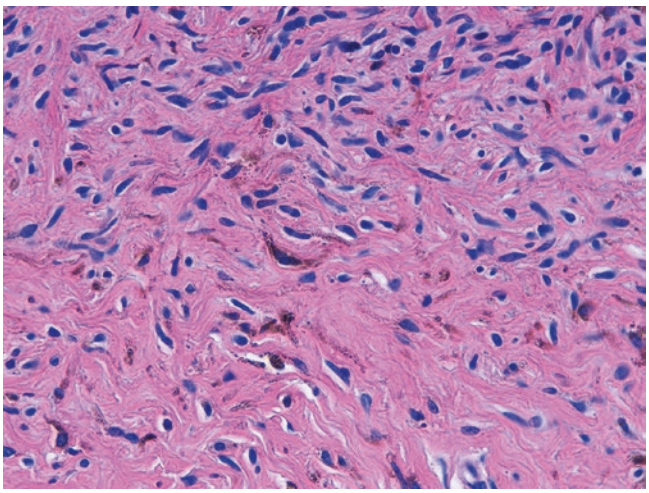

Fig. 15.53 Blue nevus. In dermis there is an admixture of spindle, heavily pigmented melanocytic cells and melanophages

infrequent form of blue nevus that forms a large bluish plaque containing multiple darker macules, as well as papules or nodules. A giant form extensively affecting the face with involvement of deep muscles has been described. Eruptive or agminated forms have also been reported.

Microscopy The common blue nevus is composed of elongated melanocytes disposed in the interstitial spaces in between collagen fibers in upper and mid-dermis. In the scalp, the lesion is sometimes deep and can affect the hypodermis, having a characteristic finger print feature. Usually, the melanocytes are heavily pigmented and there are melanophages (Fig. 15.53). The sclerosing variant shows dermal sclerosis, and sometimes myxoid changes in the stroma can be seen. It can rarely be amelanotic or hypopigmented. Epithelioid blue nevus is composed of intensely pigmented globular and fusiform cells admixed with lightly pigmented polygonal and spindle cells.

Immunohistochemistry The melanocytes in blue nevus express S100 protein, MART-1 (melan-A), microphthalmia transcription factor (MITF-1) and HMB45.

Genetics Mutations in *BRAF* gene are very rare. Chromosomic aberrations are unusual in common blue nevi [70].

Differential diagnosis In contrast with deep-penetrating nevus, blue nevus does not show maturation in deep parts of the lesion.

The coexistence of blue nevus with a common melanocytic nevus is the most frequent type of combined nevus [71], a lesion that can mimic a melanoma clinically and histologically.

Pigmented epithelioid melanocytoma has been described as an entity in between epithelioid blue nevus and animal-type (heavily pigmented) melanoma of low-grade behaviour [72, 73].

Atypical blue nevus is a blue nevus having atypical histological characteristics, including asymmetry of the lesion, density of the cellularity, nuclear atypia and mitoses. Differential diagnosis with melanoma may be challenging and should be considered as melanocytic tumors having indeterminate biological behaviour [74].

Finally, cutaneous metastases of melanoma may resemble a blue nevus clinically and histologically. Proliferating index by immunohistochemistry and the commercially available four-colour FISH probe can be useful to distinguish a blue nevus from a melanoma metastasis blue nevus-like [75].

Treatment and prognosis Blue nevus is a benign lesion, but the development of melanoma can rarely occur [76].

15.5.6 Nevus of Ota and Nevus of Ito

Definition Nevus of Ota and nevus of Ito are dermal melanocytic proliferations affecting the ophthalmic and maxillary division of the trigeminal nerve and the supraclavicular and deltoid region, respectively. Nevus of Ota has also been called oculodermal melanosis and nevus fuscoceruleus ophthalmomaxillaris. Hori's nevus is a bilateral Ota-like macule in the malar region.

Epidemiology It is frequent in the Asian population, whereas it is infrequent in white populations. There is a female predilection.

Both nevus of Ota and nevus of Ito can appear in the same patient. The presentation of bilateral nevus of Ito with nevus spilus has been reported. Pigmentation is often present at birth, but the lesion is usually apparent in childhood or adolescence. The onset in late adult life of dermal melanocytosis with distribution similar to nevus of Ito and Ota has been described.

Etiology and pathogenesis It has been suggested that the melanocytes migrate from the epidermis to the dermis over time, to form these dermal melanocytosis. Rarely, nevus of Ito can be associated with the Sturge–Weber syndrome.

Clinical aspects In nevus of Ota, there is a diffuse macular, blue or dark-brown pigmentation area, sometimes slightly speckled, which affects the skin of the ophthalmic and maxillary division of the trigeminal nerve, usually with conjunctival involvement. Ipsilateral deafness is a rare event. Lesions are rarely bilateral. Nevus of Ito is a similar condition located in the supraclavicular and deltoid regions, sometimes affecting the scapular region.

Microscopy Histologically the macular component is composed of dendritic spindle melanocytes in superficial dermis, which are usually disposed parallel to the epidermis or around follicles. In blue or dark-brown areas, the nevus is composed of nodular collections of melanocytes in dermis which resemble those of blue nevus.

Treatment and prognosis The lesions are usually stable. Malignisation is very rare [77]. Sun exposure and pregnancy can cause extensive lesions [78].

15.5.7 Deep-Penetrating Nevi

Definition Deep-penetrating nevus is a benign melanocytic proliferation that extends from superficial dermis to subcutaneous tissue, has a plexiform appearance and it is composed of spindle and epithelioid cells.

Epidemiology It is more frequent in young adults, with a slight female predominance. It is rarely multiple. It usually affects the face, upper trunk and proximal parts of the extremities.

Clinical aspects The deep-penetrating nevus usually appears as a dome-shaped papule or nodule that measures less than 1 cm. It is frequently pigmented [78].

Microscopy Histologically, there is an overlap with blue nevus and Spitz nevus. It has been regarded as a variant of congenital nevus. It is usually of compound type, but the junctional nests are only small in most cases. It may have a wedge shape (Fig. 15.54) and be composed of loosely arranged nests and fascicles of spindle cells interspersed with melanophages. Epithelioid cells are also present, usually in upper parts of the lesion. The nests extend to the deep dermis and often to the subcutaneous fat, usually surrounding hair follicles, sweat glands and nerves. Some nuclear pleomorphism can be seen, but nucleoli and mitoses are not frequent.

Immunohistochemistry Immunohistochemical studies evidence that the lesion is positive for S100 protein MART-1 (melan-A) and can be positive for HMB45. The proliferative index is low.

Genetics Deep-penetrating nevus shows *HRAS* mutations, but fails to show *GNAQ* or *GNA11* mutations [79].

Differential diagnoses Deep-penetrating nevi must be differentiated clinically and histologically from a melanoma. A high degree of atypia and abundant dermal mitoses favour the diagnosis of melanoma.

Treatment and prognosis The lesion is usually excised to rule out a melanoma. Rarely, this nevus can spread to regional lymph nodes [80].

15.5.8 Spitz Nevus

Definition This denomination recognises the contribution of Sophie Spitz in the description of this entity in 1948, in which she described the occurrence of these melanocytic tumors in children, with particular histological characteristics and a striking resemblance to malignant melanoma, but with benign behaviour [81].

Epidemiology Spitz nevus accounts for 0.5–1 % of surgically excised nevi in children and adolescents. It is usually a solitary lesion, often on the face, trunk and extremities of

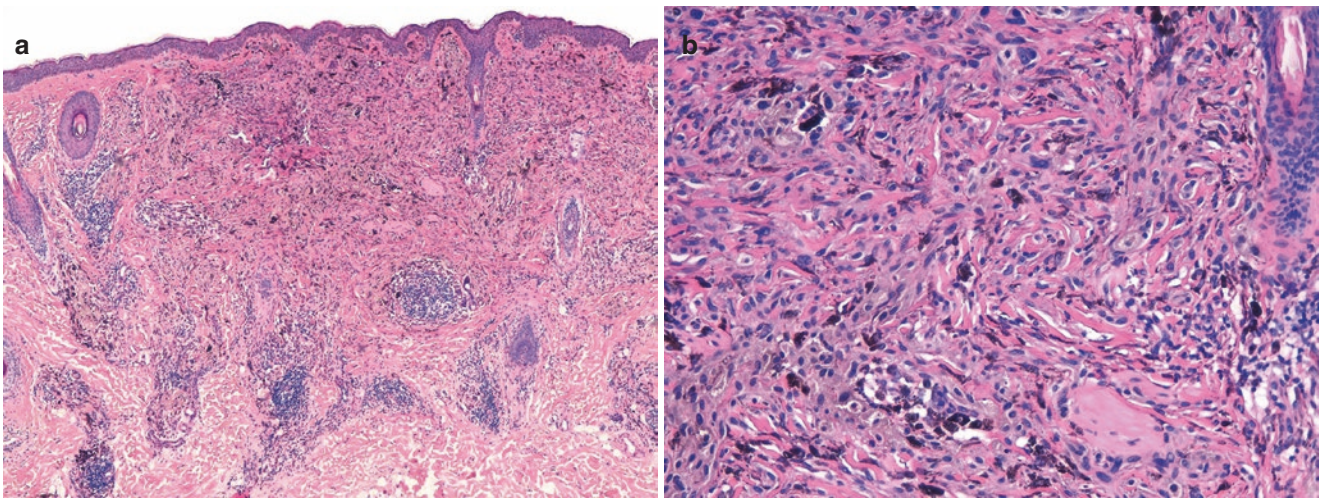

Fig. 15.54 Deep-penetrating nevus. (**a**) The lesion shows a characteristic wedge shape. This particular case is composed of spindle melanocytic cells which surround follicles and extend to the deep dermis. (**b**) The melanocytic spindle cells at a higher magnification. They lack cytologic atypia and are admixed with occasional melanophages

children or young adults [82]; they rarely affect older individuals. There is a slight predilection for females. Some rare cases arising in the tongue have been described. Multiple or agminated forms have been reported.

Clinical aspects Spitz nevus usually presents as a pink or flesh-coloured papule or nodule. Pigmented variants can also occur in about 70 % of cases.

Microscopy The vast majority of Spitz nevi are compound in type (Fig. 15.55), although 5–10 % are junctional and 20 % are intradermal. The diagnosis is based on the histological characteristics. It can be composed of spindle or epithelioid cells which usually have abundant eosinophilic cytoplasm. Nuclei are vesiculous and may show pleomorphism with conspicuous nucleolus. Multinucleated cells can be found. Mitoses can be seen but usually in the junctional component or the upper parts of the lesion. The lesion is symmetrical with no lateral extension of junctional activity beyond the limits of the dermal component, and there is maturation in deep parts of the lesion. Single melanocytes extending upward the epidermis or clusters of three or more cells in the epidermis can be seen. Other frequent histological characteristics that may help diagnosis are the presence of junctional cleavage, with separation of the epidermis from nests of nevus cells, pseudoepitheliomatous hyperplasia and absence of epidermal consumption. The presence of coalescent eosinophilic material forming globules in the dermoepidermal junction which are called Kamino bodies are frequently found (Fig. 15.56). These bodies are PAS and trichrome positive and are composed of basal membrane material. Lymphocytic inflammatory infiltrate may be seen, especially in the halo variant. Vascularisation is usually prominent. Some cases may have myxoid stroma. The desmoplastic variant shows a sclerotic stroma in the dermal component.

Some tumors have atypical features such as asymmetry, atypia or mitoses in deep parts of the lesion. Some authors have emphasised the impossibility to rule out a malignant melanoma in these atypical cases and the term atypical Spitz tumor is used, considered a melanocytic tumor of uncertain malignant potential [83].

Immunohistochemistry Spitz nevus cells usually have the immunophenotype of common melanocytic nevus cells and are positive for S100 protein and melan-A. HMB45 is usually positive only in the junctional component and superficial dermal component. Low expression of proliferative markers such as Ki67 and survivin helps to differentiate from malignant melanomas [84]. P16 immunostain intense and diffusely positive has been proposed as characteristic of Spitz nevus, instead of melanoma [84], but this immunostain is not always definitive for differential diagnosis [85].

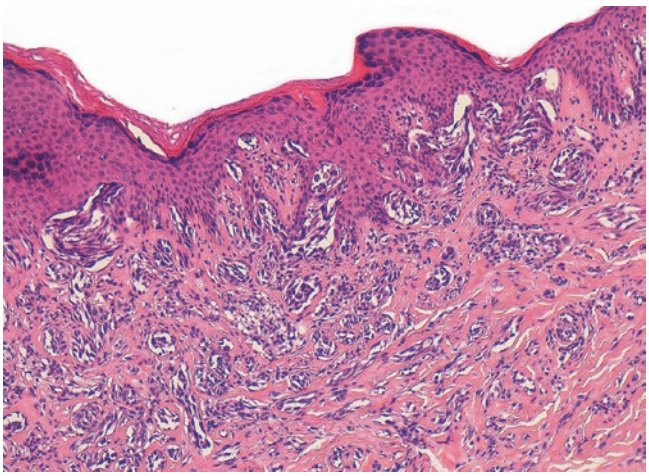

Fig. 15.55 Spitz nevus. The lesion is composed of melanocytic spindle cells in dermis with junctional activity

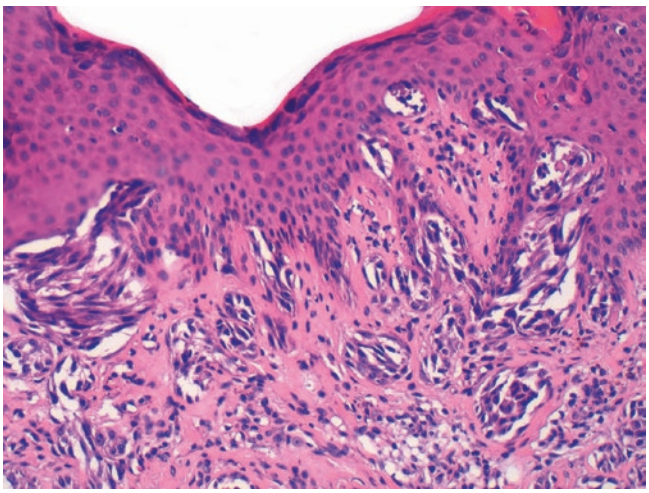

Fig. 15.56 Spitz nevus at a higher magnification. Note the epidermal hyperplasia, the junctional cleavage, with separation of the epidermis from nests of nevus cells, and presence of Kamino bodies (*upper–right*) composed of basal membrane type material in junctional situation

Genetics Molecular cytogenetic analysis shows clear differences between Spitz nevi and melanoma [86]. Up to 20 % of Spitz nevi show an isolated gain on chromosome 11p, an aberration not present in melanomas. Conversely, melanomas show frequent chromosomal aberrations that Spitz nevi do not have. One genetic alteration that could differentiate Spitz nevi from melanoma is the homozygous 9p21 deletion, which occurs in melanoma but not in Spitz nevus. This molecular alteration has been added to the commercially available four-colour FISH probe targeting 6p25, 11q13, 6q23 and centromere 6, to improve the differential diagnosis between Spitz nevus and melanoma [87]. Spitz nevus rarely shows *BRAF* or *NRAS* mutations, but *HRAS* gene copy number increase and mutations have been described more frequently [88].

Differential diagnosis Differential diagnosis of Spitz nevus with Spitzoid melanoma can be challenging. Spitz nevi are more frequent in young people, whereas melanomas are more common in older adults. Histological features that favour the diagnosis of Spitz nevus are good symmetry, uniformity of nests and presence of Kamino bodies. Fine dusty cytoplasmatic melanin, marginal or deep abnormal mitoses, dermal nests larger than junctional nests and mitotic rate in dermal component are histological features that favour malignancy. Immunohistochemical and molecular analyses can help in differentiating Spitz nevus from melanoma (see above).

Recently, a Spitz nevus type having the histological features of atypical Spitz tumor with a characteristic molecular profile showing loss of *BAP1* gene expression and *BRAF* gene mutation has been described [89]. Of interest, kinase fusions of *ROS1, NTRK1, ALK, BRAF* and *RET* have been found in Spitz tumors. They account for the majority of oncogenic aberrations in spitzoid neoplasms and have been demonstrated in 55 % of Spitz nevi, 56 % of atypical Spitz tumors and 39 % of spitzoid melanomas [90].

Treatment and prognosis The Spitz nevus is usually excised to rule out an atypical tumor or a malignant melanoma. Spread to sentinel lymph node has been observed in Spitz nevus and atypical Spitz nevus/tumor. This fact has no diagnostic or prognostic significance.

15.5.9 Architecturally Disordered Nevi and Other Atypical Melanocytic Proliferations

15.5.9.1 Dysplastic Nevus Syndrome and Dysplastic Nevi

Definition Dysplastic nevus, also called atypical or Clark's nevus, is a melanocytic tumor with distinctive clinical and histopathological characteristics that has an increased risk to develop a melanoma.

The term 'dysplastic nevus syndrome' refers to the familial or sporadic occurrence of multiple dysplastic (atypical) nevi in an individual.

Due to the controversy about the term dysplastic, the denomination 'atypical mole syndrome' was applied. Other terms have been used for dysplastic nevi, including 'nevus with architectural disorder and cytological atypia' as well as Clark nevus [91].

Familial cases of this syndrome have been called the 'B-K mole syndrome' (from the initials of two families in the first description of the entity) [92] and familial atypical mole/malignant melanoma syndrome or FAMMM syndrome [93].

Epidemiology This nevus type is found in up to 18 % of the Caucasian population. Dysplastic nevi in childhood are extremely rare; they usually appear in adolescence and adult life. However, a significant number of clinically and histologically atypical nevi in the scalp and forehead have been reported in children [94].

Although dysplastic nevi predominate in the trunk and legs, especially in females, a relationship has been reported with sun exposure.

Etiology and pathogenesis The association of dysplastic nevus syndrome and familial cutaneous melanoma is inherited as autosomal dominant with incomplete penetrance. It is associated with germinal mutations of the *CDKN2A* gene on 9p21–22 in approximately 40 % of families. *CDKN2A* encodes the tumor suppressor gene products $p14^{ARF}$ and $p16^{INK4a}$ [95]. A novel atypical nevus susceptibility gene has been identified on 7q21.3, containing a candidate gene *CDK6* [96].

Clinical aspects Clinically, dysplastic nevi usually measure more than 5 mm and show different colours, including tan, dark brown and pink [97]. However, not all clinically atypical nevi are dysplastic nevi.

Microscopy Histologically dysplastic nevi have four main histological characteristics: intraepidermal lentiginous hyperplasia of melanocytes, architectural atypia, random cytological atypia of these cells and a stromal response [98]. Lentiginous hyperplasia is a proliferation of melanocytes mainly singly along the basal layer. Nests in the sides of the elongated rete ridges as well as the tips with bridging features are frequent (Figs. 15.57, 15.58 and 15.59). There is an uneven distribution and pattern of the junctional component. The so-called shoulder phenomenon is the junctional lentiginous component that extends peripherally beyond the dermal component. A dermal

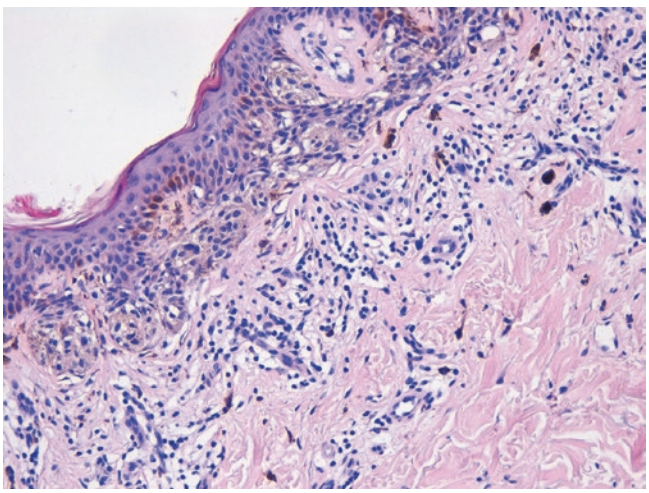

Fig. 15.57 Dysplastic nevus. Melanocytes along the basal layer aggregate in nests in the sides of the elongated rete ridges as well as in the tips with bridging features. Note the stromal response, with fibrosis, inflammation and melanophages presence

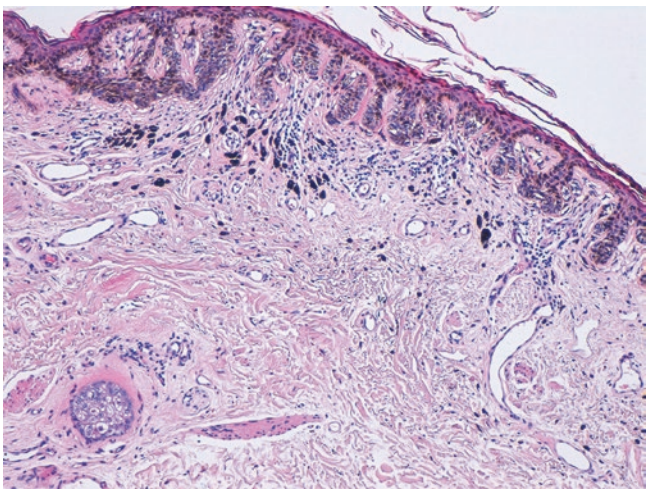

Fig. 15.58 Dysplastic nevus. Lentiginous proliferation of melanocytes showing random cytological atypia. There is prominent concentric and lamellar fibroplasia, mild lymphocytic infiltrate with numerous melanophages

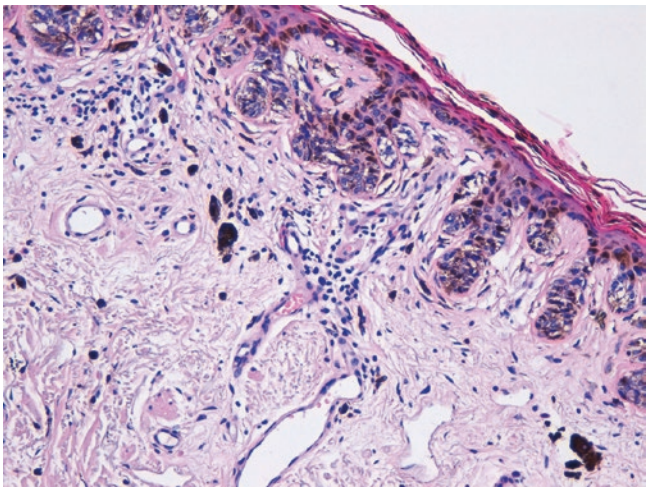

Fig. 15.59 Dysplastic nevus. Melanocytes display mild cytological atypia

melanocytic component is usually present in the centre of the lesion; these cells are small or epithelioid. The melanocytic cells in the junction usually show shrinkage artefact, with scant cytoplasm and spindle-shaped pattern. Sometimes they are larger, epithelioid, with dusty melanin. Occasional cells show enlarged hyperchromatic nuclei, sometimes with prominent nucleoli. The nuclei may be equal to those of overlying keratinocytes or are larger. The stromal response consists of lamellar fibroplasia in papillary dermis, a patchy inflammatory lymphocytic infiltrate and vascular hyperplasia. The dysplastic nevi are graded into mild, moderate or severe atypia.

Genetics In spite of mutations in *CDKN2A* on 9p21 that have been found in patients with familial melanoma, these mutations are uncommon in non-familial/sporadic dysplastic nevi [99]. *BRAF* mutations have been found in about 27 % of dysplastic nevi [100].

Differential diagnosis Cytological atypia may be found in other nevus that does not fulfil the criteria of dysplastic nevi. 'De novo intraepidermal epithelioid melanocytic dysplasia' is another condition that shows cytological atypia and pagetoid growth of melanocytes and has been considered a marker of the atypical mole phenotype. It has been considered a melanoma in situ by some authors [101].

Dysplastic nevus must be distinguished from lentiginous junctional nevus, a lesion without atypical histological features, also from lentigo maligna and from radial growth-phase (in situ) superficial spreading melanoma. Severely dysplastic nevi may show a continuum with early melanoma.

Treatment and prognosis Close clinical control of the melanocytic lesions must be performed in patients with dysplastic nevus syndrome. Complete excision of the unstable lesions is recommended, as well as in sporadic dysplastic nevus. A 5-mm tumor-free margin is, however, recommended for dysplastic nevi with severe atypia, but a narrower surgical margin can be accepted for those lesions with mild or moderate atypia [102].

The risk of developing melanoma in patients with dysplastic nevi is 10 % [103]. The risk is higher than 50 % in family members carrying the dysplastic nevus syndrome.

15.5.9.2 Dysplastic (Atypical) Lentiginous Nevus of the Elderly

Definition Dysplastic or atypical lentiginous nevus of the elderly is a pigmented lesion with lentiginous architecture which has a tendency to evolve into melanoma.

Epidemiology The prevalence of this lesion increases with age, and it is more frequent in patients over 60. However, it can affect younger patients with chronic sun-damaged skin.

It is more frequent in the back and lower parts of the legs, but can also appear in the head and neck region.

Etiology and pathogenesis It has been related to chronic sun exposure.

Clinical aspects It is usually a solitary asymmetrical pigmented flat macule with different colours that measures from 0.5 to 1 cm. It is slow growing.

Microscopy There is a lentiginous melanocytic proliferation along the dermo-epidermal junction. The rete ridges are elongated and bridging features can be seen. Nests of melanocytes located at the tips of several rete ridges can be seen, and they are irregular. Melanocytes display mild cytological atypia with irregular hyperchromatic nuclei [104]. Pagetoid

spread is usually absent. In the papillary dermis, there is usually fibrosis, pigment incontinence and a lymphohistiocytic inflammatory cell infiltrate.

Differential diagnosis Junctional lentiginous nevus is usually a small lesion measuring less than 0.5 cm, in young people and skin without sun damage. There is a regular elongation of rete ridges without cytological atypia.

Well-developed melanoma in situ of lentiginous type usually presents a higher degree of architectural and cytological atypia, and pagetoid spread is frequently present.

Treatment and prognosis The lesion must be totally excised. It is a known melanoma precursor, and in some lesions, features of melanoma in situ are seen [105].

15.5.10 Malignant Melanoma of the Head and Neck

15.5.10.1 Lentigo Maligna and Melanoma Lentigo Maligna Type

Definition Lentigo maligna (LM) is a form of lentiginous melanoma in situ, which develops in highly sun-damaged skin in the elderly. Controversy exists about the nomenclature of LM or melanoma in situ LM type. Some authors think that the terms are synonymous, whereas others think that LM is a precursor of melanoma in situ. The term lentigo maligna is synonymous with terms used in the past: 'Hutchinson melanotic freckle' and 'Dubreuilh melanosis circumscripta precancerosa'. In the last WHO edition, LM is considered a melanoma in situ [106].

Melanoma lentigo maligna type (LMM) is an infiltrating melanoma developed from a LM.

Epidemiology The incidence of malignant melanoma is progressively increasing in the Caucasian population. This increase is due to the higher incidence of melanomas, especially higher incidence of diagnosis of thin melanomas without gender and age predilection. In the head and neck region, the melanoma incidence is usually not related to recreational sun exposure, but more likely to occupational sun exposure, especially in low latitudes [107]. In the head and neck area, the most frequent melanoma subtype is lentigo maligna melanoma. It occurs equally in men and women.

Etiology and pathogenesis Risk factors for melanoma include sun exposure habits and associated pale skin, blond and red hair, the presence of numerous freckles and a tendency to burn and to tan poorly. LM and LMM arise in response to accumulated sun exposure, in contrast with other melanoma types.

Clinical aspects Clinically, LM may begin as a slowly growing small lesion, usually as a mottled light brown macule, with irregular margins. When the lesion increases in size, more variation in pigment and irregularity of borders will appear (Fig. 15.60). Nodules can appear and borders can be difficult to define. An amelanotic LM and LMM have been described. Clinically, it is a pink lesion resembling an inflammatory lesion [108].

Microscopy Histologically, LM is characterised by a predominantly junctional proliferation of atypical melanocytes with a tendency to coalesce and extend to the hair follicles and sweat gland ducts. The epidermis is atrophic, and there is usually marked elastosis in the dermis. The neoplastic cells sometimes form small, irregular tumoral nests in the junction (Fig. 15.61). Some multinucleated cells can be found. The atypical melanocytes can show a marked pleomorphism, with a variable chromatin pattern (Fig. 15.62). There is sometimes a cytoplasmic retraction due to a processing artefact, and nuclei may have a stellate morphology. There is sometimes pagetoid spread, but this feature is not frequent. Lymphocytic infiltrate and regression features can be seen in dermis. In these cases, a careful examination must be performed to rule out an infiltrating component.

Some authors differentiate LM from melanoma in situ LM type. They use the term melanoma in situ LM type when melanocytes coalesce, form small groups and have pagetoid spread or deep adnexal involvement [109]. The LMM have an invasive component that may be composed of spindle, epithelioid, small nevoid or desmoplastic cells.

Immunohistochemistry The neoplastic cells are positive for melanocytic markers (MART-1, HMB45, S100 protein) like other melanoma subtypes. These markers can be useful

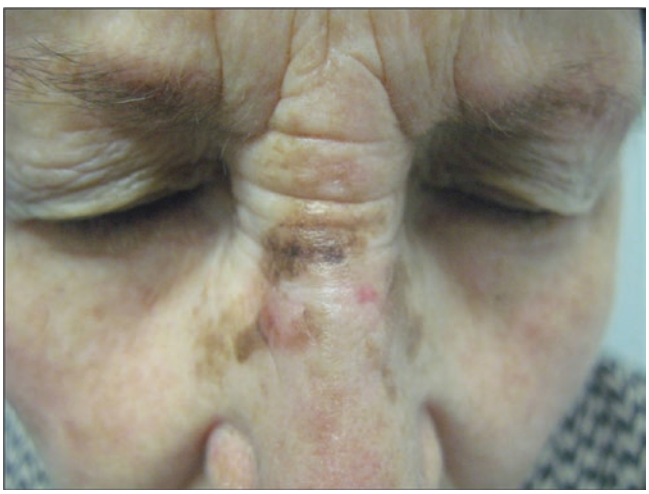

Fig. 15.60 Melanoma lentigo maligna type. Clinically the lesion may acquire a large size. It is a *brown* macule, with irregular pigmentation and margins

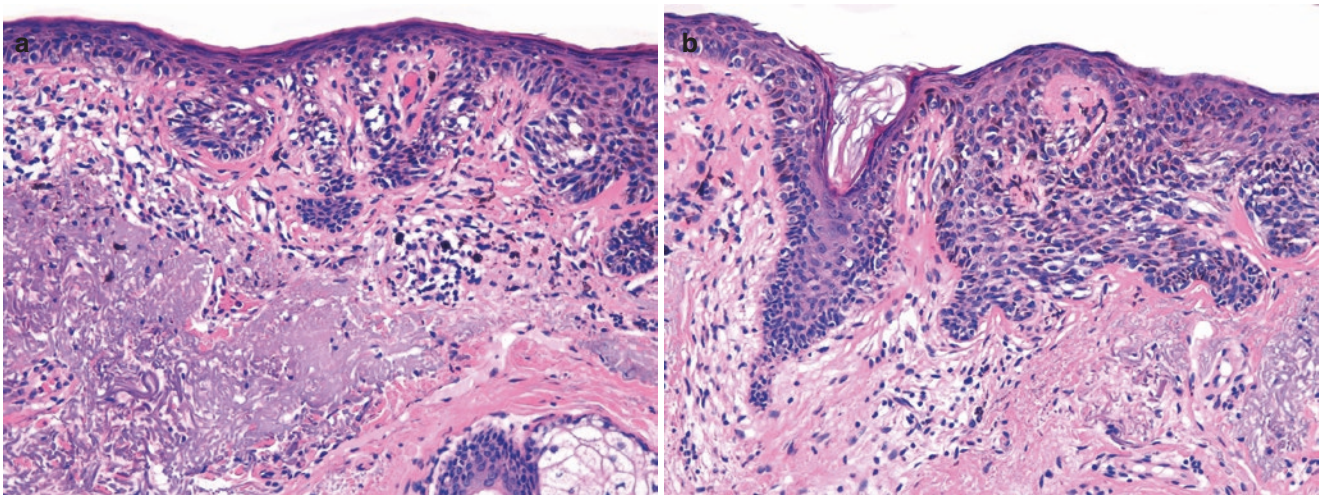

Fig. 15.61 Melanoma in situ, lentigo maligna type. (**a**) Atypical melanocytic proliferation at the junction, with characteristic infundibula spread. Note the marked dermal elastosis. (**b**) At a higher magnification, neoplastic cells show moderate nuclear atypia and hyperchromasia. Pagetoid spread is not a feature in this particular case

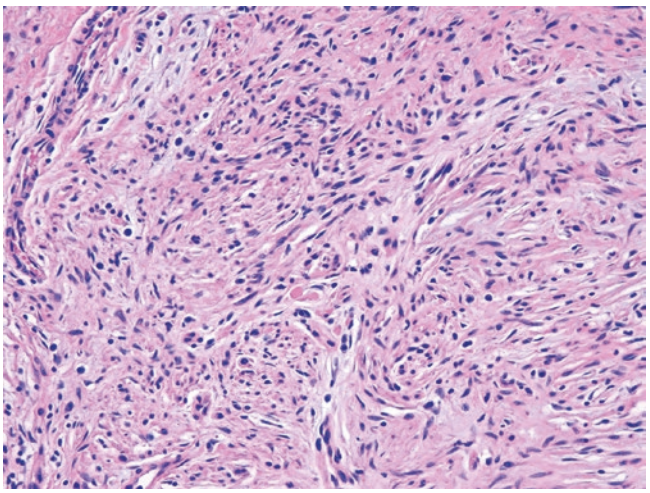

Fig. 15.62 Desmoplastic melanoma. There is a spindle cell proliferation occupying dermis in a fibrotic stroma. There are some inflammatory foci

to confirm the presence of infiltration, especially when regressing features are seen. It must be taken into account that infiltrating desmoplastic components lose melanocytic differentiation markers and usually only express S100 protein.

Genetics *BRAF* mutations are infrequent in LMM, and the *BRAF*V600K is more frequent than the *BRAF*V600E mutation [110]. *KIT* gene activation through mutation or gene copy number increase has been found in about 28 % of LMM [111].

Differential diagnosis Differential diagnoses must be done mainly with junctional melanocytic nevus. The diagnosis of a junctional nevus in sun-damaged skin must be made with caution, and LM must be always ruled out. Another differential diagnosis is actinic lentigo or pigmented actinic keratosis. However, in up to 30 % of cases, LM coexists with the other conditions associated to sun-damaged skin.

Treatment and prognosis Surgical excision with mapping, cryotherapy, topical imiquimod and radiotherapy has been used successfully for the treatment of lentigo maligna [112]. The use of treatment modalities other than surgical excision for melanoma in situ or LMM carries a significantly increased risk of local recurrence. The surgical excision of LM must be done with a margin of 0.5 cm. Treatment with imatinib has been proposed for metastatic LMM carrying KIT activation [113].

15.5.10.2 Desmoplastic Melanoma

Definition Desmoplastic melanoma is considered a melanoma that has undergone adaptive desmoplasia or a neurofibrosarcomatous transformation.

Epidemiology Desmoplastic melanoma is usually found in the head and neck. There is a slight male predominance [114]. Presentation in the lip in young people has been described [115].

Clinical aspects They are usually non-pigmented lesions and present as an indurated plaque or bulky tumor. Sometimes, there is a LMM overlying or beside the desmoplastic melanoma. In these cases, the term mixed desmoplastic melanoma has been attributed. Sometimes, a usual melanoma recurs or metastatises as pure desmoplastic melanoma.

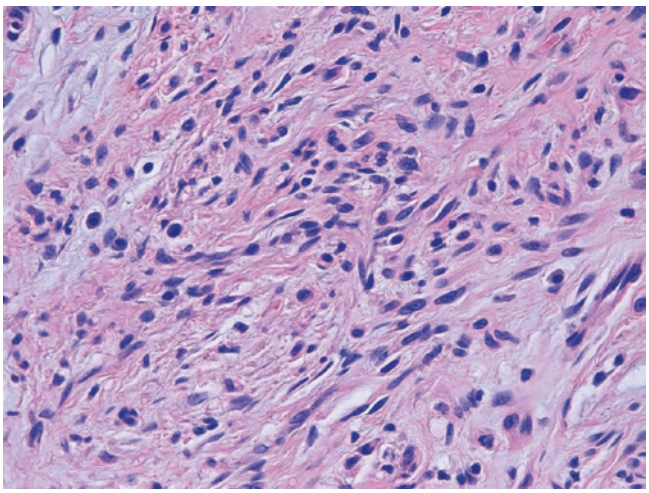

Fig. 15.63 Desmoplastic melanoma. At higher magnification, oval and spindle cells appear cytologically bland. There is myxoid degeneration

Microscopy Histologically, it is composed of strands of elongated spindle-shaped cells surrounded by mature collagen bundles (Fig. 15.62). It can represent a fibrosing variant of spindle cell melanoma. It can be pure or combined when it is mixed with other melanoma types. Areas of neural transformation and neurotropism may be seen (Fig. 15.63). There are frequently foci of mature lymphocytes and plasma cells within the tumor. Early pure variants may be difficult to diagnose. They are poorly circumscribed, with myxoid or sclerosing stroma. The cells may show moderate nuclear atypia and mitoses are infrequently found. They are usually amelanotic. Aggressive variants combined with LMM have been reported, even with osteogenic components. Desmoplastic melanoma is usually deep at diagnosis. The neurotropic variant which shows perineural invasion accounts for one-third of cases [114]. The neoplastic cells adopt a neuroma-like pattern and a tendency to display a circumferential arrangement around small nerves in deep dermis and subcutaneous tissue.

Immunohistochemistry Desmoplastic melanoma is usually positive for S100 protein and SOX-10 (Fig. 15.65) and negative for melanocytic markers, including MART-1 (melan-A), microphthalmia transcription factor (MITF-1) and HMB45 [116, 117]. The S100 protein assessment is required in order to exactly establish the tumor depth. One-third of desmoplastic melanomas can express actins. Rare cases have been described negative for S100 protein. In these cases, other markers of Schwann cell differentiation, such as p75 nerve growth factor receptor and SOX-10, can be useful. However, these markers do not differentiate desmoplastic melanoma from other tumors derived from neural crest origin.

Genetics Desmoplastic melanomas lack *BRAF* mutations [118].

Differential diagnosis Differential diagnosis must mainly be performed with a scar, which usually has positive cells for S100 protein, and desmoplastic nevus. Desmoplastic nevus is usually positive for melan-A and desmoplastic blue nevus for melan-A and HMB45. Moreover, proliferating antigen Ki-67 labelling may help in the differential diagnosis with desmoplastic nevi. A Ki-67 positivity over 5% of cells is more likely found in desmoplastic melanoma.

Other differential diagnoses include cutaneous spindle cell neoplasms, such as spindle cell squamous cell carcinomas and soft tissue neoplasias. In the latter group of tumors, nerve-derived tumors, especially malignant peripheral nerve sheath tumor, share immunoprofile with desmoplastic melanoma.

Treatment and prognosis Pure desmoplastic melanomas have better prognosis than mixed forms, with longer disease-free survival.

Desmoplastic melanoma presents a high rate of recurrences, but pure desmoplastic melanomas have a lower rate of lymph node metastases [119, 120]. Those tumors expressing N-cadherin can present a higher rate of lymph node metastases. Neurotropism leads to a bad prognosis, especially when melanoma spreads through cranial nerves.

15.5.10.3 Nevoid Melanoma

Definition Nevoid melanoma is a melanoma in vertical growth phase, composed of nevus-like cells.

Clinical aspects Clinically, most cases are verrucous or dome-shaped nodules variably pigmented, mimicking a nevus or an atypical melanocytic lesion.

Microscopy At a low magnification, nevoid melanomas can be fairly symmetrical, without an intraepidermal component (Figs. 15.64, 15.65 and 15.66), indistinguishable from a melanocytic nevus. At a higher magnification, the tumor cells are small, epithelioid melanocytes with pale staining or eosinophilic cytoplasm and round to oval vesicular nuclei with small eosinophilic nucleoli. There is usually subtle pleomorphism. The nuclear characteristics of tumor cells and presence of dermal mitoses are usually the clue for the diagnosis [121].

Immunohistochemistry Nevoid melanomas usually express immunohistochemical markers as other melanoma variants, with HMB 45 and Ki-67+ cells in deep areas of the tumor.

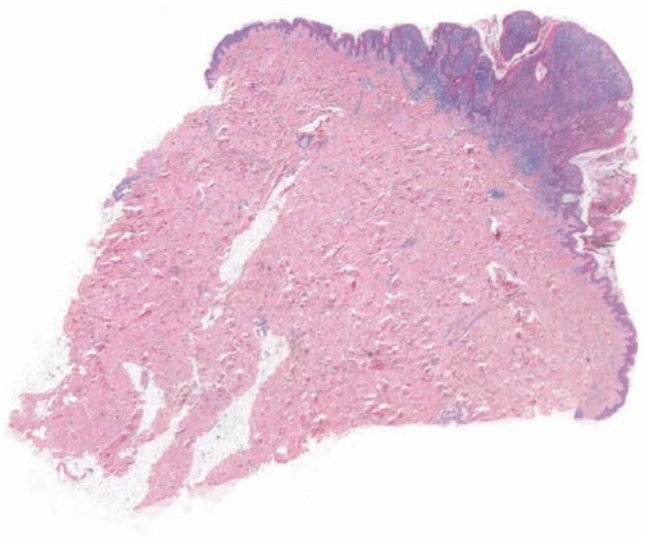

Fig. 15.64 Nevoid melanoma. At low power, nevoid melanoma shows an exophytic, verrucous, papillary configuration, resembling a common dermal nevus

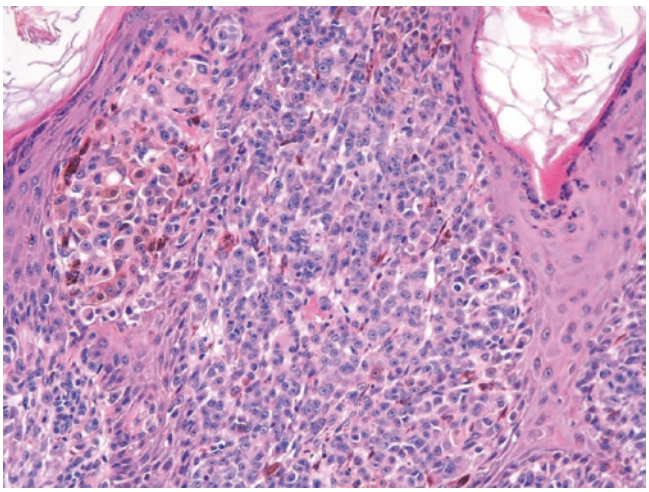

Fig. 15.65 Nevoid melanoma. There is not much intraepidermal spread. Melanocytes form expansile nests and show occasional pleomorphic nuclei

Differential diagnosis Differential diagnosis with melanocytic nevi must be performed following histological and immunohistochemical criteria. The four-colour FISH probe commercially available targeting 6p25, 11q13, 6q23 and centromere 6 is useful for differential diagnosis between nevoid melanoma and mitotically active melanocytic nevus [122].

Treatment and prognosis Nevoid melanoma must be treated as other melanoma subtypes.

A long follow-up of nevoid melanomas confirms that they have similar recurrence and mortality rates as other melanoma subtypes [121].

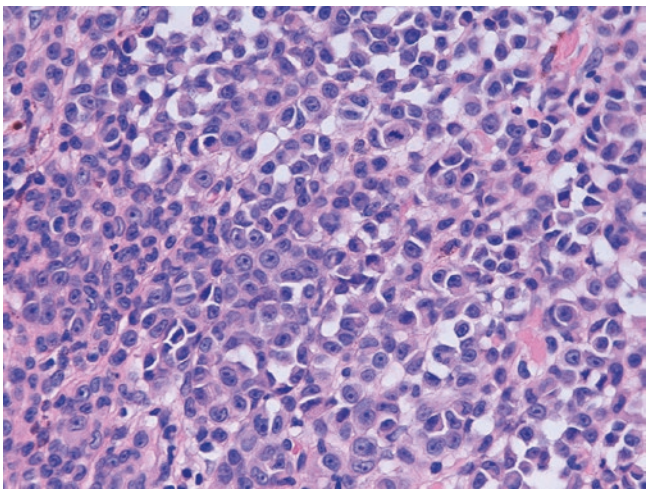

Fig. 15.66 Nevoid melanoma. Mitotic figures can be found within the dermal component

15.6 Cutaneous Lymphoid Lesions

15.6.1 B-Cutaneous Lymphoid Hyperplasia

Definition B-cutaneous lymphoid hyperplasia is a reactive cutaneous process in which there is a benign lymphoid infiltrate composed of B-lymphocytes. This process has also been called cutaneous B-cell pseudolymphoma and lymphocytoma cutis.

Epidemiology The most frequent location of the lesion is the face, including the cheek, nose and earlobe. Other locations are the trunk and upper extremities. There is a female predominance, and it can appear at any age [123, 124].

Etiology and pathogenesis *Borrelia burgdorferi* infection is a common cause in endemic regions of Europe. In these cases, *B. burgdorferi* specific DNA can be detected. However, in a large number of cases, there is not a known etiology. Other causes are insect-bite reaction, tattoos, piercings, varicella-zoster infection scars and drug reactions.

Clinical aspects It presents as a solitary, small, erythematous or bluish plaque or nodule. Ulceration is infrequent.

Microscopy Histologically, there is usually a dense lymphoid dermal infiltrate which sometimes affects subcutaneous tissue. The grenz zone is usually preserved, but sometimes there is epidermal exocytosis with spongiosis, epidermal atrophy or hyperplasia. The lymphoid infiltrate is diffuse or vaguely nodular. The lymphoid infiltrate frequently shows reactive germinal centres, with presence of centroblasts, centrocytes and macrophages. The mantle

zone is frequently compressed or absent, especially in cases due to Borrelia infection. The interfollicular areas are composed of a mixed population of small lymphocytes, centroblasts, histiocytes, plasma cells and eosinophils.

Immunohistochemistry Lymphoid follicles show follicular dendritic cell network using CD21, CD23 and CD35 antibodies. The B-lymphocytes co-express CD20, CD23, CD10 and BCL-6, but not BCL-2. B-lymphocytes and plasma cells are polyclonal and express kappa and lambda light chains of the immunoglobulins. T cells predominate in the interfollicular areas.

Genetics The immunoglobulin gene rearrangement usually shows a polyclonal pattern. However, clonality has been demonstrated in some cases [125].

Differential diagnosis The main differential diagnoses of B-cell lymphoid hyperplasia are primary cutaneous marginal zone lymphoma and cutaneous follicular lymphoma (see below) [126, 127].

Treatment and prognosis Borrelial infections must be treated with antibiotics. Most cases resolve spontaneously when the causal stimulus is removed. However, a minority of cases may have a chronic course. Some cases may evolve to B-cell lymphoma [128]. For this reason, a follow-up of the patient is required.

15.6.2 Lymphocytic Infiltrate of the Skin Jessner Type

Definition Lymphocytic infiltrate of the skin, Jessner type, is an inflammatory condition, characterised by a dense dermal lymphocytic infiltrate [129].

Epidemiology This inflammatory condition is infrequent. The lesions occur most often on the face, neck, upper chest and back. It usually affects adults, predominantly men in the third to fifth decades of life. However, rare cases in children and familial cases have been described.

Etiology and pathogenesis The etiology is unknown. Some cases have been related to sun exposure. Some authors consider this entity part of the spectrum of polymorphic light eruption or lupus erythematosus tumidus [130].

Clinical aspects The skin lesions are papules or plaques 1–2 cm. in diameter, erythematous or brownish and asymptomatic. They can be single or more often multiple.

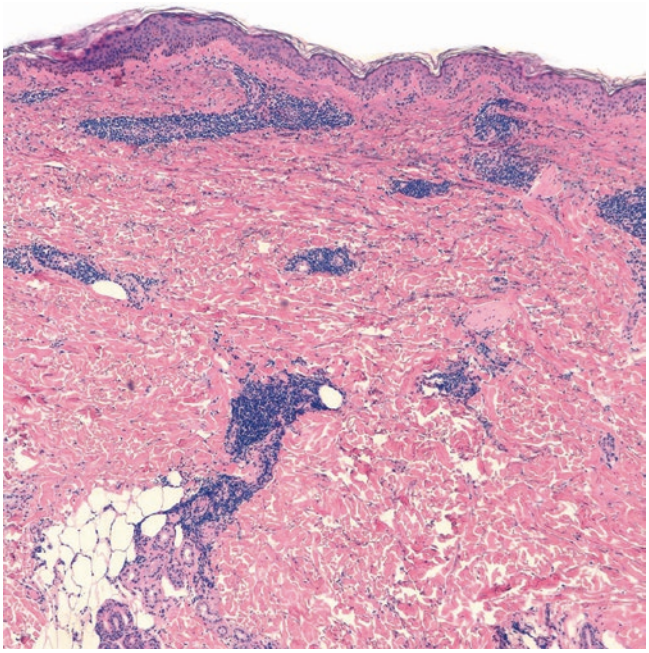

Fig. 15.67 Lymphocytic infiltrate of the skin Jessner type. There is a dense lymphocytic infiltrate in the superficial and mid-dermis, predominantly perivascular and perifollicular

Microscopy In the superficial and mid-dermis, there is a dense lymphocytic infiltrate, predominantly perivascular, but also perifollicular (Fig. 15.67). Sometimes, the lymphocytic infiltrate is deeper and affects subcutis. The epidermis is unaffected. The inflammatory infiltrate is composed of mature, small lymphocytes (Fig. 15.68). Some histiocytes and plasma cells are frequently found. Dermal mucin can be found.

Immunohistochemistry The lymphoid infiltrate consists predominantly of T cells, most often of the CD4+ helper subtype. Occasionally, CD8+ T cells predominate. Immunoregulatory T cells which express Leu 8 are usually numerous [131]. B-lymphocytes are scarce or absent. Plasmacytoid dendritic cells expressing CD123 are frequently found [132].

Differential dignosis Lymphocytic infiltrate of Jessner type can be differentiated from discoid lupus erythematosus by the absence of epidermal changes, scarring and a negative lupus band test. However, the differential diagnosis with lupus erythematous tumidus can be challenging. A low number of B cells and a high number of Leu8-expressing cells favour the diagnosis of lymphocytic infiltrate of Jessner type [131]. Dermal mucin is usually more abundant in lupus erythematosus tumidus.

Lymphocytic infiltrate of Jessner type can be histologically indistinguishable from polymorphous light eruption. In these cases, phototesting may be necessary to differentiate both entities.

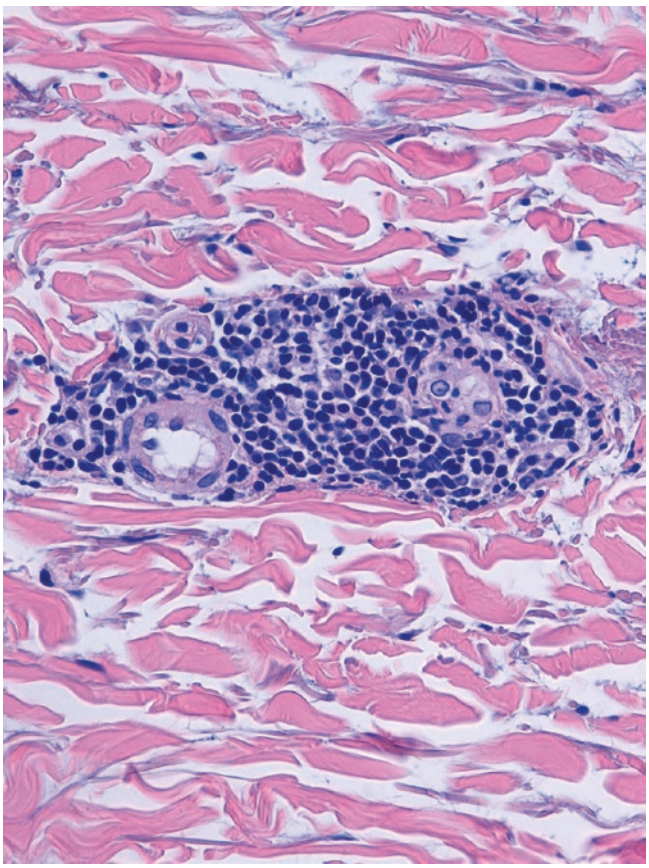

Fig. 15.68 Lymphocytic infiltrate of the skin Jessner type. The inflammatory infiltrate is composed of small, mature lymphocytes

Finally, differential diagnosis with chronic lymphocytic leukaemia/lymphocytic lymphoma can be easily performed by careful evaluation of cellular morphology and the immunophenotyping of the lymphoid infiltrate, as the latter is a B-cell lymphoproliferative condition.

Treatment and prognosis Skin lesions usually resolve within weeks or months, but relapses are frequent, and can be present over years.

15.6.3 T-Cell Pattern of Lymphoid Hyperplasia

Definition T-cell pattern of lymphoid hyperplasia is a reactive process that simulates histologically and even clinically a T-cell lymphoma. It is also called T-cell cutaneous pseudolymphoma.

Epidemiology This entity includes a large number of cases included in distinctive clinicopathological settings with a wide range of clinical presentations [123].

Etiology and pathogenesis Many cases of cutaneous T-cell pseudolymphoma are idiopathic. Some cases correspond to distinct clinicopathological entities, such as lymphomatoid drug eruptions, lymphomatoid contact dermatitis, T-cell-rich angiomatoid polypoid pseudolymphoma, pseudolymphomatous folliculitis, lymphomatoid actinic dermatitis (actinic reticuloid dermatitis), persistent insect-bite reaction and reactions to tattoos [123, 133–135]. Lymphomatoid drug eruptions can be secondary to anticonvulsants, such as phenytoin, primidone and carbamazepine or non-anticonvulsants, such as antihypertensives, antidepressants, beta-blockers, calcium channel blockers, diuretics and antibiotics. Lymphomatoid contact dermatitis represents a T-cell reaction to a contact allergen. Lymphomatoid actinic dermatitis is associated with sensitivity to ultraviolet (UV) B and UVA.

Clinical aspects The clinical presentation of T-cell pseudolymphoma depends on the etiological agent. Lymphomatoid drug reactions can present with single or multiple plaques or nodules. Anticonvulsants may induce an erythematous morbilliform maculopapular eruption with systemic symptoms such as fever, lymphadenopathies and leukocytosis. Lymphomatoid contact dermatitis presents as pruritic, usually localised scaly papules and plaques. T-cell-rich angiomatoid polypoid pseudolymphoma is a distinctive lesion, usually solitary and small, measuring less than 1 cm in diameter. Clinically, it resembles an angiomatoid lesion in the head or the trunk. Pseudolymphomatous folliculitis is uncommon and presents in the head and neck region as a solitary erythematous nodule. Chronic actinic dermatitis presents with an intensely pruritic, scaly, erythematous eruption, especially in the face.

Microscopy Drug-induced T-cell pseudolymphoma usually presents as a dense, superficial, perivascular or band-like infiltrate composed of lymphocytes and histiocytes. Atypical lymphoid cells with irregular, enlarged and hyperchromatic nuclei can be found. There is often epidermotropism, sometimes with Pautrier-like microabscesses. Eosinophils and epithelioid granulomas can be seen. The lymphoid infiltrate is sometimes nodular and deep. Large cells CD30+ can be found in the dermal infiltrate.

The angiomatoid polypoid pseudolymphoma shows, in addition, a background with numerous, small, thin-walled vascular channels.

In the pseudolymphomatous folliculitis, there is a dense folliculocentric infiltrate, usually with hyperplasia and distortion of the follicular structures.

The histological characteristics of the lymphomatoid actinic dermatitis (actinic reticuloid dermatitis) is highly variable, but can present with a very dense cellular infiltrate involving dermis and rarely subcutaneous tissue. The infiltrate is mixed,

composed of lymphocytes, histiocytes, plasma cells and eosinophils [135]. Stellate myofibroblasts and giant cells in relation to foci of elastosis are frequently found. Occasional large, hyperchromatic, cerebriform lymphoid cells can be found, as well as mitoses. Epidermal exocytosis is frequently seen.

Immunohistochemistry The infiltrate consists predominantly of T-lymphocytes, most of them $CD4^+$. There is usually no loss of pan-T-cell markers (CD2, CD3, CD5). However, loss of expression of CD7 has been described in some cases.

Genetics *TCR* genes rearrangement by PCR has been described in some cases of T-cell pseudolymphoma [136]. The duplication of clonality tests by PCR is recommended when there is not accordance with the clinical and histological results.

Differential diagnosis Differential diagnosis with a cutaneous T-cell lymphoma must be performed with the integration of the clinical, histology, immunohistochemistry and molecular results.

Treatment and prognosis Surgical excision of solitary lesions can be performed. However, lesions tend to regress spontaneously.

15.6.4 Lymphoproliferative Disorders

15.6.4.1 Mycosis Fungoides

Definition Mycosis fungoides (MF) is a cutaneous, epidermotropic peripheral T-cell lymphoma of low grade of malignancy.

Epidemiology It is the most frequent type of cutaneous T-cell lymphoma and accounts for about 50 % of cutaneous lymphomas. It affects more frequently males (2:1) and black people (2:1), between the fourth and sixth decades of life, but can present at any age. The lesions are more frequent in the trunk, but can appear elsewhere. The follicular variant of mycosis fungoides is more frequent in the elderly and has a tendency to affect the face and scalp [137].

Etiology and pathogenesis The causative agent of MF is unknown. A chronic antigenic stimulation due to infective agents has been suggested, but it has not been proved to date.

Clinical aspects The early lesions are asymmetrical erythematous patches, slightly scaly. The patch lesions can evolve into plaques and tumors. However, the disease does not always present this clinicopathological evolution, and frequently the same patient can present lesions in all evolutive phases. Other clinical presentations include the folliculotropic, localised (pagetoid reticulosis type), granulomatous, poikilodermatous (also called large plaque parapsoriasis), erythrodermic, bullous, pustular, hypopigmented and verrucous variant. The folliculotropic, pagetoid reticulosis and granulomatous types have distinctive clinicopathological characteristics.

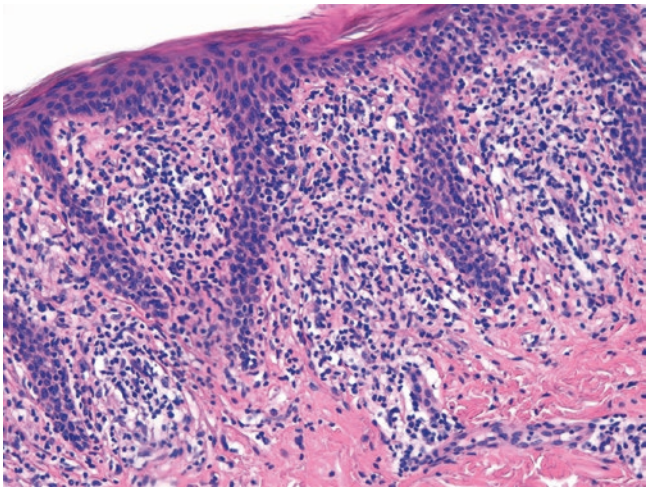

Fig. 15.69 Mycosis fungoides. The epidermis is acanthotic and shows a psoriasiform appearance. In the upper dermis there is an atypical lymphoid infiltrate with tendency to epidermotropism

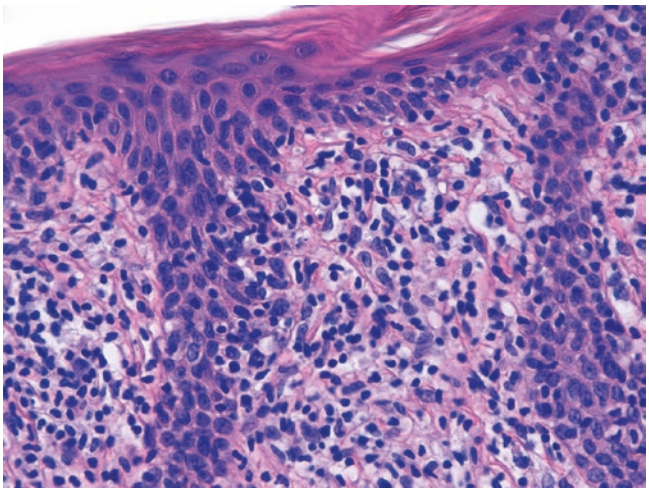

Fig. 15.70 Mycosis fungoides at a higher magnification. Small groups of neoplastic cells colonising the epidermis are seen

Microscopy In the early patch stage of MF, the histopathological changes can be subtle, and multiple biopsies are sometimes required to reach the diagnosis [138, 139]. The epidermis can be acanthotic or atrophic and can show hyperkeratosis or parakeratosis. In the plaque stage, the epidermis frequently shows a psoriasiform appearance. The neoplastic T-lymphocytes are usually seen in the upper dermis with tendency to epidermotropism (Figs. 15.69 and 15.70). Palisading of lymphocytes along the basal layer of the epidermis is a very characteristic feature. Sometimes the dermo-epidermal junction is obscured and vacuolisation of

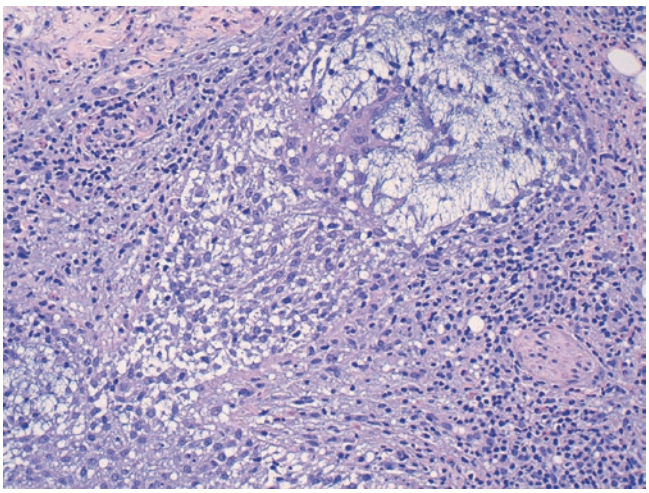

Fig. 15.71 Mycosis fungoides, folliculotropic variant. The neoplastic lymphoid cells colonise the follicular structures

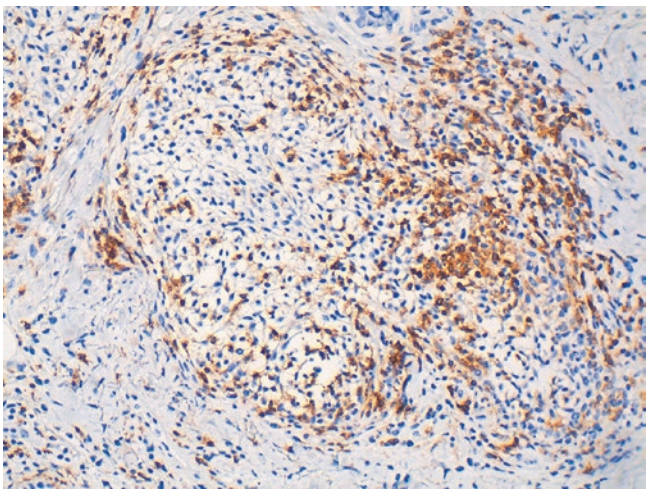

Fig. 15.73 Mycosis fungoides, folliculotropic variant. The neoplastic lymphoid cells expressing CD4 colonise the follicular structures

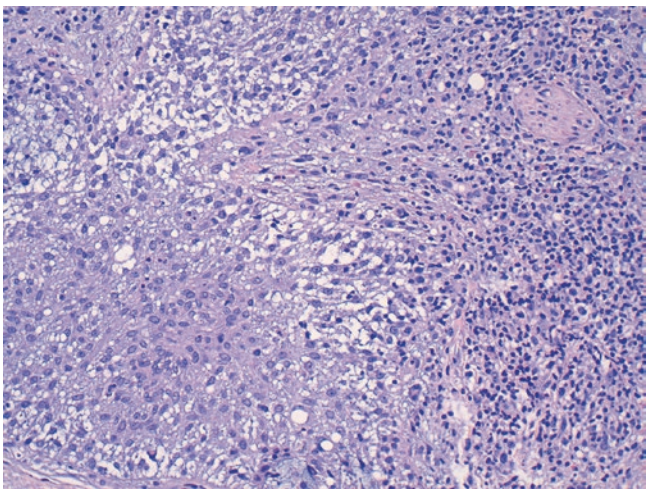

Fig. 15.72 Mycosis fungoides, folliculotropic variant. Follicular mucinosis, due to a collection of acid mucopolysaccharides in the follicular epithelium, is present in this particular case

the basal layer and even cytoid bodies' presence can be found. The small groups of neoplastic cells colonising the epidermis are called Pautrier microabscesses. In the superficial dermis, mature lymphoid cells, plasma cells and even eosinophils can be found. Syringotropism and folliculotropism can be seen. In tumor-stage MF, epidermotropism can be lost, and the lymphoid neoplastic cells are obvious and usually occupy the whole dermis, possibly affecting subcutaneous tissue. The large neoplastic cells with hyperchromatic and convoluted nuclei, called Sézary cells, can rarely be found in early lesions and are frequent in advanced stages.

In the folliculotropic variant of MF, neoplastic cells colonise the follicles (Figs. 15.71, 15.72 and 15.73). Follicular mucinosis, due to a collection of acid mucopolysaccharides in the follicular epithelium, is present in some cases.

In the pagetoid reticulosis type, the epidermis is hyperplastic, and neoplastic lymphocytes are limited to the intraepidermal location.

In the granulomatous variant of mycosis fungoides, granulomas are intermingled with the neoplastic cells [140]. Elastophagocytosis may be seen.

Immunohistochemistry MF is commonly composed of neoplastic helper/memory T-lymphocytes expressing CD4+ and CD45RO+. However, in rare occasions, the lymphoid cells are CD8+. They usually express pan-T-cell antigens, such as CD3, CD2, CD3, CD5 and CD7, as well as TCRαβ. CD7 expression is frequently lost in more than 50 % of the neoplastic cells (Fig. 15.76). The loss of CD2 or CD5 is more infrequent. Rarely, the neoplastic cell has a cytotoxic phenotype and expresses TIA-1 and granzyme B. CD30 can be expressed in some large cells, but expression in more than 25 % of neoplastic cells indicates transformation [139–141].

Genetics T-cell receptor (TCR) gene rearrangements (TCRγ and TCRβ) are identified in most cases and are useful for diagnostic purposes [142].

Differential diagnosis The main differential diagnosis must be done with inflammatory conditions. Chronic superficial dermatitis frequently shows spongiosis. Atypia and epidermotropism are not usually seen. Lymphomatoid drug reactions may show identical histological features to MF. It is important to know that immunohistochemical loss of CD7 and even T-cell rearrangements can be found in inflammatory conditions. The diagnosis must be done with the integration of clinical, histological, immunohistochemical and molecular features.

Tumor-stage MF may be differentiated from other lymphomatous infiltrates, particularly if the cells are very pleomorphic and especially if epidermotropism is absent. Again, the clinical, histological and immunohistochemical characteristics of the lesion are important to reach a correct diagnosis.

Folliculotropic MF must be distinguished from lichen planopilaris, pseudolymphomatous folliculitis, follicular lymphomatoid papulosis and follicular mucinosis/alopecia mucinosa.

The histological features in Sézary syndrome may be similar to those in MF. However, the cellular infiltrates in Sézary syndrome are more often monotonous, and epidermotropism may sometimes be absent. In contrast to MF, in Sézary syndrome, there is usually a generalised erythroderma with lymphadenopathies, and there is presence of neoplastic cells in skin, lymph nodes and peripheral blood.

Treatment and prognosis MF has a chronic, relapsing course [142]. It is usually an indolent disease, but prognosis of patients depends on the type and extent of the disease, the stage and the presence of extracutaneous spread. The folliculotropic variant of MF has a higher incidence of disease progression and has a worse prognosis.

Large-cell transformation in MF ranges from 8 to 55 % of cases and implies a worse prognosis [141, 143]. In these cases, more than 25 % of large cells must be found [144].

Patients having a patch- or plaque-stage disease can be treated with topical corticosteroids and nitrogen mustard. Phototherapy can be useful for widespread lesions. Immunomodulators, biologic agents, targeted therapies, immunosuppressants or chemotherapy have been used for refractory early stage, transformed MF and folliculotropic MF.

15.6.4.2 Indolent CD8+ Lymphoid Proliferation of the Ear

Definition It is a distinctive variant of cutaneous T-cell lymphoproliferative disorder with a characteristic clinical presentation in the ear and good prognosis.

Epidemiology Since the first description of this distinctive lymphoproliferative lesion in the ear (in the helix or the earlobe), other locations in the face, including the nose or the eyelid, have been described [145, 146]. Bilateral lesions are rare.

Etiology and pathogenesis The pathogenesis is unknown. It has been suggested that it represents a phenotypic variant of small–medium pleomorphic cutaneous T-cell lymphoma [147].

Clinical aspects It presents with asymptomatic, solitary or multiple papules, nodules or plaques on the ear or other locations in the face.

Microscopy The lymphoid proliferation usually occupies the dermis, focally with subcutaneous affectation. Epidermotropism is not a feature and the grenz zone is usually preserved. Tumor cells are monotonous medium sized. They have a blast-like appearance, with irregular nuclei, and a small nucleolus. The proliferative activity is low.

Immunohistochemistry The tumor cells express CD3, CD8, CD45RA, with a cytotoxic phenotype and expression of TIA-1 and even granzyme B. CD4 and CD30 are negative, and other T-cell markers, including CD2 and CD5, can be lost. A few reactive B cells are present in the background.

Genetics Monoclonal T-cell rearrangement of the T-cell receptor-gamma chain is usually found.

Differential diagnosis Main differential diagnosis must be performed with CD8-cytotoxic aggressive lymphoma. The latter shows a marked epidermotropism and is considered a high-grade tumor.

Treatment and prognosis Surgical treatment, radiotherapy or topical corticosteroids are usually sufficient. The behaviour is indolent with no dissemination.

15.6.5 Primary Cutaneous CD30+ T-Cell Lymphoproliferative Disorders

15.6.5.1 Lymphomatoid Papulosis

Definition Lymphomatoid papulosis (LP) is a chronic, self-healing lymphoproliferative T-cell disorder.

Epidemiology It occurs more frequently in males in the fifth decade of life. However, it can appear at any age, including children. The lesions usually affect trunk and limbs, but can develop anywhere on the body.

Etiology and pathogenesis The etiology of LP is unknown. It is a clonal proliferation of T-lymphocytes, in most cases T-regulatory subtype. Controlled growth and regression of the lesions have been attributed to TGF-β secretion and signalling.

Clinical aspects LP presents as erythematous papules, 0.5–1 cm in diameter, but sometimes larger. They can be scarce or numerous. The lesions develop over 3 or 4 weeks and become haemorrhagic and necrotic. Then they usually heal, leaving atrophic scars. It takes from several weeks to several months to regress.

Microscopy The microscopic appearance of lymphomatoid papulosis varies, correlating with the evolutive stage of the lesion biopsied. To date, five histological subtypes have been described [148–150]:

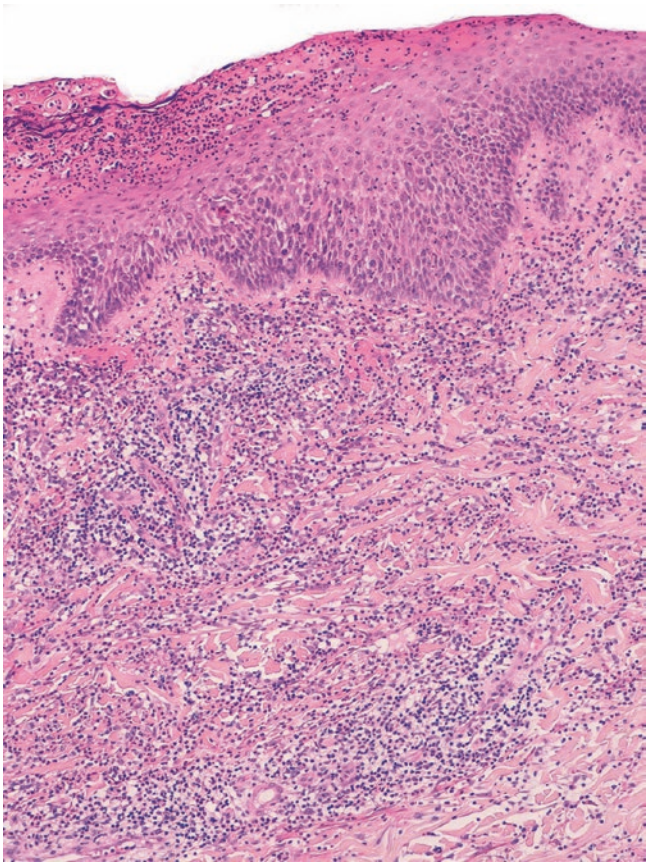

Fig. 15.74 Lymphomatoid papulosis type A: there is a wedge-shaped mixed, dense dermal cellular infiltrate. There is epidermal ulceration in this particular case

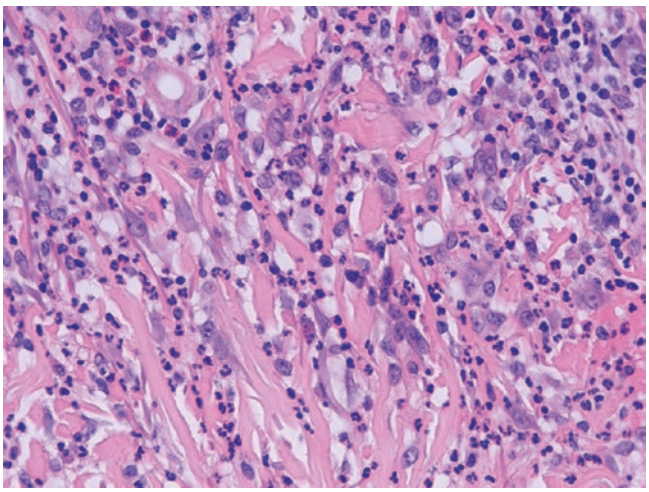

Fig. 15.75 Lymphomatoid papulosis type A: in the cellular infiltrate, large cells with pleomorphic, vesicular nuclei with prominent nucleoli are seen

Type A: By far the most frequent subtype. There is a wedge-shaped mixed dermal infiltrate (Figs. 15.74, 15.75 and 15.76). Rarely, subcutaneous tissue can be affected. In the cellular infiltrate, large cells with pleomorphic, vesicular nuclei with prominent nucleoli are present. Mitotic figures can be seen. The cellular background is composed of lymphocytes, plasma cells, histiocytes, eosinophils and neutrophils. There is epidermotropism of T-lymphocytes.

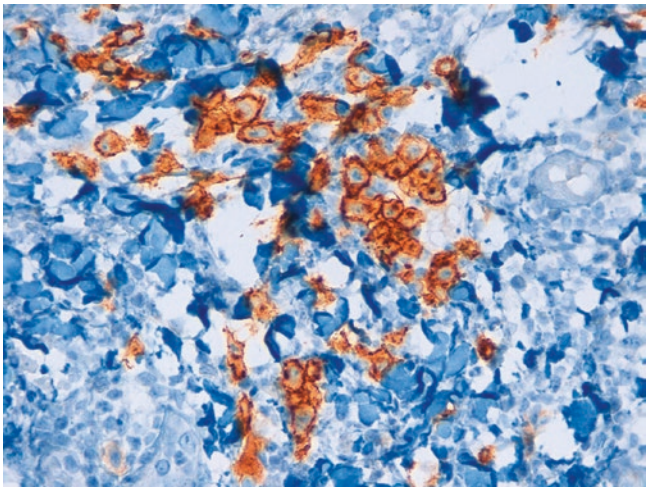

Fig. 15.76 Lymphomatoid papulosis type A: the large, pleomorphic cells express CD30

Type B: Predominantly composed of small- to medium-sized pleomorphic T-lymphocytes. These atypical cells are located in the upper dermis. Epidermotropism is prominent.

Type C: There is a nodular infiltrate predominantly composed of large pleomorphic cells.

Type D: An epidermotropic CD8-cytotoxic variant of LP.

Type E: This recently described variant is characterised by having angioinvasive growth. The atypical lymphoid cells are small- to medium-sized and express CD8 and CD30.

Immunohistochemistry The atypical lymphoid cells are usually CD4+/CD8−. Rarely, the immunophenotype of the atypical lymphoid cells can be CD4−/CD8+. Cytotoxic phenotype is frequently found, especially in LP types D and E. There is variable expression of the pan-T-cell antigens, CD2, CD3, CD5 and CD7. CD7 expression is frequently lost. The anaplastic cells express CD45 and CD30, with the exception of type B in which CD30 can be negative. Most LP (75–80 %) are also positive for MUM1, a member of the interferon regulatory factor family of transcription factors.

Genetics Clonal rearrangement of T-cell receptor genes is frequently found. Recently, the rearrangement of *DUSP22-IRF4* locus on 6p25.3 has been described in a subgroup of LP [151].

Differential diagnosis Type B of LP is histologically very similar to plaque-stage mycosis fungoides. However, Pautrier microabscesses and basal lymphocytic disposition are not seen. LP type C can be histologically indistinguishable from a large-cell anaplastic CD30+ lymphoma. LP type D can mimic pri-

mary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma, and LP type E resembles the aggressive form of angiocentric, angiodestructive and cytotoxic T-cell lymphoma. In all cases, the clinical aspect and evolution of the lesions are the clues to reach a correct diagnosis.

Treatment and prognosis LP is a chronic, sometimes relapsing, lymphoproliferative condition with a very good prognosis and a 5-year survival of 100 %. In a small number of cases, Hodgkin lymphoma, mycosis fungoides or large T-cell CD30+ lymphoma can develop. There are not risk factors to predict the lymphoma development. A close follow-up of the patients is recommended.

15.6.5.2 Primary Cutaneous Anaplastic Large-Cell Lymphoma

Definition Primary cutaneous anaplastic large-cell lymphoma (ALCL) is a neoplasm composed of large, atypical lymphocytes which express CD30 in more than 75 % of cells.

Epidemiology It usually affects patients in the sixth decade of life, with a predominance of males. It is more frequent in HIV-infected patients. It usually affects extremities and the head.

Etiology and pathogenesis Cutaneous ALCL appears de novo, and there is not a previous history of mycosis fungoides, lymphomatoid papulosis or other T-cell lymphoproliferative disorders.

Clinical aspects ALCL presents as an asymptomatic, solitary and firm nodule that presents a rapid growth and ulceration. In 20 % of cases, there are multiple lesions. Extracutaneous spread is seen in about 10 % of cases.

Microscopy Histologically, it is composed of a cellular proliferation in dermis and subcutaneous tissue. Some cases show epidermotropism. The neoplastic cells are large, with abundant cytoplasm and irregularly shaped nuclei with one or more nucleoli. Mitoses are numerous. In the background, there are small reactive lymphocytes. Some cases may show abundant neutrophils.

Immunohistochemistry ALCL is a T-cell lymphoma which usually expresses CD2, CD3, CD4, and activation markers such as CD25, CD30 and HLA-DR. Cytotoxic molecules such as TIA-1 and granzyme B are frequently expressed. CD30 must be expressed in at least 75 % of neoplastic cells. There is a variable loss of T-cell antigens CD2, CD3, CD5 and CD7. Anaplastic lymphoma-related tyrosine kinase (ALK), indicative of the 2;5 chromosomal translocation, is always negative.

Genetics Most cases show clonal rearrangement of T-cell receptor. It does not have the translocation t (2;5) (p23;q35) resulting in expression of ALK protein, characteristic of systemic anaplastic large-cell lymphoma [152].

Differential diagnosis Primary cutaneous ALCL shows similar histology and immunophenotype to primary nodal or systemic anaplastic large-cell lymphoma. However, they differ in the clinical presentation and prognosis, as well as in the molecular profile [152]. Cutaneous ALCL fails to show the translocation t(2;5) (p23;q35), characteristic of the systemic anaplastic lymphoma.

Treatment and prognosis Cutaneous ALCL has a favourable prognosis with a 5-year survival of 90 %. About 40 % of cases regress spontaneously. Spontaneous regression and age less than 60 years are associated with a better prognosis, while extracutaneous disease and older age are related to a worse outcome.

Surgical excision and radiotherapy are the most commonly used therapies for localised cutaneous ALCL. Chemotherapy is usually added for patients with multifocal or relapsing disease [153].

15.6.5.3 B-Cell Lymphomas

Primary Cutaneous Marginal Zone B-Cell Lymphoma

Definition Primary cutaneous marginal zone B-cell lymphoma (PCMZL) is an indolent lymphoma composed of small B cells. The previously used terms cutaneous immunocytoma and primary cutaneous plasmacytoma without underlying myeloma should be considered PCMZL. It forms part of the group of extranodal marginal zone B-cell lymphomas commonly involving mucosal sites, called MALT (mucosa-associated lymphoid tissue) lymphomas.

Epidemiology PCMZL usually affects extremities and trunk, but the head and neck region can be also affected [154].

Etiology and pathogenesis An association with *Borrelia burgdorferi* infection has been reported in a number of European cases, but not in cases from Asia or the United States [155].

Clinical aspects The patients usually present red papules, plaques or nodules. Ulceration is uncommon. It has a tendency to recur, but extracutaneous spread is very infrequent. The tendency to extracutaneous extension of cases from the head and neck has been reported [154].

Microscopy PCMZL form nodular or diffuse dermal infiltrates, composed of small centrocyte-like lymphocytes from marginal zone B, lymphoplasmacytoid cells and plasma cells, admixed with small numbers of centroblast- or immunoblast-like cells and many reactive T cells. Reactive germinal centres

are frequently observed. Rarely, PCMZL can show transformation into a diffuse large B-cell lymphoma.

Immunohistochemistry The marginal zone B cells express CD20, CD79a and BCL-2, but are negative for CD5, CD10 and BCL-6 [156]. Plasma cells show immunoglobulin light chain restriction, with predominantly expression of kappa chains.

Genetics There is clonal rearrangement of immunoglobulin heavy chain (IgH) genes. Some studies suggest the presence of the t(14;18)(q32;q21) involving the *IGH* gene on chromosome 14 and the *MALT1* gene on chromosome 18 and t(3;14)(p14.1;q32) involving *IGH* and *FOXP1* genes, in a proportion of PCMZLs [157, 158]. However, other translocations observed in gastric MALT lymphomas, such as t(11;18)(q21;q21) and t(1;14)(p22;q32) have not been found in PCMZL.

Differential diagnosis Main differential diagnoses must be performed with B-cell lymphoid hyperplasia and follicular lymphoma. The three conditions have a different immunophenotype of the neoplastic cells [126, 156].

Treatment and prognosis The prognosis of PCMZL is excellent. In patients with associated *Borrelia burgdorferi* infection, systemic antibiotics should be administered first.

Patients with solitary or few lesions can be treated with radiotherapy or surgical excision. Patients presenting multifocal skin lesions could benefit from chlorambucil or intralesional or subcutaneous administration of interferon alpha.

Primary Cutaneous Follicle-Centre Lymphoma

Definition Primary cutaneous follicle centre lymphoma (PCFCL) is defined as a neoplasia of follicle centre cells, usually having a mixture of centrocytes and centroblasts.

In the past, the terms 'reticulohistiocytoma of the dorsum' and 'Crosti lymphoma' were used for cases with presentation on the back.

Epidemiology The lesions appear frequently on the head or trunk. The legs are infrequently involved.

Etiology and pathogenesis The origin of this lymphoma is the follicle centre cell. This has been supported by the finding of mutated VH genes with mutation patterns reminiscent of antigen selection process.

Clinical aspects The characteristic clinical presentation of this lymphoma is a solitary lesion or several grouped plaques and indurated tumors on the scalp, the forehead or the trunk [159]. The lesions have a tendency to grow slowly, and plaques usually develop into tumoral lesions.

Multifocal lesions and extracutaneous spread are infrequent.

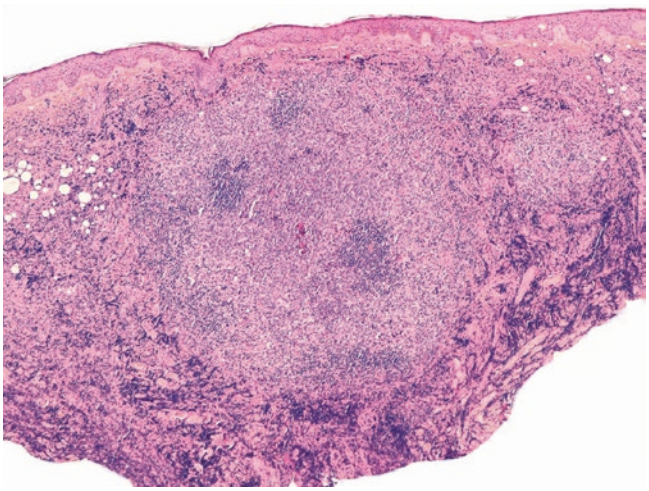

Fig. 15.77 Primary cutaneous follicular lymphoma. The neoplastic cells have a nodular growth pattern in the dermis

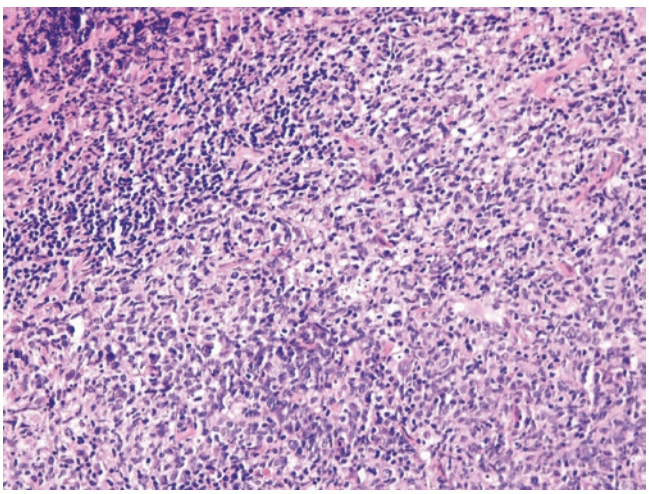

Fig. 15.78 Primary cutaneous follicular lymphoma. The tumor is composed of a mixture of small and large multilobulated centrocytes, centroblasts and immunoblasts

Microscopy PCFCL can have a nodular or diffuse growth pattern in dermis with sparing of the epidermis (Figs. 15.77 and 15.78). Lesions on the scalp usually have a better defined follicular pattern than those on the trunk. The follicles are ill defined, lack tingible body macrophages and generally have a reduced or absent mantle zone. Small and early lesions contain a mixture of centrocytes, relatively few centroblasts and many reactive T cells. Large centrocytes, often multilobated, are usually found. Tumoral skin lesions generally show a monotonous population of large follicle centre cells, generally large, multilobated centrocytes and multilobated cells and a variable admixture of centroblasts and immunoblasts. A prominent stromal component is usually present.

Immunohistochemistry The neoplastic cells express B-cell-associated antigens CD20, CD79a, PAX-5

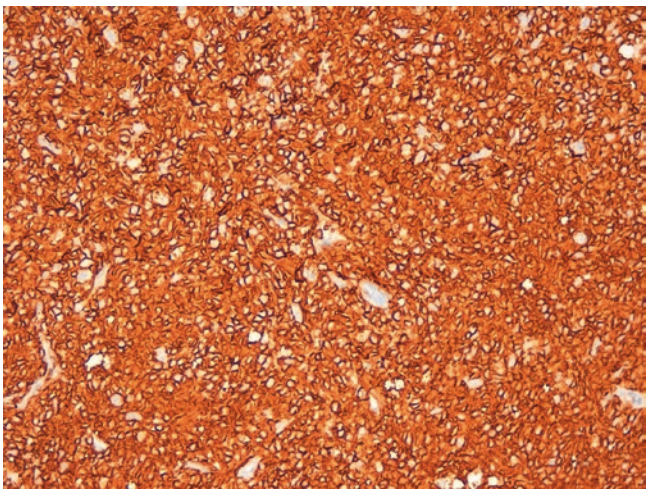

Fig. 15.79 Primary cutaneous follicular lymphoma. The neoplastic cells express B- associated antigens (CD20)

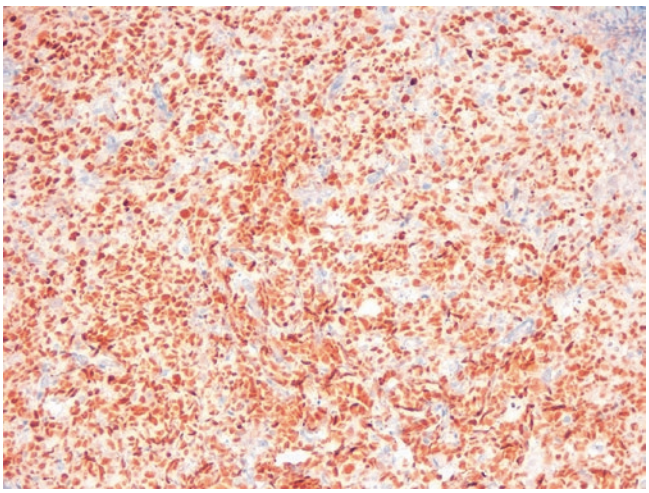

Fig. 15.81 Primary cutaneous follicular lymphoma. The neoplastic cells express BCL-6

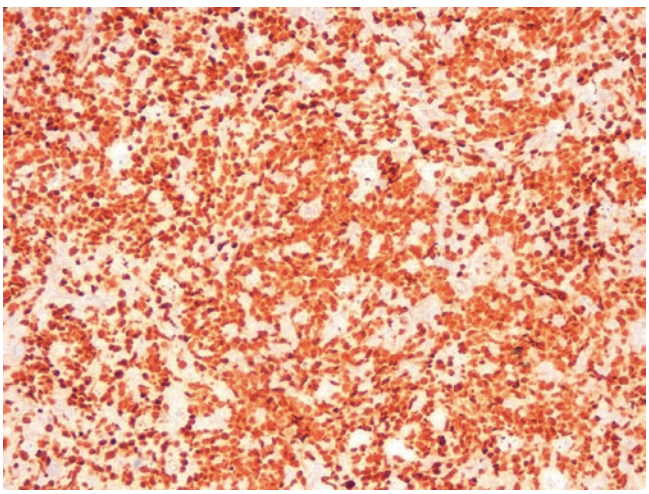

Fig. 15.80 Primary cutaneous follicular lymphoma. The neoplastic cells express PAX-5

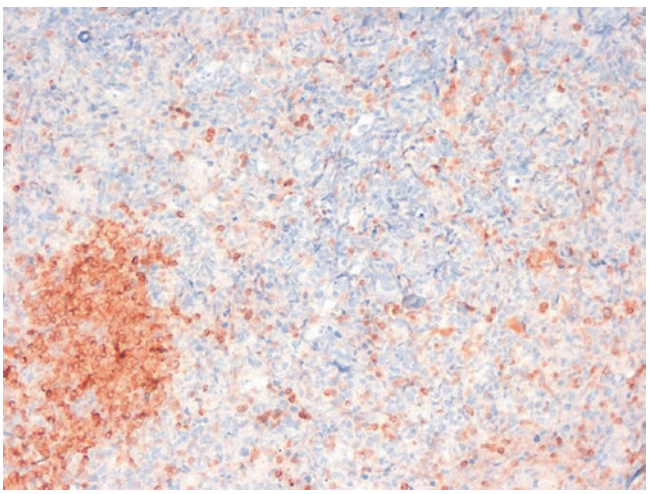

Fig. 15.82 Primary cutaneous follicular lymphoma. The neoplastic cells do not express BCL-2

(Figs. 15.79 and 15.80) and BCL-6 (Fig. 15.81) [81]. These cells are disposed in a network of dendritic cells which can be highlighted with CD21 or CD35. CD10 is positive in cases with follicular growth pattern, but can be negative in cases with diffuse growth pattern. In contrast to nodal follicular lymphoma, BCL-2 is not expressed (Fig. 15.82).

Genetics There is clonal rearrangement of immunoglobulin genes. In most studies, PCFCLs, including cases with a follicular growth pattern, do not show the t(14;18), which is characteristically found in systemic follicular lymphomas and a proportion of systemic diffuse large B-cell lymphoma [159, 161]. Inactivation of p15 and p16 tumor suppressor genes by promoter hypermethylation has been reported in about 10 % and 30 % of PCFCLs, respectively [161].

Differential diagnosis Differential diagnosis with lymphoid reactive hyperplasia and primary cutaneous marginal zone lymphoma are based on immunohistochemical profile of the lesion [156, 160].

Secondary cutaneous affectation of a systemic lymphoma has a different immunophenotype and molecular characteristics.

Treatment and prognosis PCFCLs have an excellent prognosis with a 5-year survival higher than 95 %. The histological growth pattern (follicular or diffuse), the number of blast, large cells and the extent of the lesion in the skin do not have prognostic significance.

The treatment of choice is radiotherapy, even in cases with relapse. Chemotherapy is required only in cases with very extensive cutaneous lesions or in patients with extracutaneous spread.

15.7 Cutaneous Histiocytic and Xanthomatous Infiltrates

15.7.1 Juvenile Xanthogranuloma

Definition and synonyms Juvenile xanthogranuloma represents a variant of a self-limited non-Langerhans cell (non-X) histiocytosis. The entity is not restricted to infancy and/or childhood, and the expression 'xanthogranuloma' has been suggested as the preferred terminology. Similar lesions have also been designated in the past as 'nevoxanthoendothelioma' or 'xanthoma multiplex'.

Epidemiology Xanthogranuloma represents the most common variant of non-Langerhans cell histiocytosis with an estimated incidence of 1–2 % of healthy infants and young children. Although the majority of the lesions develop within the first 5 years of life, they show predilection for infancy with about 50 % of the lesions developing within the first 6 months of life and can already be present at birth [162]. About 20 % of the lesions develop in adolescents and young adults [163, 164]. In addition, occurrence in adults and elderly patients is not uncommon – such examples have also been designated as 'late-onset' juvenile xanthogranulomas [164]. Caucasians are most commonly affected. Xanthogranulomas appear to be more common in males [162, 163]. Nevertheless, the great majority of patients (over 90 %) with multiple skin lesions are males [163]. Familial occurrence has not been reported.

Etiology and pathogenesis The etiology and pathogenesis of xanthogranuloma are unknown.

Clinical aspects Xantogranuloma usually presents as a well-demarcated, firm, round- or oval-shaped papule or nodule of yellowish to red/brownish discolouration measuring up to 1 cm in greatest diameter. Giant lesions measuring in excess of 2 cm in diameter have also been reported and show predilection for upper trunk and proximal limbs. Xanthogranuloma is generally asymptomatic, but may on occasion be associated with itching. Solitary lesions by far predominate [163]. They most commonly develop on the skin of the head and neck, followed by the trunk and upper and lower extremities [163]. Multiple skin lesions occur in less than 10 % of the patients. Involvement of mucosal sites, including oral and nasal cavity, is rare. Extracutaneous lesions are present in about 25 % of the cases, and show predilections for soft tissues, orbit and visceral organs. Visceral involvement is frequently multifocal and can be associated with multiple skin lesions.

Juvenile xanthogranuloma can be associated with neurofibromatosis type I. Such patients have 20 to 30-fold increased risk of myelomonocytic leukaemia with juvenile xanthogranuloma generally preceding the development of hematologic malignancy.

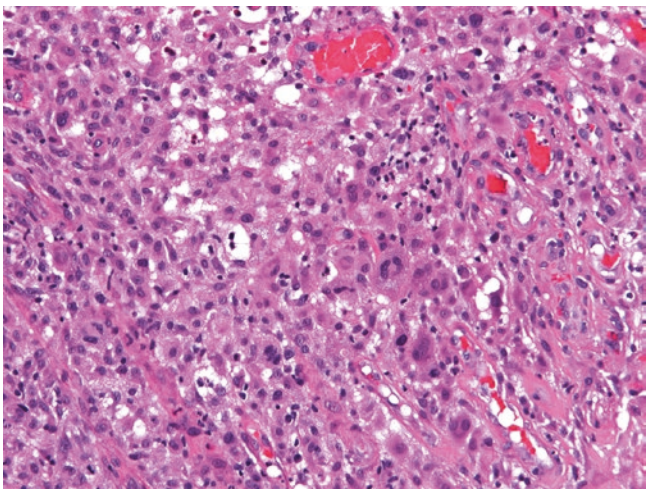

Fig. 15.83 Juvenile xanthogranuloma. Numerous foamy macrophages are admixed with mild mononuclear inflammatory cell infiltrate and rare multinucleated giant cells

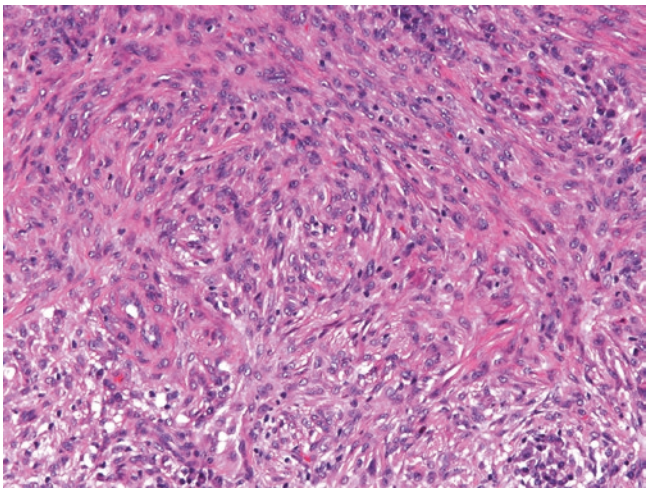

Fig. 15.84 Juvenile xanthogranuloma. Spindle cells population can represent the predominant cell type

Microscopy Xanthogranuloma is characterised by proliferation of mononuclear cells, multinucleated giant cells with or without Touton features (e.g. central wreath of nuclei and peripheral rim of eosinophilic cytoplasm) and spindle cells [163]. The cytoplasm of mononuclear and multinucleated giant cells is frequently finely vacuolated or lipidised, imparting a xanthomatised appearance (Fig. 15.83). Spindle cell population may represent the predominant cell component (Fig. 15.84). The number of Touton-type giant cells can vary from absent/very few to numerous. In the skin, lesional cells typically form well-defined nodules in the papillary and reticular dermis. Extension into the subcutis is rare. In addition,

inflammatory cell infiltrate composed of lymphocytes, eosinophilic and neutrophilic granulocytes is usually present, albeit in variable proportions. Older lesions can be associated with dermal scarring. The overlying epidermis is usually normal, but can also be atrophic with effacement of the rete ridges pattern. Extracutaneous lesions frequently lack or contain limited amounts of multinucleated Touton-type giant cells [163]. By immunohistochemistry, the lesional cells are generally positive for histiocytic/macrophage markers, including CD68 and CD163. There is a variable positivity for factor XIIIa. About 20 % of cases show weak S100 protein positivity [165], while CD1a and langerin are consistently negative.

Differential diagnosis Xanthomas generally lack background inflammatory cell infiltrate and multinucleated giant cells. Reticulohistiocytoma contains multinucleated giant cells with typical ground-glass eosinophilic cytoplasm and lacks Touton-type giant cells and xanthomised macrophages. Rosai–Dorfman disease is distinguished by emperipolesis of inflammatory cells and consistent S100 protein positivity of the lesional cells.

Treatment and prognosis The majority of cutaneous lesions display spontaneous regression over time and no treatment is generally required. Visceral involvement may prove fatal in exceptional cases [163].

15.7.2 Histiocytosis

Definition and synonyms Benign cephalic histiocytosis, originally described by Gianotti et al. in 1971, represents a variant of a self-limited non-Langerhans cell (non-X) histiocytosis [166].

Epidemiology Benign cephalic histiocytosis shows predilection for infancy and childhood, with the majority of cases presenting within the first 3 years of life [166, 167]. Although the average age of disease onset ranges from 13 to 15 months, about 50 % of the cases occur before the age of 6 months [167]. The disease is slightly more common in boys with a male-to-female ratio of 1.7:1 [167]. All ethnic groups can be affected. Congenital occurrence has not been reported.

Etiology and pathogenesis The etiology and pathogenesis of benign cephalic histiocytosis remain unknown. Nevertheless, it has been suggested that benign cephalic histiocytosis, generalised eruptive histiocytosis and juvenile xanthogranuloma likely represent a group of related diseases with overlapping clinical and histological features [168, 169].

Clinical aspects The patients typically present with asymptomatic slightly raised multiple small yellow to red-brown papules and less often with macules, measuring from 2 to 8 mm in largest diameter [166]. The number of lesions varies greatly among the patients, from a few to over 100 papules. The lesions show predilection for the face, in particular cheeks, eyelids and forehead, followed by the neck. Not uncommonly, other parts of the body may also be involved, including the trunk, limb girdles, proximal extremities and genital areas [166, 169]. New papules continue to develop within the first 2 years of disease onset [167, 169]. In addition, fusion of papules or formation of reticular growth pattern has been reported in exceptional cases. Involvement of mucosal sites, palms and soles or visceral organs is characteristically absent [167, 169].

As a rule, spontaneous regression always occurs, but the process may take time to complete (range from 9 to over 100 months, median time 37 months) [167]. The regression usually starts on the face and is characterised clinically by flattening and eventual disappearance of the papules, leaving residual areas of transient hyperpigmentation [167, 169]. Scarring is usually not a feature of benign cephalic histiocytosis, although small areas of atrophic scars have exceptionally been reported [169].

The systemic symptoms are generally lacking in benign cephalic histiocytosis, and laboratory investigations, including serum lipid levels and haematological tests, are normal [169]. Association with insulin-dependent diabetes mellitus and diabetes insipidus has been reported in exceptional cases, yet this coexistence is likely to be coincidental.

Microscopy On histology, the lesion is covered by normal or slightly attenuated epidermis with flattened rete ridges pattern. Epidermotropism is absent. In the papillary and superficial to mid-reticular dermis, a variably abundant infiltrate is seen, composed of an admixture of histiocytes, lymphocytes and eosinophilic granulocytes [166, 169]. Histiocytes typically display epithelioid morphology with hyperchromatic, oval and vesicular nuclei, prominent nucleoli and abundant eosinophilic cytoplasm. Older lesions can contain dispersed multinucleated giant cells. Nevertheless, foam cells and Touton-type giant cells are consistently absent [166, 169].

By immunohistochemistry, the lesional cells express markers of histiocytic differentiation, including CD68 and factor XIIIa [166]. They are consistently negative for CD1a and langerin. S100 protein positivity has exceptionally been reported [170].

Electron microscopy of the lesional cells reveals the presence of coma-shaped and worm-like cytoplasmic bodies, coated vesicles and desmosome-like junctions, while Birbeck granules are uniformly absent [169, 171].

Differential diagnosis The main differential diagnoses include juvenile xanthogranuloma, generalised eruptive histiocytosis and Langerhans cell histiocytosis [167, 169].

Juvenile xantogranuloma typically contains, in addition to histiocytes, foam cells and Touton-type giant cells. Not infrequently, serial sections may be necessary to demonstrate the latter two cell types. Generalised eruptive histiocytosis shows morphological overlap with benign cephalic histiocytosis, but is distinguished clinically by more widespread distribution of the lesions and involvement of mucous membranes. Langerhans cell histiocytosis can easily be distinguished by demonstration of langerin positivity by immunohistochemistry.

Treatment and prognosis Benign cephalic histiocytosis is a self-limiting disease with excellent prognosis [167, 169]. Complete regression of the lesions eventually develops and no treatment is generally necessary.

15.7.3 Multicentric Reticulohistiocytosis

Definition and synonyms Multicentric reticulohistiocytosis is a variant of multisystem non-Langerhans cell (non-X) histiocytosis characterised by development of erosive/destructive polyarthritis and papulonodular skin lesions, with frequent additional involvement of mucous membranes and visceral organs.

The entity has been designated in the past as reticulohistiocytic granuloma, giant cell histiocytosis, lipoid dermatoarthritis and giant cell reticulohistiocytosis.

Epidemiology Multicentric reticulohistiocytosis occurs more commonly in females (F:M=2–3:1) and most frequently presents in the fourth decade of life. The disease may exceptionally develop in children and is most uncommon in elderly patients. There appears to be a predilection for Caucasians, although other ethnic groups can also be affected. No familiar predisposition has been reported.

Etiology and pathogenesis The etiology of multicentric reticulohistiocytosis remains unknown. Abnormal macrophage response to different triggering factors, including various infections (e.g. tuberculosis) or immunological processes, has been proposed.

Clinical aspects The onset of multicentric reticulohistiocytosis is usually insidious. Joint involvement precedes the development of skin lesions in up to 70 % of the patients [173]. The arthritis tends to be symmetrical and predominantly affects the distal interphalangeal joints [174]. Any other joint can be involved, albeit much more infrequently, including knees, shoulders, wrists, ankles and elbows. In about 50 % of the cases, the arthritis is rapidly progressive and destructive leading to severe functional disability of the joints (e.g. destructive arthropathy).

Skin manifestations generally follow involvement of joints. Nevertheless, the development of cutaneous changes may be delayed from a few months to several years (mean delay is 3 years) [174]. Skin lesions are characterised by flesh-coloured reddish-brown papules or nodules measuring from a few millimetres to 2 cm in largest diameter. The eruption shows predilection for the face, in particular nose, paranasal sinuses, and ears, followed by hands, neck and trunk [174]. Not uncommonly, papules and nodules coalesce producing thereby a cobblestone appearance. Confluence of the lesions on the face may be associated with severe disfigurement (e.g. leonine face). The lesions on the hands typically develop on the dorsum and lateral aspects of the fingers. In addition, periungual papules are present in about 50 % with a characteristic 'coral bead' appearance [175]. Palpebral xanthelasma-like changes are present in up to one-third of the patients [172].

Much more infrequently (in about 20 %), skin lesions predate development of joint changes. Exceptionally, skin changes may represent the sole manifestation of the disease, e.g. cutaneous multicentric reticulohistiocytosis.

Mucosal lesions, including oral (predilection for lips and tongue), pharyngeal and nasal mucosa, are present in about 50 % of the patients and usually coexist with cutaneous lesions.

Multicentric reticulohistiocytosis has been associated in 5–20 % with a variety of autoimmune diseases and different malignancies in up to 25 % [174]. The most common autoimmune conditions include rheumatoid arthritis, systemic lupus erythematosus, Sjögren syndrome, hypothyroidism, diabetes mellitus, primary biliary cirrhosis, systemic vasculitis and celiac disease [172, 174, 176]. Multicentric retuculohistiocytosis may precede the diagnosis of a malignant tumor. Nevertheless, there appears to be no predominant type of tumor associated with multicentric reticulohistiocytosis since development of tumors as diverse as carcinomas, sarcomas, melanomas, mesotheliomas, lymphomas and haematological malignancies have been reported.

Constitutional symptoms including fever, weakness, weight loss and malaise can be present in about one-third of the patients [174]. Laboratory tests are generally nonspecific, but hyperlipidaemia has been reported in 30 to 58 % of the patients [174].

Visceral involvement is present in a minority of patients, manifesting as pleural effusions, pericarditis, progressive heart failure and myositis-like symptoms [174].

Microscopy The epidermis overlying the lesion is unaffected, but can on occasion be atrophic with effacement of rete ridges pattern. The typical lesion consists of a nodular proliferation of macrophages and multinucleated giant cells in the papillary and reticular dermis, admixed with a variably abundant inflammatory cell infiltrate composed of lymphocytes, plasma cells and eosinophils (Fig. 15.85). The macrophages and multinucleated giant cells display a

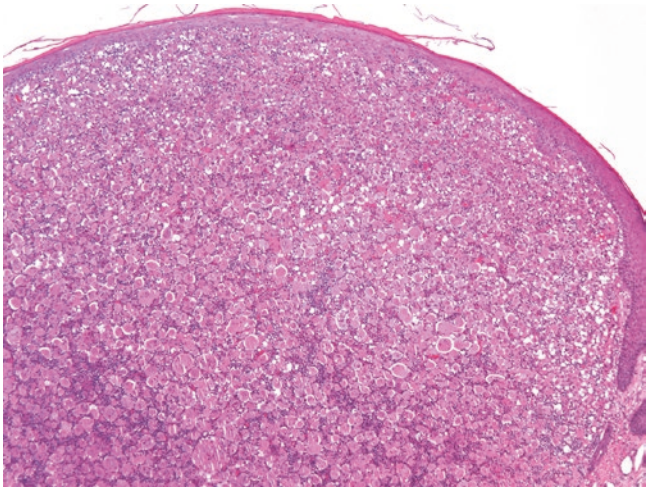

Fig. 15.85 Reticulohistiocytoma. Nodular proliferation of large macrophages is present in the dermis, intermingled with inflammatory cell infiltrate

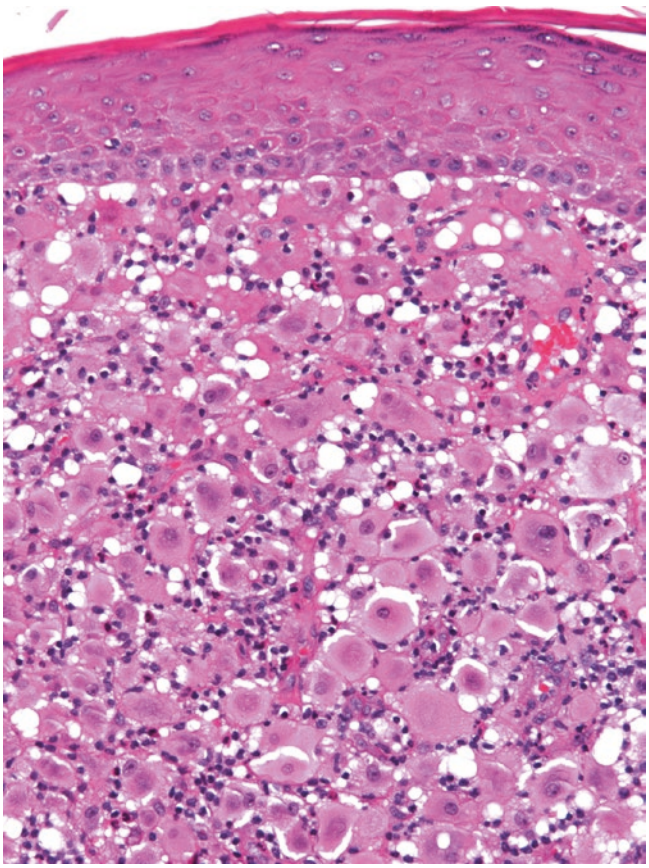

Fig. 15.86 Reticulohistiocytoma. Macrophages have abundant amphophylic cytoplasm and unremarkable nuclei. Eosinophils and lymphocytes can be appreciated in the background

characteristic eosinophilic to amphophylic 'ground-glass' cytoplasm (Fig. 15.86) [177]. The nuclei of multinucleated giant cells can be arranged haphazardly or are seen aligning towards the periphery or the centre of the cells. Foam cells are generally absent. The earlier lesions are characterised by the predominance of mononuclear cells and eosinophilic granulocytes, while an increasing number of giant cells and areas of fibrosis are typically observed in the later stages.

By immunohistochemistry, macrophages and multinucleated giant cells express markers of macrophage/histiocytic differentiation, including CD68, CD163 and mostly also factor XIIIa [178]. The giant cells are PAS-D+. Aberrant CD10 expression has been detected in the multinucleated giant cells. The lesional cells lack S100 protein, CD1a and langerin expression, although S100 protein positivity has been reported in exceptional cases.

Differential diagnosis Solitary reticulohistiocytoma essentially displays similar morphology and is distinguished clinically by representing a single/solitary lesion [177]. Juvenile xanthogranuloma is characterised by the presence of lipidised cells and Touton-like giant cells, which are lacking in reticulohistiocytosis. Melanocytic proliferations can be easily distinguished by expressing markers of melanocytic differentiation (e.g. S100 protein, melan-A, HBM45 and tyrosinase) [179]. Epithelioid fibrous histiocytoma, which is now considered unrelated to to conventional fibrous histiocytoma and histological variants typically displays ALK positivity in the majority of cases [179].

Treatment and prognosis The treatment is aimed at preventing the development of destructive changes and must be introduced early in the course of the disease. Several treatment options have been proposed, including immunosuppressive and cytotoxic drugs, albeit with variable success. Recently, anti-TNF-alpha agents proved to be particularly effective. Without treatment, the disease generally follows a rapidly progressive yet self-limiting course with stabilisation of the disease process within 5–10 years.

15.7.4 Xanthomas

Definition and synonyms Xanthomas represent a group of related disorders characterised histologically by local accumulation of lipid-laden macrophages. Not infrequently, they can be associated with abnormalities of lipid metabolism and may represent the initial manifestation of the underlying process.

Discussed below are only those xanthoma variants that can be encountered in the head and neck area, including verruciform xanthoma, xanthoma disseminatum and xanthelasma.

15.7.4.1 Verruciform Xanthoma

Definition and synonyms Verruciform xanthoma is a benign proliferation characterised by verrucous epithelial hyperplasia associated with accumulation of lipid-laden macrophages within the stromal papillae.

Epidemiology Verruciform xanthoma shows predilection for adult patients and most commonly presents in the fourth to sixth decade of life [180]. There appears to be no gender preference, although some studies suggest a slight male predominance.

Etiology and pathogenesis Etiology of verruciform xanthoma has not been clearly elucidated. A local trauma to the squamous epithelium has been suggested due to the various conditions (e.g. mechanical trauma, local inflammation in the background of some autoimmune diseases or graft versus host disease), leading to breakdown of the cells with a release of lipid material and subsequent accumulation of lipids within the macrophages [181, 182].

Clinical aspects Verruciform xanthoma presents as an asymptomatic solitary slowly growing lesion with predilection for the alveolar ridge, gingiva, followed by buccal mucosa, palate, floor of the mouth and lip [181]. Multiple lesions within the oral cavity are exceptionally rare. Most common extraoral sites represented include vulva, scrotum and penis. Serum lipid levels in patients with verruciform xanthomas are normal.

Microscopy Squamous epithelium displays prominent hyperplasia and is frequently covered by a parakeratotic layer (Fig. 15.87). The surface can be flat, verrucous or, less often, more papillary [180, 181]. Quite distinctively, collections of neutrophilic granulocytes can be present on the surface. Dysplasia of the squamous epithelium is absent. Rete ridges are typically elongated. Numerous macrophages with foamy cytoplasm are present within the superficial stroma, filling stromal papillae, and generally do not extend below the deepest portions of the rete ridges (Fig. 15.88). Multinucleated giant cells are not seen.

By immunohistochemistry, foamy macrophages are CD68+. Faint cytoplasmic S100 protein staining can occasionally be observed. CD1a and langerin are negative.

Differential diagnosis Verruciform xanthoma has distinctive morphology and there is no true differential diagnosis.

Treatment and prognosis Verruciform xanthoma does not show the tendency for spontaneous regression. Complete local surgical excision is curative. A very superficial biopsy may be mistaken for viral warts.

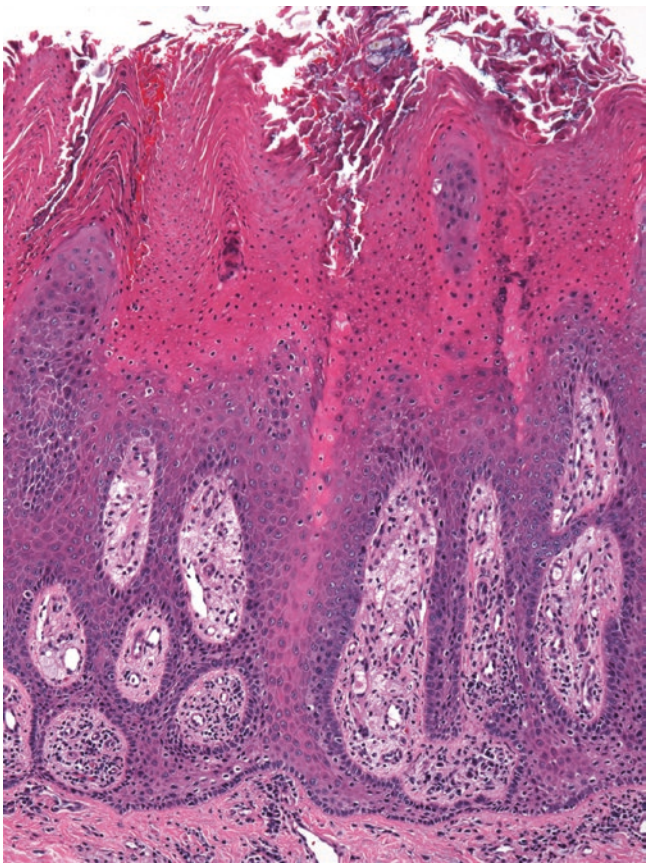

Fig. 15.87 Verruciform xanthoma. Squamous epithelium with prominent papillomatosis and parakeratosis. Collections of foamy macrophages can be seen in the subepithelial stroma/dermis

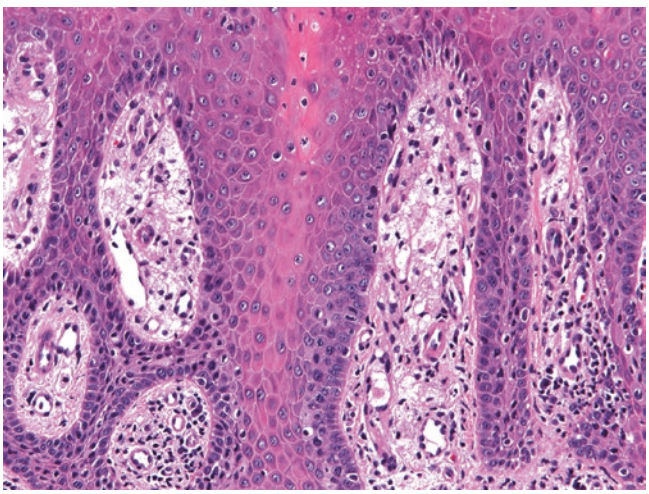

Fig. 15.88 Verruciform xanthoma. Numerous foamy macrophages are present within the stromal papillae

Xanthelasma

Definition and synonyms Xanthelasma is a benign disorder characterised morphologically by accumulation of foamy macrophages in the dermis.

Epidemiology Xanthelasma represents the most common variant of xanthoma and shows predilection for adult patients between 40 and 60 years of age [183]. There appears to be a slight female predominance.

Etiology and pathogenesis Xanthelasma can indicate an underlying disorder of lipid metabolism with increased risk of ischaemic heart disease. Up to 50 % of the patients with xanthelasma display elevated levels of total serum cholesterol and/or low-density lipoproteins [183]. Importantly, however, xanthelasma can also develop in normolipemic patients.

Clinical aspects Xanthelasma presents as multiple symmetrical asymptomatic slowly growing yellowish plaques or papules, measuring from 2 to 4 mm in diameter. Not uncommonly, they show clustering or coalescence into larger plaques or groups of papules. Plaques and papules can be either soft or hard on palpation. The sites of predilection include upper eyelids and periorbital skin, in particular in the area of the inner canthus [184].

Microscopy On histology, accumulation of macrophages with foamy or vacuolated cytoplasm is present in the superficial and mid-reticular dermis, most commonly in perivascular or periadnexal distribution (Fig. 15.89) [185]. Infiltration of the underlying skeletal muscle is rare. The changes can on occasion be quite subtle and may easily be overlooked. As a rule, other inflammatory cells and multinucleated giant cells are absent, and dermis typically lacks fibrosis. Occasionally, scattered mast cells can be found in between xanthomised macrophages.

Differential diagnosis Xanthogranuloma is distinguished from xanthelasma by the presence in the former of foamy macrophages, admixed with multinucleated giant cells and variably abundant inflammatory cell infiltrate.

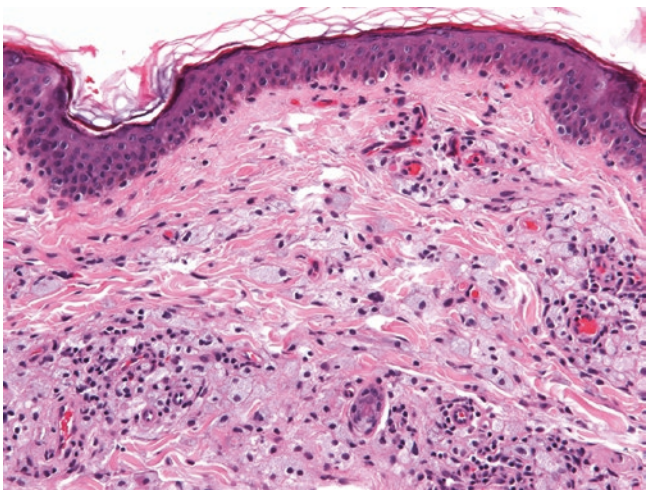

Fig. 15.89 Xanthelasma. Accumulation of macrophages with foamy or vacuolated cytoplasm is present in the superficial and mid-reticular dermis

Treatment and prognosis No spontaneous regression of xanthelasma is observed. Functional problems, such as obstructed vision, rarely develop, and the majority of lesions are removed for cosmetic reasons. Possible treatment options include surgical excision, ablation by laser or chemical substances and/or electrocoagulation. Recurrences are common [185].

Xanthoma Disseminatum

Definition and synonyms A rare cutaneous histiocytic disorder, characterised by widespread mucocutaneous lesions associated with systemic involvement developing in normolipemic patients.

Epidemiology Xanthoma disseminatum shows predilection for children and young adults with about 60 % of patients presenting before the age of 25 years [186]. Males are affected slightly more commonly than females.

Etiology and pathogenesis The etiology and pathogenesis of xanthoma disseminatum have not yet been elucidated.

Clinical aspects Xanthoma disseminatum typically presents as widespread yellow to brown/reddish plaques, papules or nodules with predilection for the scalp, face, trunk, extremities and flexural sites [186]. The lesions may eventually become generalised. In about 50 % of the cases, cutaneous lesions are associated with mucosal involvement, most frequently oral cavity (gums, tongue) and upper respiratory tract (larynx, epiglottis, trachea and bronchi) [186]. Development of xanthomas in the respiratory tract may be associated with dyspnoea, necessitating tracheostomy in extreme examples [186]. Diabetes insipidus is present in about 40 % of the patients due to the involvement of the hypothalamus and/or pituitary region. Additional involvement of conjunctiva and cornea is not uncommon and develops in about 20 % of the patients. Involvement of bones and liver is very rare. Associated diseases include multiple myeloma and Waldenström's macroglobulinemia [187, 188].

Microscopy Superficial and mid-reticular dermis contains histiocytes and foam cells growing in small groups, sheets or nodules, admixed with variable proportions of giant cells (including Touton-like giant cells). Inflammatory cell infiltrate is usually sparse. Established or advanced lesions frequently contain spindled histiocytes forming a vague storiform growth pattern.

By immunohistochemistry, the lesional cells are CD68 positive and negative for S100 protein, CD1a and langerin.

Differential diagnosis Xanthogranuloma may be undistinguishable on histological grounds alone.

Treatment and prognosis Depending upon clinical features and prognosis, three main variants of the disease can be distinguished: a self-healing variant with spontaneous resolution, a persistent variant (most common) and a rare progressive variant with organ dysfunction and central nervous system involvement. Treatment options include surgical excision, laser ablation, electrodessication and various systemic drugs including corticosteroids, anti-lipemic drugs and/or chemotherapy [189].

15.7.5 Langerhans Cell Histiocytosis

Definition and synonyms Langerhans cell histiocytosis is a proliferative disease of cells displaying phenotypic and immunohistochemical characteristics of Langerhans cells, associated with a myriad of clinical presentations. Although Langerhans cell histiocytosis has been traditionally separated into different clinical forms, including Letterer–Siwe disease (acute disseminated disease with/without visceral involvement), Hand–Schüller–Christian disease (chronic multisystem disease), eosinophilic granuloma (solitary lesion) and congenital self-healing histiocytosis (Hashimoto–Pritzker disease), it has been clearly established that they represent a spectrum of a single disease process, namely, Langerhans cell histiocytosis, alternatively designated also Langerhans cell disease [190, 191].

Epidemiology Langerhans cell histiocytosis is an uncommon disease with a predilection for children. The prevalence in children has been estimated to be up to 10/1,000,000 per year and in adults from 1 to 2/1,000,000 per year [192, 193].

Etiology and pathogenesis Although the majority of Langerhans histiocytosis cases displays clonal proliferation of Langerhans cells, both neoplastic process and reactive condition due to the deranged immune system regulation have been suggested as possible pathogenetic mechanisms [190].

Clinical aspects The World Health Organization's Committee on Histiocytic/Reticulum Cell Proliferations categorised Langerhans cell histiocytosis into two main groups with significant differences in prognosis and treatment: single system and multiple system disease [191]. Single system Langerhans cell histiocytosis includes single site (e.g. bone, lung, skin or lymph node) or multifocal disease (multiple lesions confined to the single site). Multisystem variant is further subdivided into lesions with two or more organs involved at the time of the diagnosis, without or with organ dysfunction [191].

Isolated involvement of the skin appears to be more common in children than adults and has been detected in 20–60 % [194]. Patients with isolated skin lesions can have associated mucosal involvement in about 20 % [194]. Skin lesions can be solitary (papule, nodule, ulcer), multiple or generalised. They show predilections for scalp, trunk, flexural (intertriginous) sites and genital areas. Eczematous lesions, mimicking seborrheic dermatitis, are not uncommon. Lesions in the skin folds and anogenital area are frequently ulcerated.

Microscopy Langerhans cells are characterised by folded or reniform nuclei, prominent nuclear membrane, indistinct nucleoli and moderately abundant eosinophilic cytoplasm (Fig. 15.90) [195]. The nuclei frequently display longitudinal groove and have been designated as coffee bean-shaped nuclei. Mitotic activity is usually not prominent. Langerhans cells frequently grow in clusters or sheets and are admixed with variably prominent inflammatory cell infiltrate, composed of eosinophilic granulocytes, lymphocytes, plasma cells, macrophages (including foam cell macrophages) and multinucleated giant cells. Although eosinophilic granulocytes are generally abundant, they can also be scarce or completely absent. Early lesions typically display predominance of Langerhans cells admixed with eosinophilic and neutrophilic granulocytes. Established lesions frequently display epidermotropism with formation of Langerhans cell microabscesses. Later lesions show predominance of macrophages and in addition display variable degrees of fibrosis.

Immunohistochemistry Langerin, an antibody-targeting transmembrane protein associated with Birbeck granules, is the most specific and sensitive marker of Langerhans cells (Fig. 15.91). In addition, Langerhans cells are also positive for S100 protein and CD1a [195].

Genetics Recently, the activating, oncogenic *BRAF*V600E gene mutation has been demonstrated, strongly supporting the neoplastic origin of the disease [196], and the presence of

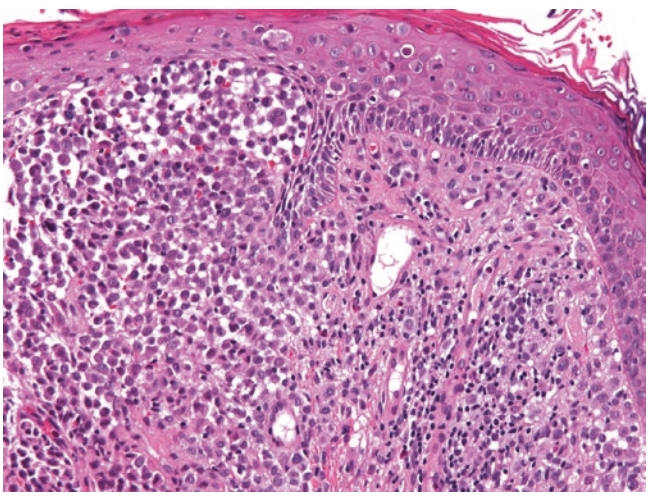

Fig. 15.90 Langerhans cell histiocytosis. Numerous Langerhans cells are present within the dermis. They focally infiltrate the epidermis

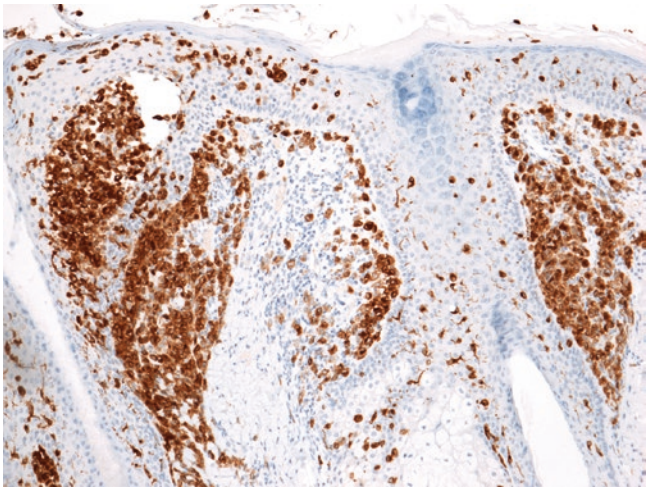

Fig. 15.91 Langerhans cell histiocytosis. Langerin is a sensitive and specific marker of Langerhans cells

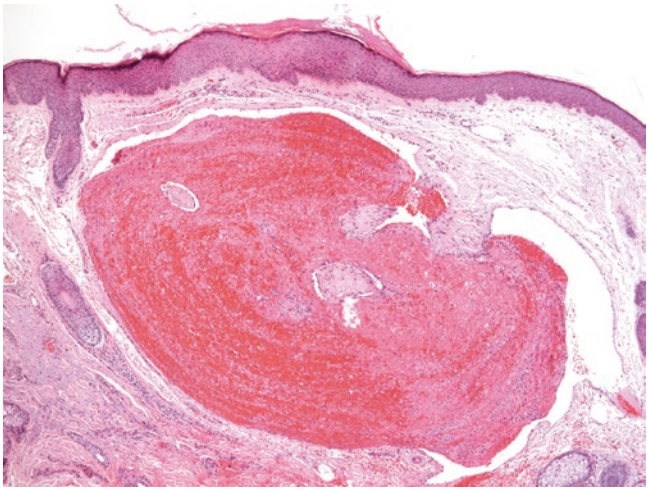

Fig. 15.92 Venous lake. Dilated and congested vein is present in the papillary dermis

such mutation may select patients who may potentially benefit from new targeted therapies which may provide better disease control and prognosis.

Electron microscopy Demonstration of Birbeck granules has traditionally been regarded as a diagnostic feature of Langerhans cells. Nevertheless, they may be altogether absent in a considerable number of cases.

Differential diagnosis Non-Langerhans cell histiocytoses can be distinguished by the lack of langerin positivity.

Treatment and prognosis A single-system Langerhans cell histiocytosis is generally associated with excellent prognosis and a high incidence of spontaneous regression. On the other hand, multisystem disease with involvement of one or more of the risk organs with organ dysfunction, including lungs, liver, spleen and haematopoietic system, usually signifies poor outcome. Solitary lesions can be treated with complete surgical excision. Generalised cutaneous involvement with/without systemic involvement requires more aggressive treatment, including systemic corticosteroids, radiation therapy and chemotherapy.

15.8 Vascular, Fibrohistiocytic and Smooth Muscle Tumors of Skin and Subcutis

15.8.1 Venous Lake

Definition and synonyms Venous lake is a vascular lesion distinguished by dilated and congested vein in the superficial dermis without obvious increase in the number of blood vessels. The surrounding dermis frequently displays solar elastosis.

Epidemiology No gender predilection has been observed. The majority of patients are adults.

Etiology and pathogenesis Not known, likely related to structural abnormalities in the superficial dermis around dilated veins

Clinical aspects Venous lake presents as a soft and compressible bluish to violaceous papule, usually measuring less than 1 cm in largest diameter. The lesion predominantly occurs on the sun-damaged skin, including the head and neck area and distal extremities. Mucosal sites can also be affected. The majority of the lesions are asymptomatic. Bleeding upon minor trauma is not uncommon.

Microscopy Venous lake is characterised by dilated and congested pre-existent veins in the papillary dermis, lined by a single layer of flat endothelial cells (Fig. 15.92). No vascular proliferation is seen. Solar elastosis is typically present. Mild perivascular fibrosis is occasionally seen. Inflammatory cell infiltrate is usually absent.

Differential diagnosis Angiokeratoma represents the main differential diagnosis and is distinguished from the venous lake by the presence of epidermal changes in the latter, e.g. hyperkeratosis and acanthosis.

Treatment and prognosis No therapy is usually required. Treatment options can include cryotherapy, laser therapy, sclerosing agents or surgical excision.

15.8.2 Intravascular Papillary Endothelial Hyperplasia

Definition and synonyms Intravascular papillary endothelial vascular hyperplasia is a reactive proliferation of endothelial

cells representing an organising thrombus [197, 198]. Synonyms include Masson's pseudotumor or Masson's tumor.

Epidemiology The lesion shows predilection for middle-aged adults with a slight female predominance.

Etiology and pathogenesis Intravascular papillary endothelial hyperplasia represents a reactive/reparative condition due to organisation of the thrombus or bleeding.

Clinical aspects Intravascular papillary endothelial hyperplasia presents as a solitary violaceous papule or nodule of less than 2 cm in diameter. Multiple lesions or eruptive development are exceptionally encountered. The most common sites of occurrence are head and neck, extremities and trunk. Mucosal surfaces can also be affected.

Microscopy Three different variants of an intravascular papillary endothelial hyperplasia can be separated: (1) primary or pure, occurring within a pre-existent normal vessel, usually a vein; (2) secondary or mixed, typically developing in the background of a pre-existent vascular lesion (e.g. malformation or tumor) and (3) third or undetermined, occurring outside blood vessels and representing an organising haematoma.

The salient histological features include arborising and interconnecting papillary projections, forming a meshwork of cleft-like spaces with cores composed of fibrin, hypocellular collagen and capillaries (Fig. 15.93) [199]. Papillary projections are lined by a single layer of bland endothelial cells. Nevertheless, mild nuclear pleomorphism and regular mitoses can be seen.

Differential diagnosis Well-differentiated angiosarcoma is distinguished by nuclear pleomorphism, mitotic activity and endothelial multilayering.

Treatment and prognosis Intravascular papillary endothelial hyperplasia is a reactive lesion and no treatment is required. Nevertheless, complete local excision is curative.

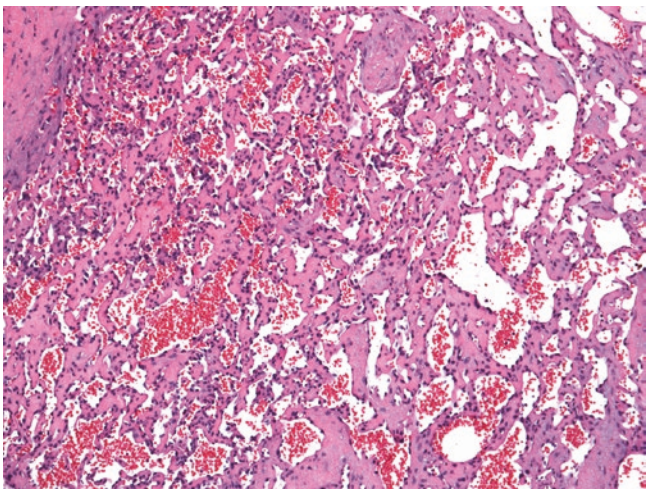

Fig. 15.93 Intravascular papillary endothelial hyperplasia. Interconnected papillary projections lined by a single layer of flat endothelial cells. The vessel wall can be appreciated on the *upper left* corner

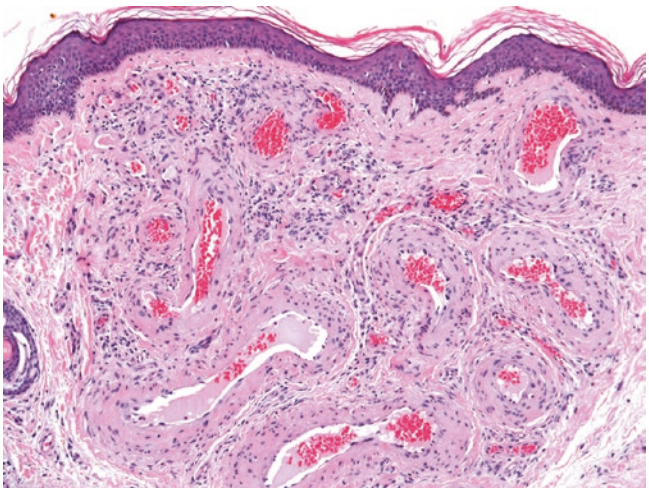

Fig. 15.94 Cirsoid aneurism. A well-demarcated proliferation of thick-walled blood vessels is present in the dermis

15.8.3 Arteriovenous Hemangioma

Definition and synonyms Arteriovenous hemangioma also designated 'cirsoid aneurysm' or 'acral arteriovenous tumor', represents a form of vascular malformation [200].

Epidemiology Arteriovenous hemangioma most commonly occurs in adults in their fifth and sixth decades of life [201]. Both sexes are equally affected.

Etiology and pathogenesis Arteriovenous hemangioma represents a spectrum of vascular malformations.

Clinical aspects Arteriovenous hemangioma usually presents as a solitary small reddish to bluish papule or nodule, measuring less than 1 cm in diameter. The lesion is asymptomatic, but may on occasion be painful, or associated with intermittent bleeding. Multiple lesions are uncommon and can present in an eruptive or agminate pattern. The most common site of occurrence is head and neck area, followed by extremities. Mucosal site, such as oral cavity or vulva, can also be affected.

Microscopy The lesion is composed of an admixture of thick- and thin-walled vascular channels resembling arteries and veins in the dermis (Fig. 15.94). The veins frequently predominate or represent an exclusive component of the lesion (Fig. 15.95). Such lesions are probably best regarded as venous hemangiomas. A small ascending feeding vessel,

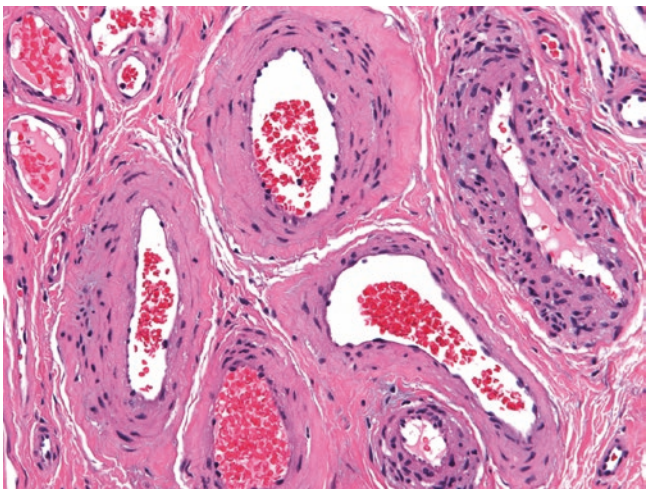

Fig. 15.95 Cirsoid aneurysm. The veins frequently predominate and can represent the exclusive component of the lesion

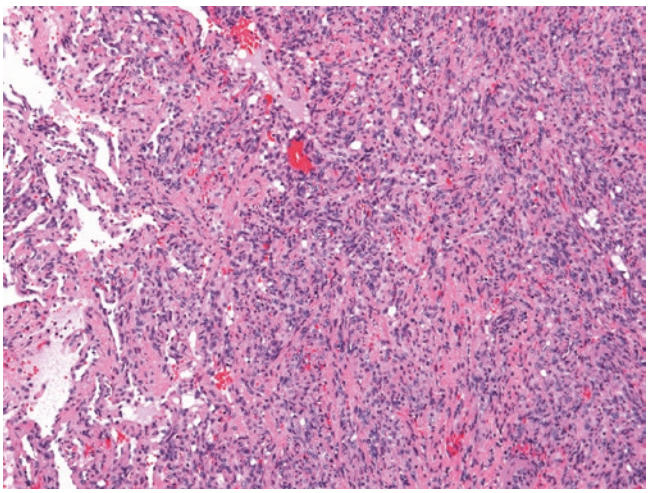

Fig. 15.96 Spindle cell hemangioma. Cavernous hemangioma-like areas (*left*) blending with a cellular component, composed of an admixture of spindled and epithelioid cells (*right*)

typically coiled, can frequently be appreciated in the lower parts of the lesion, usually at the border between the dermis and subcutis. Secondary changes can occasionally be seen, including intraluminal microthrombi, dystrophic calcifications and perivascular inflammatory cell infiltrate.

Differential diagnosis There is no true differential diagnosis.

Treatment and prognosis Complete local excision is curative.

15.8.4 Spindle Cell Hemangioma

Definition and synonyms Spindle cell hemangioma is a vascular proliferation, originally reported as a spindle cell hemangioendothelioma [202]. The subsequent change in terminology reflects a uniform benign behaviour of the lesion [203]. Interestingly, about 70 % of the spindle cell hemangiomas display somatic mutations of the *isocitrate dehydrogenase 1* gene [204].

Epidemiology Spindle cell hemangioma has a wide age distribution and most commonly develops in the fourth decade of life. There is no gender predilection.

Etiology and pathogenesis Mutations of the *isocitrate dehidrogenase 1 gene* have been detected in about 70% of spindle cell hemangiomas.

Clinical aspects The lesion presents as a solitary reddish to brownish nodule, measuring up to 2 cm in diameter. Synchronous or subsequent occurrence of additional nodules in the same anatomic area develops in up to 50 % of the cases and is characteristic of the entity. The preferred sites of occurrence are distal extremities, but the head and neck area and mucosal sites are not uncommon.

The majority of spindle cell hemangioma are sporadic lesions. Nevertheless, about 5 % of the cases are associated with the Maffucci's syndrome (multiple enchondromas and spindle cell hemangiomas) [204].

Microscopy Spindle cell hemangioma is a well-demarcated proliferation in the dermis and subcutis [202, 203, 205]. Importantly, about 60 % of the lesions occur completely or partially intravascular, usually within the vein. The defining histological features are (1) cavernous hemangioma like areas and (2) cellular or solid component, composed of spindled and epithelioid cells, representing collapsed cavernous vessels and intervening stroma (Fig. 15.96).

The cavernous hemangioma-like areas are characterised by dilated thin-walled cavernous vascular spaces, lined by a single layer of flattened endothelial cells. Vascular spaces are congested and frequently contain thrombi in different stages of organisation.

The cellular component is delineated by an admixture of spindled and epithelioid endothelial cells. The spindle cells form short interlacing fascicles or are arranged randomly. The nuclei of spindle cells are elongated or plump. Mitotic activity can be present, but is never pronounced. The spindle cells represent an admixture of various cell types, including fibroblasts, pericytes, smooth muscle cells, macrophages, primitive mesenchymal cells and cells with primitive endothelial differentiation. The epithelioid cells usually represent a minor cell population, being present either singly or in small groups. Their nuclei are round to oval, mitoses absent or sparse and the cytoplasm typically moderately abundant and eosinophilic. Intracytoplasmic vacuoles representing abortive vascular lumina formations are characteristic (Fig. 15.97).

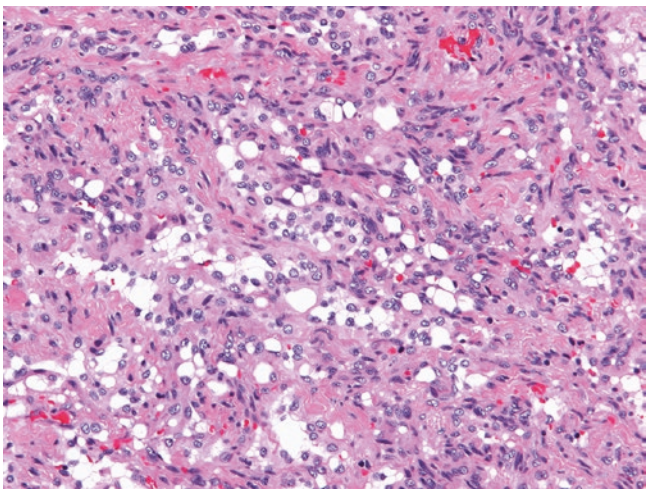

Fig. 15.97 Spindle cell hemangioma. Epithelioid cells are characterised by round to oval nuclei and moderately abundant eosinophilic cytoplasm typically containing intracytoplasmic vacuoles

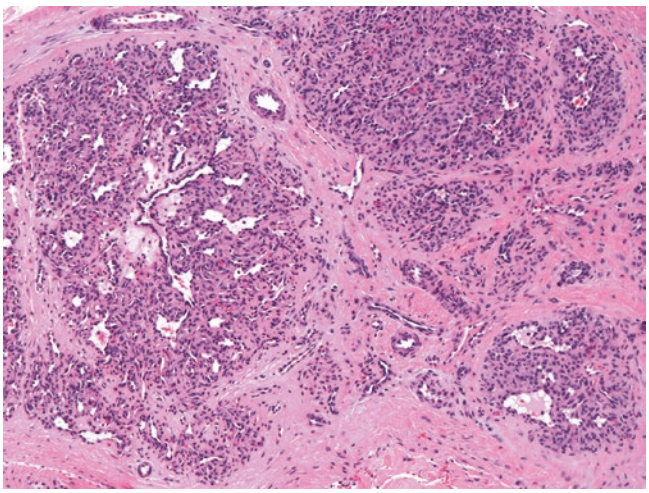

Fig. 15.98 Lobular capillary hemangioma. Multilobular proliferation of capillaries, separated by fibrous septa. Note the absence of inflammation

Additional histological features include large and thick-walled blood vessels, usually veins in the vicinity of spindle cell hemangioma.

By immunohistochemistry, the spindle cells display variable positivity for smooth muscle actin, desmin, HHF35 and CD68, while endothelial markers are typically negative. The epithelioid cells stain for markers of vascular differentiation, including CD31, CD34 and factor VIII-related antigen. Both cell types lack cytokeratin, S100 protein and HHV-8 positivity.

Differential diagnosis Nodular Kaposi's sarcoma can be distinguished by the lack of cavernous hemangioma like areas and by consistent HHV-8 positivity.

Treatment and prognosis Surgical excision is the preferred treatment. Although non-destructive local recurrences have been reported to be present in about 60 %, they likely reflect multifocal growth, or intravascular extension of the lesion. No loco-regional or distant metastases have been reported.

15.8.5 Capillary Hemangioma and Variants

Definition and synonyms Capillary hemangioma is a vascular proliferation composed of capillaries and small veins. The entity has also been invariably designated as a pyogenic granuloma. Variants of capillary hemangioma include granuloma gravidarum, subcutaneous or deep and intravenous capillary hemangioma.

Epidemiology Capillary hemangioma has a wide age distribution [206–208]. The lesion is most frequently encountered in the second and third decade of life. About 40 % of capillary hemangiomas occur prior to the age of 5 years. Granuloma gravidarum develops in less than 5 % of pregnancies. There is a slight male predominance for cutaneous capillary hemangiomas while mucosal and intravascular variants display female predilection.

Etiology and pathogenesis It likely represents a benign vascular tumor, although the true nature of the lesion is still controversial.

Clinical aspects Capillary hemangioma presents as a solitary red to violaceous papule or nodule [206]. Surface ulceration and bleeding is common. The lesion typically displays a rapid initial growth. The most common sites of occurrence include skin of the trunk, followed by head and neck and extremities. Granuloma gravidarum shows predilection for gingival mucosa.

Microscopy Capillary hemangioma is characterised by (multi)lobular proliferation of capillaries and small veins in the dermis (Fig. 15.98), with possible extension into the subcutaneous fatty tissue. The individual lobules are separated by fibrous septa, displaying various degrees of oedema and myxoid change. Capillaries and veins are lined by a single layer of endothelial cells, which can be flat or more rounded (epithelioid). Mitotic activity can be brisk. A focal cytological atypia is not uncommon and represents a degenerative phenomenon.

Ulcerated lesions tend to be associated with a variably intense inflammatory cell infiltrate, composed of an admixture of granulocytes, lymphocytes, macrophages and plasma cells.

Differential diagnosis Pyogenic granuloma can be indistinguishable on histological grounds from bacillary angio-

matosis. Special stains (Giemsa or Warthin–Starry) are necessary to demonstrate the presence of an infectious agent.

Treatment and prognosis Various treatment procedures have been proposed, among others surgical excision, application of topical agents, cryotherapy and laser therapy. With the exception of granuloma gravidarum, capillary hemangioma does not show tendency for spontaneous regression. Recurrences are frequent.

15.8.6 Congenital Hemangioma

Definition and synonyms Congenital hemangioma collectively refers to the group of vascular proliferations that are fully developed at birth by definition. They can be further subdivided on the basis of clinical evolution of the lesion into the rapidly involuting congenital hemangiomas (RICH) and non-involuting congenital hemangiomas (NICH), the latter group likely representing the form of vascular malformation [209, 210]. It is important to emphasise a certain degree of morphological overlap between RICH and NICH, but also with the so-called infantile hemangioma referred to as a juvenile hemangioma or strawberry nevus [211]. The latter entity represents a distinctive vascular proliferation with a characteristic nuclear expression of GLUT-1.

Epidemiology RICH and NICH show no gender predilection [209].

Etiology and pathogenesis NICH likely represents a vascular malformation.

Clinical aspects RICH and NICH usually present as a pink to violaceous plaque or nodule with predilection for head and neck, followed by lower and upper extremities [209, 212]. The size of the lesion generally does not exceed 5 cm. The lack of subsequent growth after delivery is typical of RICH. Following a short interval of standstill, the RICH enters into the phase of spontaneous regression, which is usually complete within the first 2 years of life. In exceptional examples, RICH can be associated with thrombocytopaenia and self-limited coagulopathy [213]. Surface ulceration with subsequent bleeding is uncommon.

In contrast, NICH does not involute but rather increases in proportion with the child's growth [212].

Microscopy RICH is characterised by a lobular proliferation of capillaries in the dermis and subcutis (Fig. 15.99). Capillaries can be focally dilated and are typically surrounded by a layer of pericytes. Thin-walled draining vessels can be present, especially in the centre of the lobules. Larger vessels corresponding to veins, arteries and lymphatics can also be seen. Nevertheless, thick-walled anomalous blood vessels, including arteriovenous shunts, are generally absent. Involuting lesions are distinguished by progressive fibrosis of the lobules.

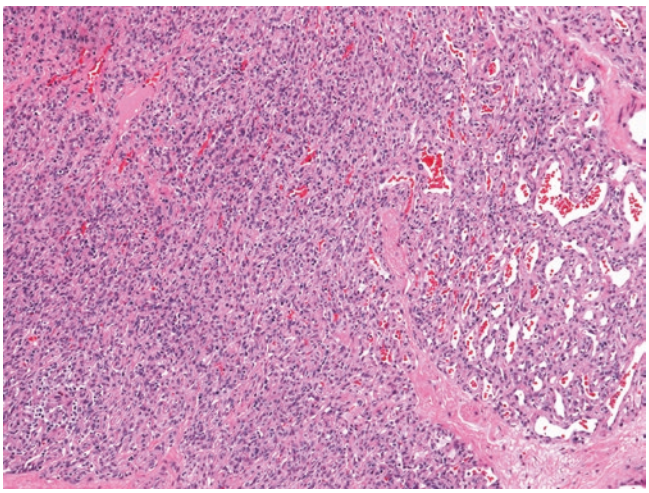

Fig. 15.99 Rapidly involuting congenital hemangioma. Early lesions are characterised by solid proliferation of capillaries

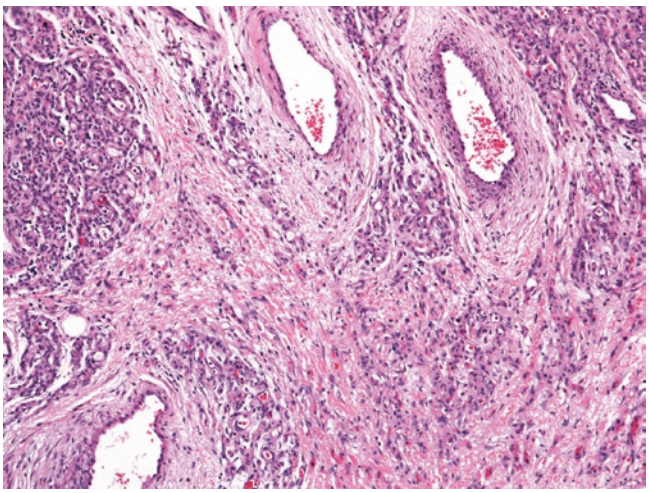

Fig. 15.100 Non-involuting congenital hemangioma. Anomalous blood vessels in the centre of the lesion, associated with fibrosis. Note lobular proliferation of capillaries at the periphery

NICH shows morphological overlap with RICH (see above). In addition, arteriovenous fistulas are common as thickened anomalous blood vessels suggestive of vascular malformation (Fig. 15.100).

Differential diagnosis RICH and NICH can be difficult to separate on histological grounds alone. Anomalous blood vessels with thickened walls are suggestive of NICH. Infantile hemangioma can be separated by GLUT-1 nuclear positivity.

Treatment and prognosis RICH: No treatment is generally necessary as a great majority of the lesions will completely regress spontaneously within the first 2 years of life. The residual fibrovascular stalk can be surgically removed.

NICH: No spontaneous regression is seen. Complete surgical excision is curative, but may be difficult to achieve.

15.8.7 Cavernous Hemangioma

Definition and synonyms Cavernous hemangioma represents a benign vascular proliferation distinguished by dilated and congested cavernous vascular spaces.

Epidemiology Cavernous hemangioma shows predilection for neonates and children. There is an even gender distribution.

Etiology and pathogenesis A considerable subset of cavernous hemangiomas likely represents a vascular (venous) malformation. There is also an overlap with a non-involuting congenital hemangioma [214].

Clinical aspects Cavernous hemangioma usually presents as a solitary bluish to violaceous soft and compressible papule or nodule with a predilection for head and neck area as well as limbs. Prominence upon physical activity is typical and results from increased blood flow through the lesions. Adult patients can also be affected.

Multiple cavernous hemangiomas can be associated with Maffucci's syndrome (cavernous hemangiomas enchondromas, increased risk for chondrosarcomas), blue rubber bleb nevus (hemangiomas in the oral cavity, gastrointestinal tract, liver and central nervous system) and Kasabach–Merritt syndrome (consumption coagulopathy).

Microscopy Cavernous hemangioma is a dermal-based vascular proliferation composed of increased numbers of large, thin-walled, dilated and congested vascular channels lined by a single layer of flattened endothelial cells, characteristically lacking mitotic activity and cytological atypia (Fig. 15.101). Lobular pattern of proliferation, as seen in capillary hemangioma is generally absent. However, blending with capillary hemangioma is common, especially towards the superficial aspects of the proliferation. Intraluminal thrombi in various stages of organisation with/without dystrophic calcifications can also be present.

Differential diagnosis Sinusoidal hemangioma represents a morphological variant of cavernous hemangioma [215]. The defining features of sinusoidal hemangioma are large, thin-walled, dilated and congested vascular channels, arranged in a back to back pattern imparting a sieve-like architecture.

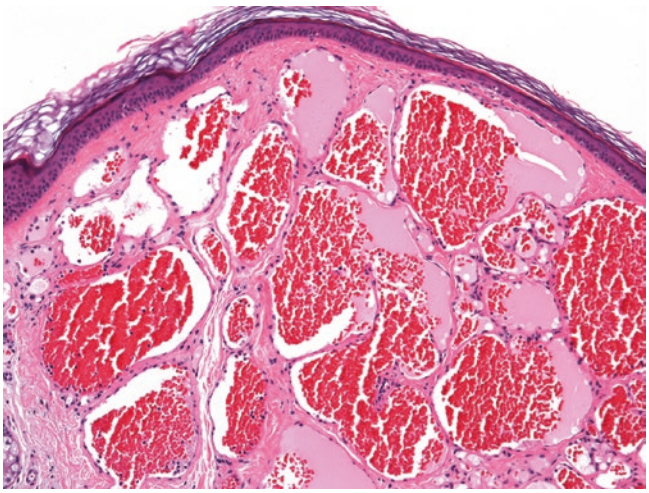

Fig. 15.101 Cavernous hemangioma. Numerous large, dilated, congested and thin-walled vascular spaces in the dermis

Treatment and prognosis Complete surgical excision is curative. Cavernous hemangioma does not regress spontaneously [214].

15.8.8 Papillary Hemangioma

Definition and synonyms Papillary hemangioma is a distinctive variant of hemangioma reported by Suurmeijer and Fletcher in 2007. Only a limited number of cases have been reported since the original description [216].

Epidemiology Papillary hemangioma shows wide age distribution, but most commonly develops in the sixth decade of life. Males are more commonly affected.

Etiology and pathogenesis Currently unknown.

Clinical aspects Papillary hemangioma presents as a solitary non-painful skin-coloured to bluish papule or nodule measuring about 1 cm in diameter. All reported lesions developed in the head and neck area, with a predilection for the face.

Microscopy Papillary hemangioma is a well-circumscribed and non-encapsulated vascular proliferation in the dermis, with possible extension into the superficial subcutis. Dilated vascular spaces, usually veins, are seen containing branching papillary projections with stromal cores and capillaries. Papillary projections are characteristically not arranged in an organoid or glomeruloid pattern and are lined by a single layer of endothelial cells. Quite characteristically, endothelial cells at the luminal surfaces of papillae contain numerous intracytoplasmic hyaline globules, representing giant lysosomes [217].

Differential diagnosis The main differential diagnosis is with glomeruloid hemangioma a distinctive vascular proliferation generally occurring in the setting of POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M-protein, skin changes) [218]. Papillary hemangioma characteristically lacks association with POEMS syndrome, glomeruloid architecture of vascular proliferation and thick basement membrane-like material surrounding pericytes.

Treatment and prognosis Complete excision is curative. Recurrences following incomplete or marginal excision are most uncommon.

15.8.9 Epithelioid Hemangioma

Definition and synonyms Epithelioid hemangioma is a preferred expression for a benign vascular proliferation composed of variably sized blood vessels lined by epithelioid endothelial cells and admixed with inflammatory cell infiltrate. The entity was originally reported by Wells and Whimster in 1969 as an angiolymphoid hyperplasia with eosinophilia [219]. Nevertheless, several different names have been used in the past for epithelioid hemangioma including angiolymphoid hyperplasia with eosinophilia, histiocytoid hemangioma papular angioplasia, atypical pyogenic granuloma, pseudopyogenic granuloma, inflammatory angiomatous nodule, inflammatory arteriovenous hemangioma and intravenous atypical vascular proliferation [219]. Although epithelioid hemangioma has initially been considered as representing a spectrum of Kimura's disease, epithelioid hemangioma and Kimura's disease have now been regarded as unrelated entities [220].

Epidemiology Epithelioid hemangioma shows predilection for young to middle-aged adults in their third to fourth decade of life (mean age 30 years). A slight female predominance has been observed. Occurrence in infancy, childhood or elderly patients is infrequent. Any ethnic groups can be affected, but there appears to be predilection for Asians and Caucasians.

Etiology and pathogenesis Epithelioid hemangioma has initially been regarded a reactive vascular proliferation. Associated conditions and/or proposed triggers include scabies, previous vaccination, pregnancy, nephritic syndrome, mycosis fungoides, arteriovenous malformations, port-wine stain, coexistence with Kimura's disease, cutaneous squamous cell carcinoma and antecedent burns. Nevertheless, such an association is likely coincidental, and a neoplastic origin of epithelioid hemangioma has recently been favoured.

Clinical aspects Epithelioid hemangioma most commonly presents as a pink to red brown plaque, papule or nodule measuring from 0.5 to 3 cm in largest diameter [221, 222]. The lesion is usually asymptomatic, but can on occasion be itchy and painful. About 80 % of the patients develop a single lesion. Although rare, multiple lesions usually occur in the same anatomic area. Generalised variants of epithelioid hemangioma involving multiple body parts are exceptional. Epithelioid hemangioma shows predilection for the head and neck area, in particular periauricular skin, forehead and scalp [221, 222]. Other cutaneous sites as well as mucosal surfaces are rarely involved. Extracutaneous presentation is exceptional and includes liver, spleen, heart, blood vessels and peripheral nerves [223]. Blood eosinophilia is present in up to 20 % of the patients [221].

The majority of epithelioid hemangioma develop within the dermis and subcutis. A minor subset arises within the deep soft tissues or bones.

Microscopy Epithelioid hemangioma is a non-encapsulated well-circumscribed and lobular proliferation composed of two main components: variably sized blood vessels and inflammatory cell infiltrate (Fig. 15.102). In contrast with the subcutaneous locations, lesions in the dermis tend to be less circumscribed and less lobular.

The proliferating blood vessels consist of capillaries and venules, frequently containing thickened vessel walls. Well-formed lumina are typically seen at the periphery of the proliferation, while central areas frequently contain compressed vessels with collapsed lumina imparting the lesion with a more solid growth pattern. The defining histological features are epithelioid endothelial cells with round to oval nuclei,

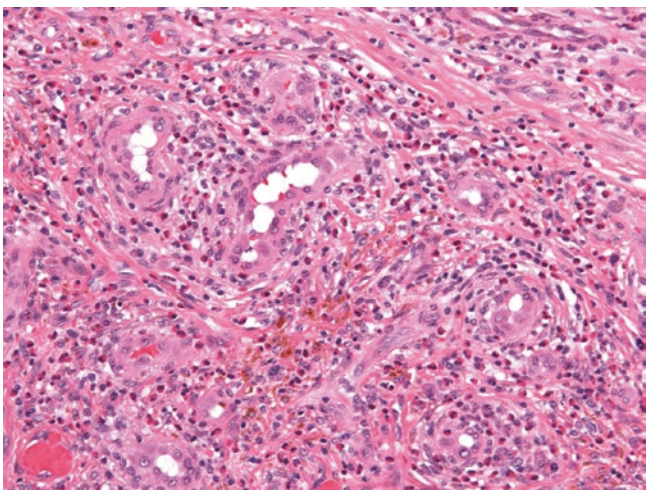

Fig. 15.102 Epithelioid hemangioma. Lobular proliferation of capillaries lined by epithelioid endothelial cells, admixed with inflammatory cell infiltrate containing numerous eosinophilic granulocytes

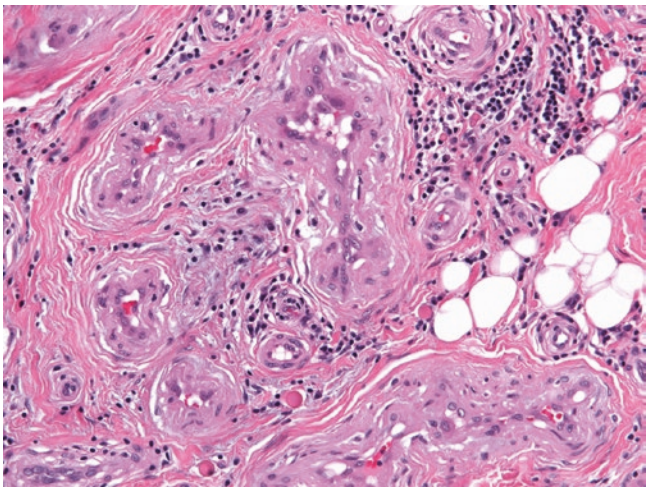

Fig. 15.103 Epithelioid hemangioma. Inflammatory cell infiltrate is less pronounced in this example. Note epithelioid endothelial cells lining the vascular spaces

delicate chromatin and abundant eosinophilic or amphophylic cytoplasm. They protrude into the vascular lumina giving the lesion a hobnail or 'tombstone' appearance and typically form a single layer of endothelial cells. Mild variation in nuclear size is not infrequent. Mitoses are generally absent or limited in numbers. Atypical mitoses are absent as a rule. Intracytoplasmic vacuoles, representing abortive lumina formation as an expression of primitive vascular formation are not uncommon. Although occasionally lesional blood vessels are lined by flat endothelial cells, the epithelioid morphology of endothelial cells generally predominates.

The inflammatory cell infiltrate is variably abundant and is mainly composed of an admixture of lymphocytes, plasma cells and eosinophils. The inflammation is most prominent at the periphery of the lesion and at the subcutaneous location. Nevertheless, the inflammatory cell infiltrate can be can sparse or completely absent, usually in the pure dermal proliferations (Fig. 15.103). The absence of inflammation does not preclude the diagnosis of epithelioid hemangioma as long as the majority of endothelial cells display epithelioid morphology.

By immunohistochemistry, epithelioid endothelial cells infrequently display focal cytokeratin positivity, which might represent a potential diagnostic pitfall. Inflammatory cell component can contain scattered reactive CD30+ cells, occasionally forming small clusters [224]. Although clonal T-cell proliferation has been detected in a subset of epithelioid hemangiomas, the significance of this phenomenon is at present unknown [225].

Differential diagnosis The main differential diagnosis includes Kimura's disease, epithelioid angiomatous nodule and angiosarcoma. As a general rule, Kimura's disease lacks epithelioid endothelial cells and predominantly occurs in young males of Asian descent [224]. Epithelioid angiomatous nodule is characterised by poorly vasoformative and mainly solid proliferation of polygonal epithelioid endothelial cells with predilection for trunk and extremities. Angiosarcoma is distinguished by proliferation of atypical endothelial cells with characteristic multilayering, frequent mitotic activity and the presence of atypical mitoses.

Treatment and prognosis Epithelioid hemangioma is characterised by persistence of the lesion(s), while spontaneous regression appears to be exceptional [221, 222]. Various treatment options have been suggested, including complete surgical excision, cryotherapy, treatment by laser, intralesional or systemic steroids and oral retinoids, albeit with variable success. Incomplete or marginal excision is associated with a high recurrence rate, which is reported to be between 33 and 50 % [221, 222].

15.8.10 Cutaneous Epithelioid Angiomatous Nodule

Definition and synonyms A benign vascular proliferation, likely representing a morphological spectrum of epithelioid hemangioma originally described by Brenn and Fletcher in 2004 [226].

Epidemiology Cutaneous epithelioid angiomatous nodule displays a wide age distribution, most commonly presenting in young to middle-aged adults [226, 227]. There is a slight male predominance.

Etiology and pathogenesis Not known. Likely represents a reactive vascular proliferation rather than neoplastic growth.

Clinical aspects Cutaneous epithelioid angiomatous nodule usually presents as a violaceous to bluish solitary papule or nodule, measuring less than 2 cm in diameter. The lesion grows rapidly over the course of weeks to months. The sites of predilection include head and neck area, trunk and extremities. Mucosal surfaces can also be affected. Multiple lesions are rare.

Microscopy The defining histological feature includes well-circumscribed, non-encapsulated nodular proliferation of epithelioid endothelial cells in the papillary and upper reticular dermis, with possible extension into the deep dermis. Characteristically, epithelioid endothelial cells grow in solid sheets and are poorly vasoformative (Fig. 15.104). Open vascular spaces with erythrocytes are usually present only focally, especially at the periphery of the proliferation, and are lined by a single layer of epithelioid endothelial

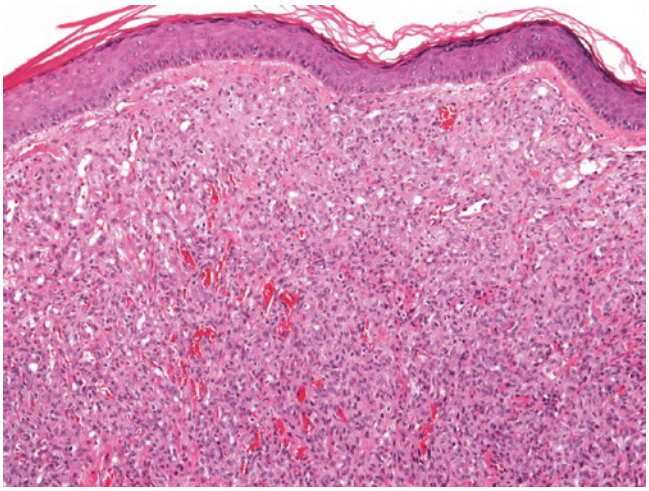

Fig. 15.104 Epithelioid angiomatous nodule. Nodular poorly vasoformative vascular proliferation in the dermis

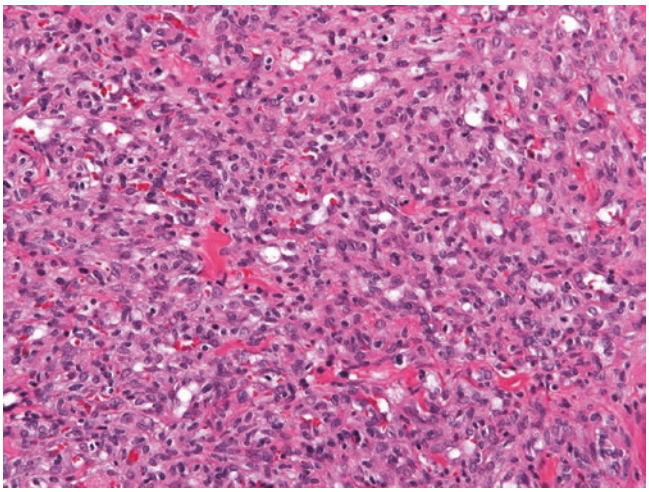

Fig. 15.105 Epithelioid angiomatous nodule. Open vascular spaces are lacking in the central aspect of the proliferation. Intracytoplasmic vacuoles representing abortive lumina formation are frequently present

cells. Endothelial cells contain abundant eosinophilic cytoplasm with occasional intracytoplasmic vacuolisation, representing abortive vascular lumina formation (Fig. 15.105). Normal mitoses are not uncommon, but nuclear pleomorphism is generally absent. In the background, deposition of haemosiderin pigment as well as a variably intense inflammatory cell infiltrate composed of lymphocytes, plasma cells and eosinophils are frequently seen.

The overlying epidermis occasionally forms collarette at both sides of dermal proliferation.

Differential diagnosis Cutaneous epithelioid angiomatous nodule likely represents a morphological spectrum of epithelioid hemangioma [227]. At present, the former is distinguished by being poorly vasoformative and predominantly solid and nodular proliferation of endothelial cells. Epithelioid angiosarcoma is poorly circumscribed and typically displays significant cytological atypias and frequent mitoses, including atypical ones.

Treatment and prognosis Cutaneous epithelioid angiomatous nodule follows a benign clinical course. Complete excision is usually curative.

15.8.11 Kaposi's Sarcoma

Definition and synonyms Kaposi's sarcoma is a multifocal vascular proliferation causally related to human herpes virus 8 (HHV-8) infection [228]. The entity was originally described by Moritz Kaposi in 1872 in an elderly male patient who presented with pigmented cutaneous plaques on the lower extremities [229].

Epidemiology and clinical features Four distinct clinical subtypes of Kaposi's sarcoma can be distinguished: classic (sporadic), immunosuppression associated, acquired immune deficiency syndrome (AIDS)-related and African (endemic) Kaposi's sarcoma [230].

Classical (sporadic) Kaposi's sarcoma shows predilection for elderly patients and occurs predominantly in patients from the Mediterranean, but also Eastern European and Middle Eastern descent [231]. Although initially considered to be up to 15 times more common in males, the male-to-female ratio appears to be somewhat lower (up to 4:1). The classic (sporadic) Kaposi's sarcoma most commonly occurs on lower extremities and generally starts as a unilateral lesion, eventually becoming bilateral and multifocal. Involvement of mucous membranes is common, in particular oral cavity and gastrointestinal tract. About 10 % of the patients have lesions in the visceral organs.

Immunosuppression associated Kaposi's sarcoma has equal gender distribution and generally follows a similar site distribution as classical (sporadic) Kaposi's sarcoma. However, mucosal sites, lymph nodes and visceral involvement can be affected in up to 50 % of the patients. The risk of Kaposi's sarcoma development is highest within the first 2 years following transplantation [232, 233].

Acquired immune deficiency syndrome (AIDS)-related Kaposi's sarcoma shows predilection for homosexual males [234, 235]. It typically develops on the trunk and has a tendency for rapid dissemination. Extracutaneous spread with visceral involvement is common.

African Kaposi's sarcoma predominates in sub-Saharan Africa [236]. Two clinical variants have been recognised: the cutaneous variant is similar to classic (sporadic) Kaposi's sarcoma, while the lymphadenopathic variant predominantly affects lymphatic tissue. The cutaneous variant mainly

occurs in young adults, while lymphadenopathic shows predilection for children.

Three clinical and pathological stages of Kaposi's sarcoma can be separated regardless of the clinical subtype and may coexist in a single patient, representing a morphological continuum: patch, plaque and nodular stage. Kaposi's sarcoma generally presents with bluish-red well-demarcated painless macules. Macular lesions subsequently progress to form larger flat plaques which eventually become elevated. Hard nodules subsequently develop and may be complicated by erosions, ulcerations and bleeding.

While individual lesions may show the tendency to regress, the new lesions continue to develop.

Etiology and pathogenesis Infection with HHV-8 has been regarded a necessary yet not sufficient step in the development of Kaposi's sarcoma. Additional factors thought to be important include genetic, immunologic and environmental factors [230]. Whether Kaposi's sarcoma represents an unusual reaction of endothelial cells to the above-mentioned cofactors or a true neoplasm has not been resolved at present [237]. It has been demonstrated that Kaposi's sarcoma starts as a polyclonal proliferation with subsequent evolution into the mono/oligoclonal disease [238]. Nevertheless, Kaposi's sarcoma has been grouped among vascular neoplasms with intermediate grade of malignancy in the recent WHO classification of tumors of soft tissue and bone [239].

Microscopy Kaposi's sarcoma is composed of irregular vascular channels admixed with spindled endothelial cells. Both endothelial cells lining irregular channels and spindle cells forming fascicles are fairly uniform and lack pleomorphism. Intracellular and extracellular PAS+ and diastase-resistant hyaline globules representing degenerated erythrocytes are typical, although not specific, features of Kaposi's sarcoma. Additional features include extravasation of erythrocytes, deposition of haemosiderin pigment and inflammatory cell infiltrate composed predominantly of lymphocytes and plasma cells. The quantity of individual components varies significantly between the three histological stages of Kaposi's sarcoma, namely, patch, plaque and nodular stage.

Patch stage Kaposi's sarcoma represents the earliest form of cutaneous Kaposi's sarcoma. At this stage, the salient features of Kaposi's sarcoma can be missed as the changes can be very subtle. Low-power magnification typically shows a mild superficial and deep perivascular and periadnexal inflammatory cell infiltrate, composed of lymphocytes and plasma cells, thereby mimicking an inflammatory dermatosis (Fig. 15.106). Higher magnification additionally reveals in the superficial and mid-reticular dermis a mild increase in the number of irregular, jagged and slit-like vascular spaces

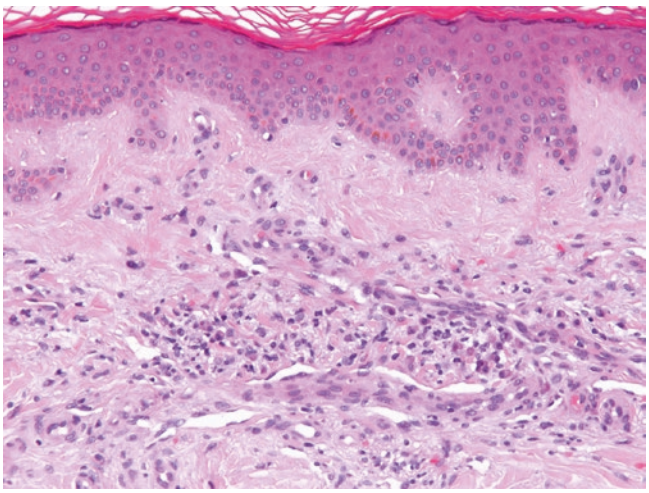

Fig. 15.106 Patch stage Kaposi's sarcoma. Mild increase in the number of blood vessels in the superficial and mid-dermis. Note perivascular inflammatory cell infiltrate composed of lymphocytes and plasma cells

lined by a single layer of endothelial cells. The newly formed vascular channels appear empty and show a tendency towards parallel orientation with the epidermis. They characteristically grow in a dissecting pattern among dermal collagen bundles, skin adnexa and pre-existing vascular channels. Due to the dissection pattern of vascular proliferation, these structures seem to float within the empty spaces, a feature generally regarded as a promontory sign. Although characteristic for Kaposi's sarcoma, a promontory sign is by no means specific and can also be found in other vascular proliferations, including angiosarcoma. The spindle cell proliferation is absent or very limited in a patch-stage Kaposi's sarcoma. Extravasation of erythrocytes, haemosiderin deposition and hyaline globules are either absent or scarce and focal.

Plaque-stage Kaposi's sarcoma is delineated by more diffuse proliferation of thin-walled blood vessels through the entire thickness of the dermis (Fig. 15.107), with possible extension into the subcutis. The vascular lumina display variations in size and shape and are lined by fairly uniform endothelial cells. In addition, spindle cell proliferation with formation of short or haphazardly arranged fascicles becomes apparent and can represent the predominant component of the lesion, representing the hallmark of plaque-stage Kaposi's sarcoma. The nuclei of spindle cells can be hyperchromatic, but no significant nuclear or cytological atypia is readily apparent and mitoses are generally sparse. Hyaline globules are not uncommon. Primitive vascular clefts start to form within the spindle cell areas. Perivascular inflammatory cell infiltrate, composed of lymphocytes and plasma cells, becomes more prominent. Extravasation of erythrocytes and deposition of haemosiderin pigment around blood vessels are characteristically present and can be prominent (Fig. 15.108).

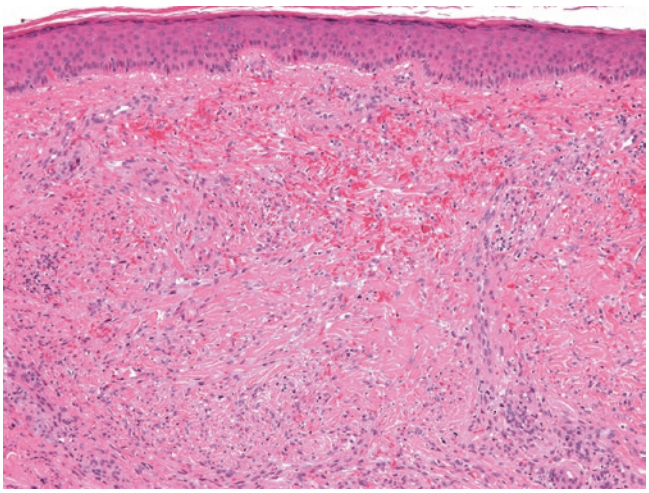

Fig. 15.107 Plaque-stage Kaposi's sarcoma. More diffuse proliferation of blood vessels throughout the dermis. Note extravasation of erythrocytes and mild inflammatory cell infiltrate

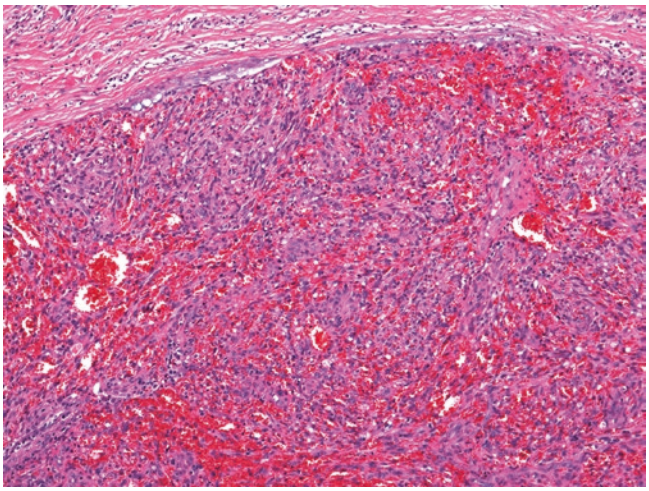

Fig. 15.109 Nodular Kaposi's sarcoma. Well-demarcated nodule in the dermis

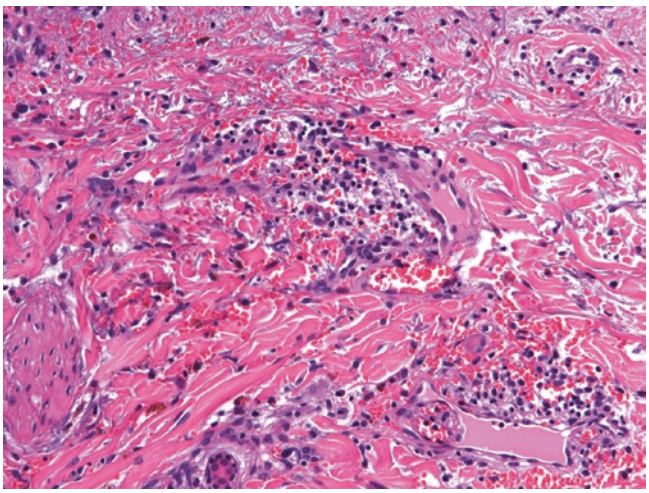

Fig. 15.108 Plaque-stage Kaposi's sarcoma. Extravasation of erythrocytes and deposition of haemosiderin pigment can be prominent

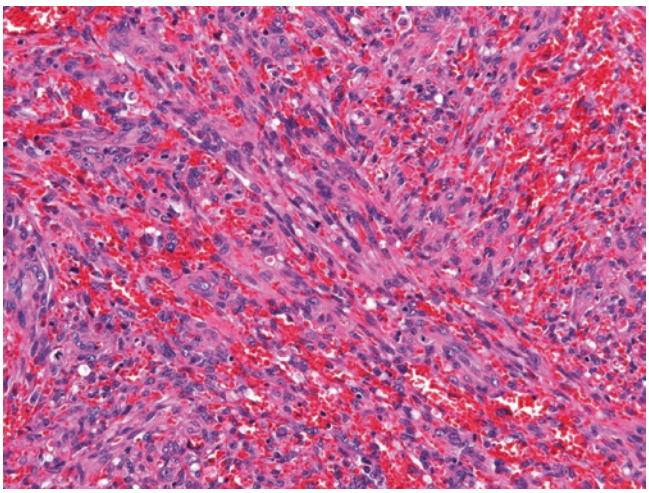

Fig. 15.110 Nodular Kaposi's sarcoma. The nodule is typically composed of spindle cells forming fascicles

Nodular-stage Kaposi's sarcoma represents an advanced stage of the disease with formation of a well-demarcated nodule in the dermis (Fig. 15.109) with possible extension into the subcutis. The nodule is typically composed of spindle cells forming fascicles, separated by slit-like channels occasionally containing erythrocytes, but generally lacking endothelial lining (Fig. 15.110).

Several histological variants of Kaposi's sarcoma have been reported, including anaplastic, lymphoedematous, lymphangioma-like, lymphangiectatic, bullous, telangiectatic, hyperkeratotic (verrucous), keloidal, micronodular, pyogenic granuloma-like, ecchymotic, intravascular, glomeruloid, ecchymotic, with myoid nodules, and pigmented Kaposi's sarcoma [240].

By immunohistochemistry, endothelial cells lining slit-like spaces and spindle cells display CD34 and CD31 positivity, with CD31 usually giving a stronger immunoreactivity. The defining immunohistochemical feature of Kaposi's sarcoma is nuclear positivity for the latent nuclear antigen 1 of the HHV-8 in virtually all cases (Fig. 15.111). The HHV-8 nuclear positivity is associated with high sensitivity and specificity for Kaposi's sarcoma. The lesional cells typically stain with podoplanin, suggesting a lymphatic origin.

Differential diagnosis Patch-stage Kaposi's sarcoma can be confused with inflammatory dermatosis. The presence of plasma cells in a perivascular distribution should prompt investigation for distinctive HHV-8+ vascular proliferation

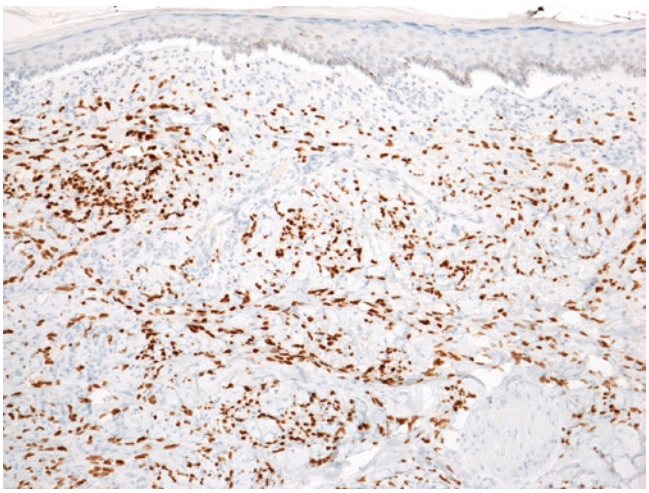

Fig. 15.111 Kaposi's sarcoma. Nuclear positivity for the latent nuclear antigen 1 of the human herpes virus 8 is characteristically present in nearly all Kaposi's sarcomas

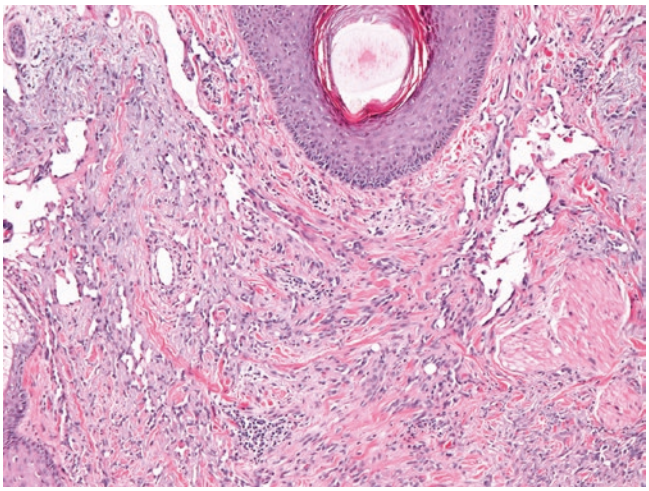

Fig. 15.112 Cutaneous angiosarcoma. Widely infiltrative tumor composed of dissecting, anastomosing and ramifying vascular channels

in the superficial dermis. Hobnail hemangioma also designated targetoid haemosiderotic hemangioma shows predilection for the limbs and trunk, typically has a wedge-shaped growth pattern on histology, has endothelial cells that protrude into the vascular lumina and it uniformly lacks HHV-8 positivity. Kaposiform hemangioendothelioma can resemble nodular stage Kaposi's sarcoma but is consistently negative for HHV-8. Angiosarcoma is distinguished by atypia of endothelial cells, multilayering, and mitotic activity.

Treatment and prognosis Prognosis varies from indolent disease with spontaneous regression to rapidly progressive fatal multifocal illness. Classical Kaposi's sarcoma, a cutaneous variant of African Kaposi's sarcoma and immunosuppression associated Kaposi's sarcoma, generally pursues a slowly progressive and chronic indolent course. In contrast, acquired immune deficiency syndrome (AIDS)-related Kaposi's sarcoma and lymphadenopathic variant of Kaposi's sarcoma follow an aggressive behaviour with rapid dissemination, eventually leading to the death of the patient.

15.8.12 Cutaneous Angiosarcoma of the Head and Neck

Definition and synonyms Angiosarcoma of the head and neck, also designated 'idiopathic' or 'sporadic' angiosarcoma, is a highly aggressive malignant vascular tumor, accounting for about 1 % of head and neck malignancies and representing about 10 % of soft tissue sarcomas occurring in the head and neck area [241]. The term idiopathic angiosarcoma will be used throughout this chapter for cutaneous angiosarcoma of the head and neck.

Epidemiology Idiopathic angiosarcoma most commonly develops in white elderly patients in their seventh or eighth decade of life [242]. It is distinctly uncommon in black patients and appears to be more prevalent in males [242].

Etiology and pathogenesis Not known at present.

Clinical aspects Idiopathic angiosarcoma usually starts insidiously as an isolated or multiple painless ill-defined bruise-like macule(s), eventually progressing to larger plaque(s), papule(s) and nodule(s), which can be ulcerated on the surface [242]. In about 50 % of the patients, the idiopathic angiosarcoma grows multifocally within a particular anatomic area [242]. As a rule, the tumor is locally much more extensive than considered clinically. Idiopathic angiosarcomas typically display a rapid growth with lateral spread of the tumor and subsequent involvement of larger areas, making complete excision difficult or impossible to achieve. Idiopathic angiosarcoma shows predilection for the scalp, forehead and central face.

Microscopy Three main histological patterns can be observed depending on the degree of tumor differentiation and are usually present within the same tumor: (1) vascular channel formation, (2) sheets of tumor cells with absent or very limited formation of vascular channels and (3) tumor cells with undifferentiated morphology lacking any features of vascular differentiation.

Well-differentiated angiosarcoma is a widely infiltrative tumor composed of dissecting, anastomosing and ramifying vascular channels of irregular size and shape (Fig. 15.112). The tumor infiltrates through the dermal collagen, around skin adnexa, and grows diffusely into the underlying subcutaneous fatty tissue (Fig. 15.113). The vascular channels are

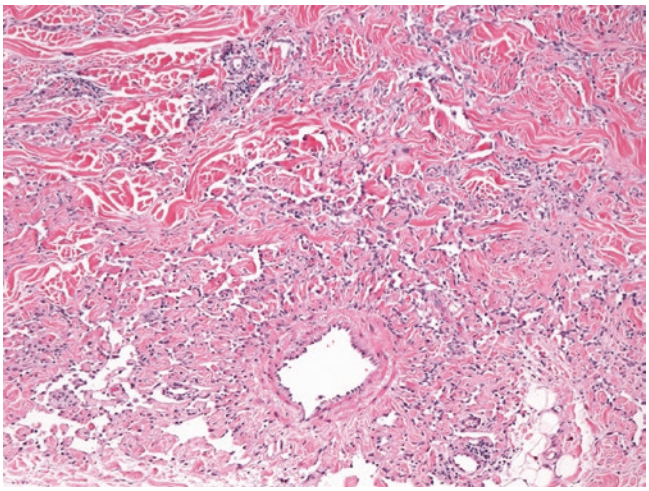

Fig. 15.113 Cutaneous angiosarcoma. Diffuse dissecting growth in the dermis with extension into the subcutis

lined by a single or multiple layers of endothelial cells, always displaying some degree of cytological atypia. The endothelial cells are typically plump, protruding into the vascular lumina. Their nuclei are hyperchromatic and enlarged. Mitoses are present, including atypical ones, but their number can vary. Small intraluminal papillary projections of endothelial cells are not uncommon.

Less differentiated areas of angiosarcoma are composed of more solid sheets of endothelial cells. The endothelial cells increase in size. There is usually a focal hint of abortive vascular differentiation, either in the form of dissecting vascular channels likely to be present at the periphery of the tumor, or as a formation of intracytoplasmic lumina. Such areas can be altogether absent in undifferentiated angiosarcomas. Mitoses are usually easily identified at this stage and nuclear pleomorphism is readily apparent.

Focal epithelioid change is not uncommon in idiopathic angiosarcoma and can on occasion be prominent. Importantly, the presence of epithelioid cells in idiopathic angiosarcoma should not result in classifying the tumor as an epithelioid angiosarcoma. Namely, epithelioid angiosarcoma has been regarded as a distinctive variant of angiosarcoma occurring outside the classical clinical setting of angiosarcoma, which includes idiopathic angiosarcoma of the head and neck, lymphoedema-associated angiosarcoma and postirradiation angiosarcoma [243]. In addition, more than 80 % of the tumor should be composed of epithelioid cells by definition [243, 244]. Epithelioid morphology is characterised by polygonal or rounded cells with abundant eosinophilic cytoplasm, large vesicular nuclei and prominent nucleoli [243, 244].

Unusual morphological variants of angiosarcoma include signet ring cell, pseudoglandular, syncytial, foamy cell (histiocytoid) and granular cell [245].

Additional histological features include the presence of sparse and patchy perivascular inflammatory cell infiltrate composed of an admixture of lymphocytes, plasma cells, histiocytes and granulocytes. However, inflammatory cell infiltrate can on occasion be prominent, thereby masking the vascular proliferation in the background and giving the lesion a pseudolymphomatous appearance.

By immunohistochemistry, angiosarcoma shows consistent nuclear positivity for ERG, a sensitive marker of endothelial differentiation [246]. In addition, other vascular markers including CD31, CD34, factor VIII-related antigen and FLI-1 are also positive to a variable extent, with CD31 being regarded the most sensitive and specific of the latter group [243]. Importantly, up to two-thirds of angiosarcomas, in particular those with epithelioid morphology, co-express cytokeratin(s), representing a potential diagnostic trap [243].

Differential diagnosis Well-differentiated angiosarcoma can be misdiagnosed as a hemangioma. The distinction can be particularly challenging in small and superficial biopsies. Angiosarcoma always displays pleomorphism of endothelial cells, which can, however, be mild. Additional helpful features include multilayering, increased mitotic activity, and the presence of atypical mitoses. Poorly differentiated angiosarcoma lacks vasoformation, and, this being a local phenomenon, thereby mimics a myriad of tumors, including poorly differentiated carcinoma, melanoma and haematological malignancies. Additional immunohistochemistry with a broad panel of antibodies should resolve the issue.

Treatment and prognosis Surgery represents the mainstay treatment of idiopathic angiosarcoma. However, clearance of excision margin is difficult to achieve due to the extensive local infiltration. Recurrences are detected in over 80 % of the patients [247]. Surgical treatment is generally supplemented by radiotherapy, chemotherapy and immunotherapy [247].

Idiopathic angiosarcoma has traditionally been regarded as a tumor with dismal prognosis, with a 5-year survival rate ranging from 10 % to 20 % [248]. Nevertheless, recent studies have demonstrated a far better survival for patients aged less than 70 years, smaller tumor size (less than 5 cm), absence of epithelioid morphology and tumoral necrosis, amounting to 48 % of disease-free survival for this so-called low-risk group of patients [249]. In contrast, none of the patients with high-risk features (age above 70 years, tumor size >5 cm, the presence of necrosis or epithelioid morphology) was alive at the 5-year follow-up interval [249]. The most common sites of metastases include loco-regional lymph nodes and lungs [249].

15.8.13 Fibrous Papule

Definition Fibrous papule (FP) is a hamartoma characterised by spindled to stellate fibroblasts, dense collagenous stroma and ectatic blood vessels.

Epidemiology FP occurs more frequently on the nose or central face of middle-aged patients.

Etiology and pathogenesis Etiopathogenesis is not known. Recently, it has been shown that, similar to tuberous sclerosis complex (TSC)-associated angiofibromas, FP are characterised by activation of mTOR and subsequent activation of p70 ribosomal protein S6 kinase (p70S6K) and ribosomal protein S6 (S6) by phosphorylation.

Clinical aspects It appears as a solitary dome-shaped, flesh-coloured papule. It may clinically mimic basal cell carcinoma or melanocytic nevus.

Microscopy FP is composed of cytologically bland, spindle-shaped to stellate, multinucleated fibroblasts embedded in dense collagen (Fig. 15.114). Ectatic thin-walled blood vessels are present. Several histopathological variants have been described, including clear cell FP (tumor cells have abundant clear vacuolated cytoplasm), epithelioid FP (sheets and small groups of epithelioid tumor cells) and granular cell FP (tumor cells have granular eosinophilic cytoplasm).

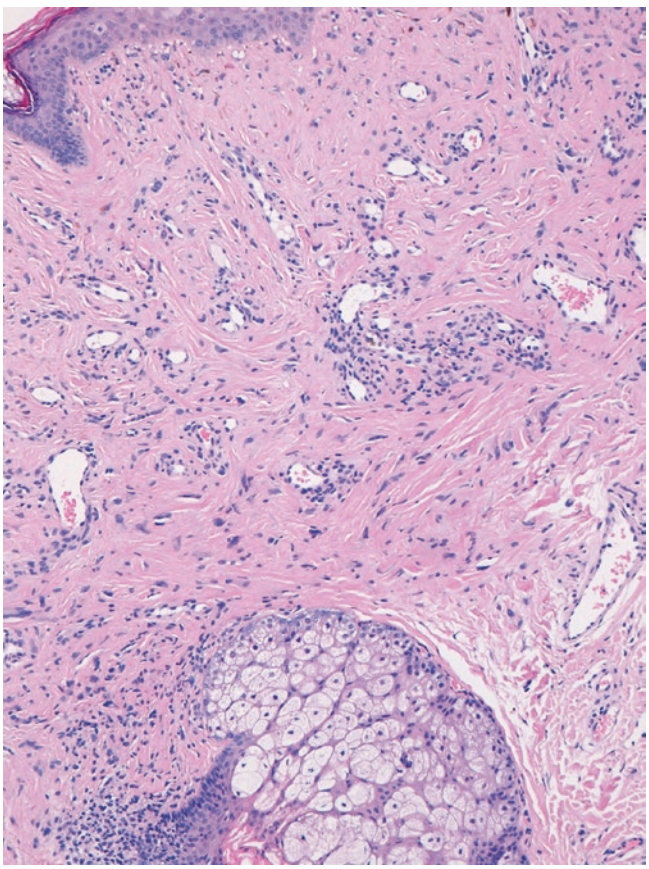

Fig. 15.114 Fibrous papule. Bizarre stellate cells and dilated vessels are seen in the superficial dermis

Immunohistochemistry Tumor cells are positive for vimentin; variably positive for CD34, factor XIIIA and CD68, while cytokeratins, S100 protein, HMB45 and melan-A are negative.

Differential diagnosis Differential diagnosis includes DF, which is uncommon on the face and shows a storiform cellular proliferation of spindled cells with overlying epidermal hyperplasia. Clear cell and epithelioid fibrous papule variants can be confused with xanthogranuloma. Intradermal nevus shows cohesive nests of melanocytes positive for melanocytic markers.

Treatment and prognosis FP is a benign lesion and no treatment is necessary.

15.8.14 Atypical Fibroxanthoma

Definition Atypical fibroxanthoma (AFX), also known as superficial malignant fibrous histiocytoma (MFH) or superficial pleomorphic sarcoma, is a cutaneous mesenchymal neoplasm characterised by morphological features of malignancy but indolent clinical behaviour.

Epidemiology It generally arises in elderly males, in the seventh decade. Presentation at a younger age is exceptional but may be seen in the setting of xeroderma pigmentosum. The sun damaged skin of the head and neck region (scalp predilection) in general is the most commonly affected area.

Etiology and pathogenesis UV exposure and previous radiation therapy have been advocated. The tumors carry the UV-signature mutation in the *TP53* gene. AFX may also occur in immunosuppressed patients.

Clinical aspects Clinically, it appears as an asymptomatic, ulcerated, rapidly growing skin nodule, measuring <2 cm in diameter.

Microscopy AFX shows no positive discriminatory morphological or immunohistochemical features, and its diagnosis remains one of exclusion, requiring adequate tissue sampling to exclude other neoplasms [250]. AFX is composed of a dermal proliferation of atypical, pleomorphic spindled and epithelioid cells (Figs. 15.115 and 15.116). No invasion of subcutis is generally observed. Nuclei are mark-

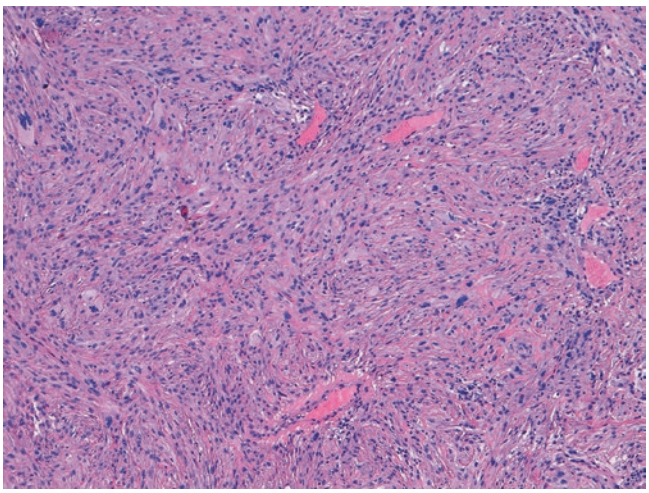

Fig. 15.115 Atypical fibroxanthoma. Fascicles of atypical spindle and pleomorphic cells within the dermis

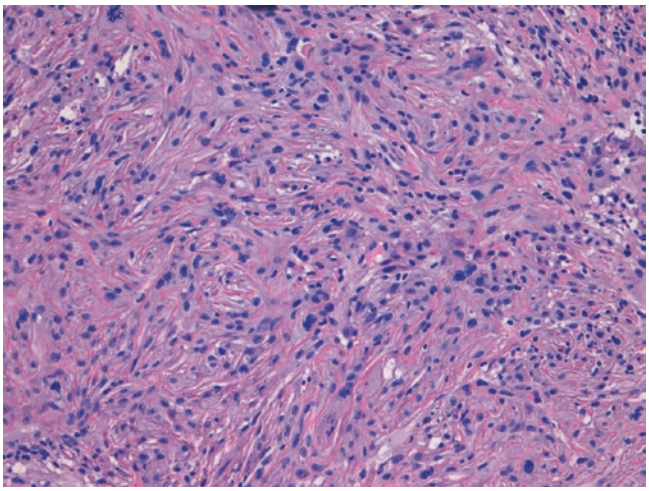

Fig. 15.116 Atypical fibroxanthoma. At higher magnification, there is marked pleomorphism and numerous mitotic figures are seen

edly enlarged, hyperchromatic, often bizarre and show irregular nuclear borders and prominent nucleoli. Scattered multinucleated giant cells may be seen. Numerous mitoses, including atypical mitoses, can be appreciated. There is frequent epidermal ulceration. Spindle cell AFX, the most common variant, is characterised by a predominance of atypical but relatively monomorphic spindle cells in a fascicular arrangement.

Immunohistochemistry In AFX, tumor cells are positive for CD68, CD10, CD99 and vimentin, while they are negative for melanocytic markers, cytokeratins, p63, muscle and vascular markers [251].

Differential diagnosis Differential diagnosis mainly includes sarcomatoid carcinoma, leiomyosarcoma and melanoma, and immunohistochemical analyses generally allow correct identification.

Treatment and prognosis AFX should be treated with wide surgical excision. Less than 10 % of cases may locally recur. The vast majority of cases do not metastasise.

15.8.15 Dermatofibroma

Definition Dermatofibroma (DF) (also known as dermal dendrocytoma, benign fibrous histiocytoma) is a common benign tumor composed of a mixture of collagen, fibroblasts, dermal dendrocytes and histiocytic cells.

Epidemiology DF is more common in females (male-to-female ratio of 1:4). It can occur in any age, but usually develops in the fourth and fifth decades. Although the distal extremities are the most common sites affected, any cutaneous site might be involved.

Etiology and pathogenesis Although originally attributed to traumatic insult to the skin (e.g. insect bite), its precise etiology is unknown. No inciting event is identified in the majority of cases. The chronic nature and the clonal growth of the proliferative lesion strongly suggest a neoplastic nature rather than a reactive process. A study of eruptive dermatofibromas in relatives suggests that a genetic component may exist.

Clinical aspects DF usually appears as an asymptomatic, slowly growing, solitary, small brown or reddish-brown, 0.5- to 1-cm firm nodule, fixed to the skin surface and freely movable over the subcutis on palpation. The characteristic tethering of the overlying epidermis to the underlying lesion with lateral compression, called the dimple sign, may be a useful clinical sign. Multiple DFs have been described in immunosuppressed patients.

Microscopy DF is a dermal-based non-encapsulated spindle cell tumor located within the mid-dermis (Fig. 15.117). It is typically composed by whorling fascicles of spindled to histiocytoid-appearing cells with collagen deposition. Early lesions typically show more histiocytes and lymphocytes, while established lesions show greater cellularity and spindled cells, and more fibrosis. Cytologic atypia and pleomorphism are usually minimal, but can be focally present. There are often jagged borders and collagen trapping at the periphery. The subcutis is typically preserved, although in deep DF, it may be involved. Most lesions display a grenz zone of normal papillary dermis overlying the tumor. The overlying epidermis is usually acanthotic and displays basal pigmentation. Follicular induction by DF has also been reported as mimicking overlying basal cell carcinoma.

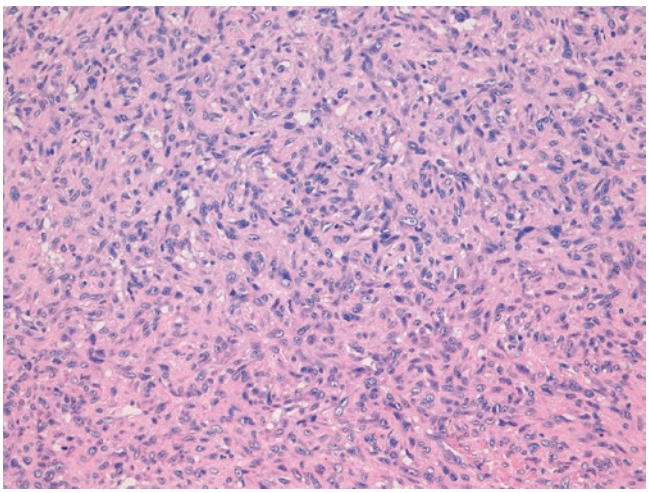

Fig. 15.117 Dermatofibroma. Classical storiform pattern

Numerous histopathological variants have been described. The aneurysmal variant (haemosiderotic) shows pseudovascular spaces, haemosiderin and reactive spindled and epithelioid cells and may mimic vascular tumors. The cellular variant is uncommon; it is often a large, deeply penetrating tumor with occasional mitoses and multinucleated cells; focal central necrosis is seen in 10 % of cases. This subtype is the most likely to recur (up to 30 % in some studies). Many other rare variants are described, including atypical/pseudosarcomatous/'DF with monster cells', lipidised, granular cell, clear cell, histiocytic/xanthomatous, osteoclastic, myxoid, keloidal/scar-like, palisading, deep penetrating (may mimic DFSP) and lichenoid.

Immunohistochemistry Factor XIIIa labels dermal dendritic cells, while anti-macrophage antibody MAC 387, which labels histiocytes, shows less consistent results. HSP47, a recently used marker for skin fibroblasts, stains frequently positive in DF. CD34 is typically negative but may show focal staining, especially at the periphery of the lesion.

Differential diagnosis DFSP is the main differential diagnosis. DFSP shows a monomorphous proliferation of spindled cells and typically extends deeply along septae of subcutaneous fat with honeycombing fat entrapment. Antibodies towards factor XIIIa and CD34 are usually useful in distinguishing both tumors. Factor XIIIa is mainly expressed in DF, while CD34 diffusely stains DFSP. Nestin is expressed in 13 % of DF and 94 % of DFSP [252]. Although ambiguous tumors sharing histopathological and immunohistochemical features of DF and DFSP have been described, the absence of COL1A1-PDGFB chimeric transcripts in one of these tumors favours DF. The differential diagnosis includes atypical fibroxanthoma (AFX), which typically occurs in heavily sun-damaged skin (especially head and neck area) of elderly patients. Both AFX and DF are positive for non-specific markers, including CD68, CD10 and vimentin.

Treatment and prognosis DF has been classically considered a benign lesion and complete excision is curative [253]. If incompletely excised, recurrences (up to 30 %) are likely (8). A few case reports of highly cellular, large and deep and locally recurrent metastatic dermatofibroma have been reported [254].

15.8.16 Dermatofibrosarcoma Protuberans

Definition Dermatofibrosarcoma protuberans (DFSP) is the most common type of cutaneous sarcoma. It is a low-grade malignant spindle cell tumor of skin characteristically showing prominent storiform pattern. The pigmented variant is also known as Bednar tumor.

Epidemiology DFSP is an uncommon tumor that shows a slight male predominance in young to adult patients. It most often occurs on trunk or extremities and more rarely on head and neck (10–16 % of cases).

Etiology and pathogenesis The etiology is unknown in most cases. Rare cases have been reported in association with previous trauma, burns or arsenic exposure.

Clinical aspects It appears as a slowly growing indurated plaque, papule or nodule, 1–10 cm in diameter. The cut surface is usually grey white; large tumors may show haemorrhage and cystic changes. In some cases, however, it may shrink into an atrophic and/or sclerotic plaque.

Microscopy DFSP shows slender monomorphous bland spindle cells that appear uniformly embedded in the collagen stroma. DFSP generally shows involvement of the dermis and subcutaneous fat. It is characterised by a proliferation of monomorphic spindle-shaped cells arrayed in storiform or cartwheel patterns (Fig. 15.118). High cellularity and irregular, short, intersecting bands of tumor cells forming a storiform or 'herringbone' pattern are generally present in the bulk of the lesion (while at the periphery and towards the subcutaneous fat, cells grow in a diffuse infiltrative way, forming a honeycomb pattern). Lesional cells typically lack significant pleomorphism. Mitoses are usually infrequent (<4/10 HPF) and not atypical. A component of high-grade sarcoma (usually fibrosarcoma) is occasionally found in 15 % of the cases of DFSP. Fibrosarcoma arising from DFSP shows areas of increased cellularity, atypia and mitoses (>5/10 HPF). Spindle cells are typically arrayed in prominent fascicles with 'herringbone' appearance, and there is usually loss of CD34 expression.

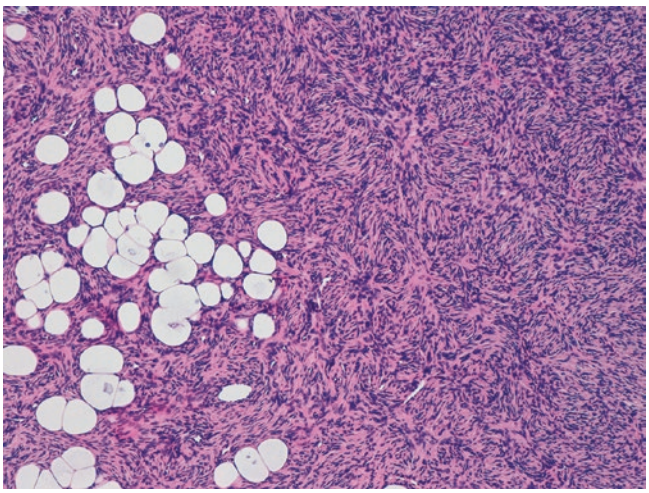

Fig. 15.118 Dermatofibrosarcoma protuberans. Deep involvement of reticular dermis and subcutis with a typical storiform or cartwheel arrangement

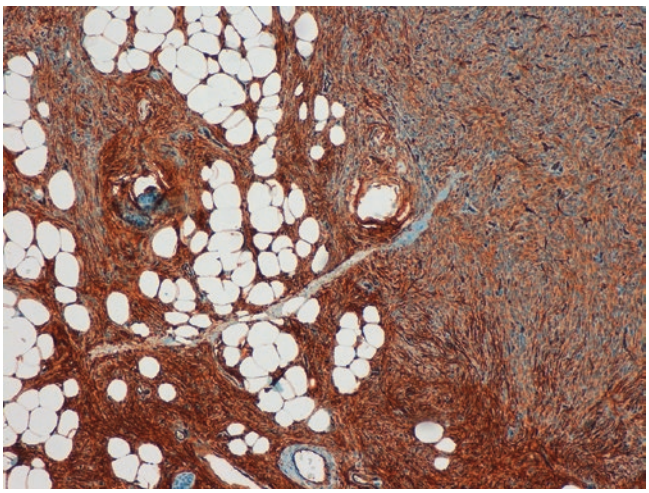

Fig. 15.119 Dermatofibrosarcoma protuberans. Immunohistochemical stain with anti-CD34 antibody shows diffuse and strong positivity in neoplastic cells

Several variants of DFSP have been reported including pigmented DFSP, also known as Bednar tumor, in which melanin-containing dendritic cells are scattered between the neoplastic spindle-shaped cells. In myxoid variant of DFSP, large areas of interstitial mucin are present.

Immunohistochemistry By immunohistochemistry, CD34 is typically, strongly and diffusely positive (sensitivity 84–100 %) (Fig. 15.119). S100 protein can rarely be positive in a few (dendritic) cells, while it may decorate pigmented cells in Bednar tumor.

Genetics Rearrangements of *collagen 1A1 (COL1A1)/platelet-derived growth factor B (PDGFB)* have been described and a characteristic t(17;22) is detected in most cases. Reverse transcriptase polymerase chain reaction (RT-PCR) and fluorescence in situ hybridisation (FISH) are suggested as screening tools for the presence of *COL1A1-PDGFB* fusion gene prior to initiation of oral imatinib molecular-targeted therapy [253, 255]. DFSP tumors lacking the classic t(17,22) translocation mutation seem to respond poorly to imatinib.

Diagnosis Cellular DF shows more pleomorphic cell types; both spindle-shaped fibroblastic cells and histiocytoid-appearing cells are present. DF may show superficial involvement of fat, but there is no deep, honeycombing fat entrapment as in DFSP. Immunohistochemistry is also helpful showing FXIIIA(+), CD68(+), CD163(+) and CD10(+); CD34(−) in DF. DFSP should also be differentiated from AFX, spindle variant. AFX occurs in sun-damaged skin of the elderly (typically head and neck region) and is a dermal-based proliferation of markedly atypical and pleomorphic-appearing tumor cells. There is no storiform pattern. Spindle cell/desmoplastic melanoma is S100 protein positive, lacks areas of storiform pattern and overlies melanoma in situ present in majority of cases (>70 %). Fibromatosis is characterised by a proliferation of β-catenin+/actin (SMA/+/CD34-elongated spindle cells associated with dense collagenous stroma. Cutaneous leiomyosarcoma (LMS) shows long fascicles of enlarged, atypical, eosinophilic-staining spindle cells that lack storiform pattern.

Smooth muscle markers, including actin and desmin, are positive, while CD34 is typically negative.

Treatment and prognosis Optimal treatment is complete surgical excision. Radiation therapy (RT) is recommended in those patients with positive resection margins. DFSP commonly extends far beyond the clinical margins, and it behaves as a locally aggressive tumor with a high recurrence rate (ranging from 11 to 53 %), but with a relatively good prognosis. Prognosis is excellent in most cases. The metastatic potential is very low (5 %) and essentially occurs only in cases with fibrosarcomatous transformation. Imatinib has been used for locally extensive and metastatic disease with complete response reported in up to 50 % of cases.

15.8.17 Cutaneous Leiomyoma

Definition Most cutaneous leiomyomas (with the exception of those originating in the genital tract) are benign tumors originating from the arrector pili smooth muscle elements.

Epidemiology Most develop in adolescence or early adulthood. It most commonly occurs on the extremities, trunk and head and neck.

Etiology and pathogenesis Some cases are familial.

Clinical aspects Most often, they appear as multiple painful pink or brown small papules, which may coalesce in nodules.

Microscopy Leiomyoma is composed of well-differentiated smooth muscle cells arranged in bundles and fascicles in the dermis. Cells have abundant fibrillary pink cytoplasm and oval, blunt-ended (cigar shaped) nuclei. As a rule, there is no cytological atypia, and occasional mitotic figures (up to 1 per 10 HPF) are acceptable while higher mitotic activity, diffuse atypia, necrosis and subcutaneous extension suggest leiomyosarcoma.

Immunohistochemistry Tumor cells are strongly positive for desmin, smooth muscle actin, calponin and h-caldesmon.

Differential diagnosis Dermatomyofibroma appears as a solitary plaque lesion usually on shoulder or trunk. It is characterised by a fascicular spindle cell proliferation parallel to epidermis without effacement of adnexa. Tumor cells typically express SMA, but not desmin or h-caldesmon. Superficial leiomyosarcoma generally shows more cytological atypia, necrosis and mitotic activity than leiomyoma.

Treatment and prognosis Surgery is the recommended approach for localised and symptomatic lesions.

15.8.18 Cutaneous Leiomyosarcoma

Definition Cutaneous leiomyosarcoma (LMS) is a malignant skin neoplasm composed of cells exhibiting smooth muscle differentiation. To be deemed cutaneous, the major portion of the tumor should be located within the dermis, with minor extensions into the superficial subcutis. Since LMS confined to the dermis may rarely recur locally but have no metastatic potential, Hornick and Fletcher [256] have suggested to designate purely dermal smooth muscle tumors with mitotic activity and cytological atypia as 'atypical smooth muscle tumors' and adopting the term LMS only for tumors involving the subcutis. In their view, the term 'atypical smooth muscle tumors of the dermis' would avoid an unnecessary and worrisome label of malignancy.

Epidemiology Although the age range is wide, cutaneous LMS is predominantly a tumor of adulthood, with male predilection.

Etiology and pathogenesis The development of cutaneous LMS has been associated with infections, previous radiation, Epstein–Barr virus (EBV) and immunosuppression.

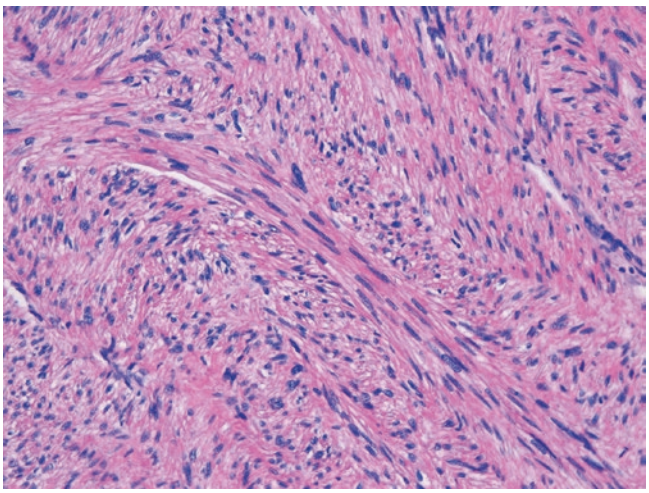

Fig. 15.120 Cutaneous leiomyosarcoma. The tumor is composed of interlacing fascicles of plump atypical cells with eosinophilic cytoplasm and eccentric, blunt-ended nuclei

Clinical aspects Cutaneous LMS presents as a single nodule or plaque-like tumor measuring up to 5 cm.

Microscopy LMS is composed of intersecting fascicles made up of spindle cells featuring eosinophilic, fibrillary cytoplasm, along with blunt-ended, occasionally indented nuclei and nuclear atypia (Fig. 15.120) [257]. Some tumors may show a pilar-type architectural pattern, characterised by interlacing bundles of smooth muscle cells ramifying in an orderly fashion among collagen fibres in the centre of the reticular dermis and extending more irregularly between collagen bundles at the periphery. Pilar-type tumors are generally low-grade lesions, with an overall pattern suggestive of pilar leiomyoma but featuring variable mitotic activity and cytological atypia.

Immunohistochemistry Smooth muscle differentiation in LMS can be demonstrated by immunohistochemical positivity for at least two out of three smooth muscle markers, e.g. SMA, desmin and heavy caldesmon (h-caldesmon). Tumor cells may label with keratins (up to 50% of cases). LMS show complex variable karyotypes. All are EBV-encoded RNA (EBER) positive.

Differential diagnosis Differential diagnosis includes cellular DF, leiomyoma, sarcomatoid carcinoma and AFX. Cellular DF shows a distinctly fascicular growth pattern similar to smooth muscle tumors, frequently extending to the subcutaneous fat, thus posing more diagnostic difficulty than conventional DF. Approximately half of cellular DF demonstrate staining for muscle actin or SMA due to their myofibroblastic phenotype and for this reason may be erroneously considered cutaneous LMS. However, spindle

cells in cellular DF lack the presence of distinctive eosinophilic fibrillary cytoplasm and almost never express desmin and/or h-caldesmon. Another frequent mistake is to misinterpret spindle cell (sarcomatoid) carcinoma as cutaneous LMS, especially for partially ulcerated lesions in the head and neck of elderly individuals. Distinction of spindle cell (sarcomatoid) carcinoma from LMS is aided by careful evaluation of the patient's clinical history, evidence of atypia in the overlying surface epithelium and adjacent solar keratosis. Immunohistochemistry can be difficult, as spindle cell carcinoma often exhibits SMA expression, and smooth muscle cells may rarely express cytokeratins.

Treatment and prognosis Cutaneous LMS should be surgically excised. Tumors confined to the dermis may rarely recur locally but have no metastatic potential. Up to one-third of subcutaneous tumors metastasise, and 10–20% of patients with subcutaneous lesions die of the disease. Radiation and chemotherapy are administered for metastatic tumors.

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Cytology of Head and Neck Lesions

16

Fernando C. Schmitt, Esther Diana Rossi, Carmela Iglesias, and Guido Fadda

Contents

F.C. Schmitt, MD, PhD, FIAC (✉)
Institute of Molecular Pathology and Immunology of Porto University-IPATIMUP, Medical Faculty of Porto University, Porto, Portugal

Department of Medicine and Pathology, Laboratoire National de Santé, 1, rue Louis Rech BP 72, 3401 Dudelange, Luxembourg
e-mail: fernando.schmitt@lns.etat.lu; fernando.schmitt@ipatimup.pt

E.D. Rossi, MD, PhD, MIAC • G. Fadda, MD, MIAC
Institute of Anatomic Pathology and Histology, Faculty of Medicine, Catholic University of Sacred Heart, Fondazione Universitaria Policlinico A. Gemelli,
Largo Francesco Vito 8, Rome 00168, Italy
e-mail: esther.rossi@rm.unicatt.it; guidofadda@rm.unicatt.it

C. Iglesias, MD
Department of Anatomic Pathology, Vall d'Hebron University Hospital, Universitat Autonoma of Barcelona,
Passeig Vall d'Hebron, 119-129, 08035 Barcelona, Spain
e-mail: ciglesias@vhebron.net

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16.1 Introduction

Fine needle aspiration cytology (FNAC) is a well-established tool for the initial evaluation of head and neck masses as well as to follow patients with previous diagnosed head and neck cancers. The cytological examination allows us to determine if a process is benign (reactive, inflammatory, or a benign neoplasia) or malignant and if possible gives a specific diagnostic condition. Masses in the head and neck are especially good targets for needle aspiration because many are superficially located or otherwise accessible to puncture. FNAC is a simple, rapid, and cost-effective technique, requiring minimal equipment. The main advantage of FNAC is the avoidance of a surgical biopsy and its attendant risks, which include scarring, potential tumor seeding, increased hospital stay, and increased costs. Correlation of clinical, ultrasound, or other imaging modalities with cytological findings is quite important to achieve correct results and to reduce the rates of inadequate samples.

In this chapter, we will revise the role of FNAC in the workup of patients with head and neck masses and describe the main cytomorphological features of the different conditions that can affect the head and neck region. Ancillary techniques that can be applied on cytological material will be also discussed. In this chapter, we emphasize only the cytological aspects because all the entities described here were described in detail in the other chapters of this book.

16.2 Basic Principles of Cytology of Head and Neck

16.2.1 Clinical Indications, Complications, and Contraindications

FNAC is now the first-line investigation for the diagnosis of palpable and even non-palpable head and neck masses of all types. Coupling the technique with ultrasound allows a better assessment of the lesion and enhances an appropriate cytological sampling [1]. A neck mass that is present for longer than a week should be considered pathological until proven otherwise and if possible examined by FNAC [2]. Concordant findings between clinic, image, and cytology may guide appropriate management and avoid unnecessary tests. Knowing beforehand if a lesion is malignant or benign will aid in planning surgery and may prompt or postpone decisions for surgical intervention [3]. In some regions of the world, tuberculosis lymphadenitis is highly prevalent in cervical nodes, and FNAC allows a quick diagnosis with possibility to introduce therapy immediately. Lumps in all structures of the head and neck can be accessible to the fine needle, and patients of all age groups, including pediatric patients [4], can be subjected to the procedure. FNAC is well-tolerated by the patients and practically exempt of any severe complication. A meta-analysis evaluating 3,459 aspirates from head and neck lesions studied by FNAC, irrespective of anatomical site, shows that the method has a sensitivity of 89.6 %, a specificity of 96.5 %, a false-positive rate of 3.5 %, a false-negative rate of 10.3 %, and an accuracy of 93.1 %. The predictive positive value was 96.2 %, and the negative predictive value was 90.3 % [5]. In most of these studies, the cytological sampling was not image guided, so these values probably can be improved.

The cytological diagnosis of most head and neck lesions can be made with a high degree of accuracy, but awareness of the diagnostic pitfalls is essential for safe practice [1, 5]. FNAC is a method that has some problems and limitations. Nondiagnostic aspirates, subclassification of lymphomas, inaccurate diagnosis of low-grade lymphomas, and the inability to distinguish thyroid follicular adenoma from follicular carcinoma are among the common problems faced. In case of salivary gland tumors, there are specific potential pitfalls due to the morphological variability of these tumors, which makes the interpretation highly dependent upon adequate sampling [3]. Cystic lesions also carry their own limitations [1]. Multiple aspirates may be required to obtain diagnostic material, and the use of ultrasound to target solid or peripheral parts of the cyst may be essential to establish the diagnosis. If nonspecific cyst contents are obtained and a residual mass is present, the aspirations should be repeated. In addition, difficulties occur to analyze FNAC samples derived from patients who have previously been treated with radiotherapy where granulomatous response and excessive fibrosis are common [5].

Complications occurring in aspiration of head and neck lesions are extremely rare and, when they occur, are usually bleeding with a resulting hematoma. This can be avoided if, after aspiration, firm directed pressure is placed over the puncture site. Lesions near or abutting onto large vessels need to be aspirated under ultrasound guidance and with thin needles. Vessels should be approached tangentially to avoid penetration and hemorrhage. Vascular lesions on thyroid and salivary glands should be aspirated gently without so many movements. Fewer passes may be advised in some instances where blood is drawn at the first pass.

There are no formal contraindications for FNAC; however, we need to avoid the procedure in patients with history of anticoagulation. The pain associated with FNAC is more often triggered by its anticipation than the actual pain due to needle penetration. In our experience, in most of the cases a clear explanation of the procedure in advance may relieve this type of pain.

16.2.2 Technical Aspects (Including FNA Technique)

The equipment necessary to perform a FNAC is simple. In our practice, FNAC is performed in an outpatient service offered to patients with lesions that require investigation. Patients are usually booked in advance by letter of referral or telephone. The minimum staff and equipment required are an assistant (especially if the FNAC was image guided), an examination couch, a writing desk, a work surface, an examination tray for instruments, and a good lighting. The aspirator will need a bench, preferentially with a sink, cotton, and an antiseptic solution to perform a quick antisepsis of the skin overlying the lesion; gloves, a syringe, a needle, and a syringe holder for the aspiration procedure; glass slides for smear preparation; liquid fixatives, preferentially 95 or 100 % alcohol; a small bandage; and a disposal box to put the potentially infected material. As one can see, it is easy to assemble these materials in a small box and place it on a small table or bench during the FNA procedure at the radiologist's room, doctor's clinical office, or ward, if there is no designated room for the FNAC clinic. Preferentially, the pathologist who performs FNA should have a room at the laboratory with the appropriate conditions to perform the FNAC and also a microscope to examine the smears after quick staining, in order to ensure adequate quality and quantity of the sample.

Most aspirations are performed with 23G–27G needles. Experienced cytopathologists advocate that aspirations performed with needles thicker than 23G cannot be considered FNAC. Small diameter needles are usually well tolerated by the patients, referring to the procedure as almost painless; they also result in low rates of complications such as hemorrhage. The adequate length of the needle depends on the target lesion. Most of head and neck lesions are superficial and can be reached with a 25 or 30 mm 23G needle. Sterile and disposable syringes made of transparent plastic are preferred for FNAC. Most pathologists and clinicians use 10 ml syringes, but the 20 ml ones can also be used, mainly in FNAC of large cystic lesions, in order to empty the cyst with less needle punctures. It is important to remember that larger syringes will not provide larger or better samples; on the contrary, in highly vascularized lesions, larger syringes may result in more hemorrhagic, and thus inadequate, samples. In summary, syringe choice should be based on availability and personal preference. Commonly a metal or plastic pistol-grip syringe holder is used during aspiration. Such syringe holder permits suction and release of suction of the syringe to be accomplished with one hand, while the other hand can be made available in locating and stabilizing the palpable lesions. The authors prefer the metal syringe holder, which weighs less than 200 g and has higher durability. The suction in the syringe is enough to make the plunger returns to its original position at the end of aspiration. Moreover, in the majority of aspirations, needle placement and application, as well as quality of material, are not compromised by the use of the syringe holder. One has to remember that the good quality aspiration sample fills the needle hub and not the syringe.

Palpable and non-palpable head and neck lesions can be submitted to FNAC procedure. Although aspiration should preferably be done guided by imaging, it is largely accepted that palpable lesions can be sampled using palpation as a guide for their localization. The best approach to fix the nodule appropriately is to use the middle and index fingers, instead of the commonly used thumb and index finger, for immobilization [6].

Currently, many FNAC procedures performed in routine practice are guided by ultrasound, even if the lesion is palpable. In head and neck FNAC, there are many advantages to use ultrasound assessment:

1. Head and neck region has a complex anatomy and the ultrasound enables more accurate localization of lesions and evaluation of the clinical findings than is possible for palpation. For example, clinically solitary lesions on thyroid, lymph nodes, and salivary glands can be multiple at ultrasound;
2. Non-palpable lesions may be detected and sampled for cytology;
3. Structures such as large vessels may be avoided, although sometimes inadvertent puncture of a large artery is inevitable. This has not lead to serious complication;
4. It may be possible to choose an aspiration route avoiding muscles because transfixing muscles can be painful for the patient;
5. The aspirating needle can be observed on the ultrasound monitor in real time and enables aspiration of the correct target with confidence;
6. The ability to evaluate cystic lesions is enhanced enabling to sample solid areas if present.

Depending on the site of the lesion, there are two guidance methods when we perform FNAC under ultrasound control. In the first one the transducer probe locates the lesion in the middle of the ultrasound field and makes a mark, by skin pressure, circumscribing the puncture site; the aspirator, then, passes the needle through the skin and advances it slowly into the lesion, in a way that the transducer probe can be placed perpendicular to the needle and guides the needle's movements (Fig. 16.1). In the other method, the FNAC procedure is guided step by step, so that the ultrasound field visualizes the needle's movements since the aspirator passes the needle through the skin until the needle is removed from the lesion. The transducer probe locates

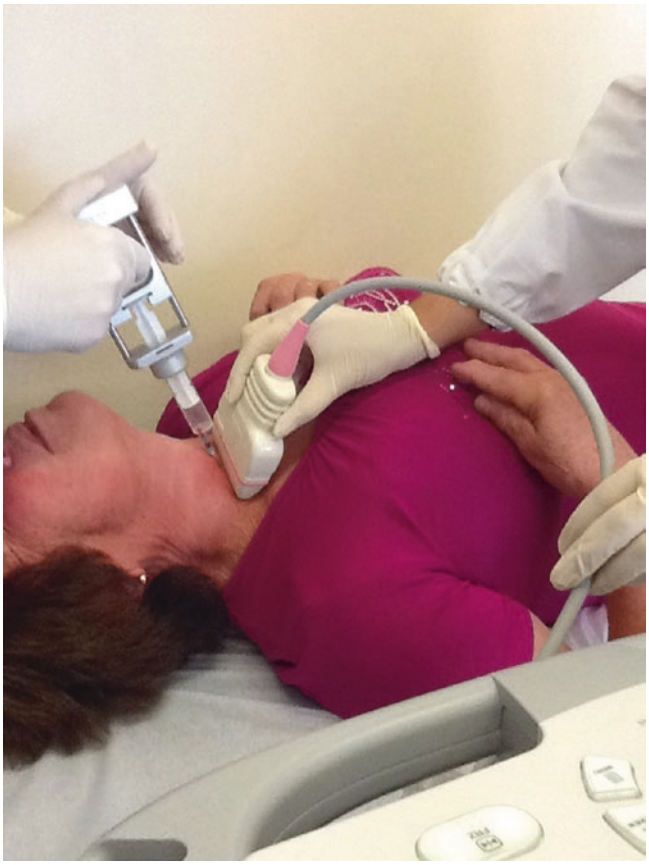

Fig. 16.1 Fine needle aspiration of thyroid guided by ultrasound

the lesion in one of the edges of the ultrasound field; the aspirator passes the needle through the skin, in parallel with the transducer probe, as well as with the sound waves, in the edge where the lesion is located in. It is possible, then, to guide the needle into the lesion, avoiding passing through vascular structures that might be in the way of the needle to the lesion.

Aspiration of most lesions is painless, and the patient feels only the initial pinprick through the skin. Anesthesia is not required for most aspirations. One of the keys to performing an adequate aspiration is the immobilization of the lesion by the aspirator's free hand as better cutting or coring of the mass can be achieved. The needle, with syringe and holder attached, is then inserted into the mass. The syringe plunger is pulled back, creating negative pressure, as the needle is advanced forward and backward. It is not the suction that directly results in obtaining a sample but rather the cutting action of the needle. The suction helps to pull tissue into the cutting path of the needle and to move the resulting fragments up into the needle's shaft. Pumping the syringe plunger does not enhance sampling; in fact, sampling can be reduced as a result of increased bleeding. In general, needle movements that are more frequent, longer in length, and kept within the tumor during the entire aspiration yield more tissue. Movement and frequency will depend on the size of the lesion and the aspirator's ability to maintain control. Typically, 30–50 excursions with the needle are made over a 10–20 s period. A blood-tinged specimen will appear in the hub of the needle. Suction is then released and the needle is withdrawn. Although little bleeding occurs in a fine needle aspiration, it is best to avoid all bleeding, as cellular yield decreases and lesion localization/demarcation is less distinct with increased soft tissue hemorrhage. Therefore, after withdrawing the needle, applying a gauze pad with one's fingertips pressing directly over the puncture site for 1–3 min is recommended (a longer time is applied for individuals with an easy bruising history or for those currently taking blood-thinning agents).

After removing the needle from the patient, the syringe is detached from the needle, filled with air, and reattached. The aspirator expresses a drop of the sample acquired onto one or more slides. The drop is then smeared, fixed with 95 % ethanol or air-dried, and then stained for interpretation. A modification of this aspiration technique is the acquisition of tissue using a needle only, the so-called capillary method. The syringe and the syringe holder are not used in this method. This modification allows greater sensitivity for the aspirator. Fingers are placed on the hub of the needle, allowing for a heightened appreciation of tissue density. Virtually no bleeding occurs. Because of the lack of suction, frequently no material is seen in the needle hub. Therefore, the aspiration is voluntarily stopped after 15–20 s. The needle is withdrawn, and an air-filled syringe is attached. Material is expelled onto a slide. Disadvantages of this method include the low cellular yield with fibrotic and sparsely cellular lesions and the rapid leakage of fluid from the end of the needle due to the pressure associated with cysts. When using the needle-only method (no attached syringe), the aspirator should always have a cup or syringe immediately available to capture the fluid which will otherwise rapidly leak onto the patient. Cysts require complete drainage. The drained area should be reevaluated by palpation or imaging for any residual solid mass, which will in turn require a separate needle aspiration.

16.2.3 Specimen Preparation (Including LBC)

The glass slides should be clean and ready to use. The slides should be label preferentially with a pencil at the frosted end. The labeling can include patient's name (initials), identification number, or the site of aspiration, being safer if at least two identifiers are used.

Preparation of high-quality smears is one of the most important parts of the aspiration procedure itself [7]. It does not matter how adequate is the specimen aspirated or how experienced is the cytopathologist, if the smears are not

interpretable, then a reliable diagnosis cannot be made. In a smear, cells should be spread over the slide surface by a gentle pressure, so that the cells are not crushed. The aim of preparing a smear is to obtain a homogenous layer of well-preserved cells, concentrated in a small area of the slide, which makes the microscopic analysis easier and quicker. Remember that a FNAC smear is not a blood cell analysis smear. One does not need the thinnest smear, but a smear that can maintain some of the lesion architecture, without being thick or crushing the cells.

There are two basic methods of smearing: one-step and two-step method [7]. The one-step method is preferentially used on small-volume specimens obtained from solid lesions. The specimen should be placed near the slide label. The slide that contains the specimen droplet is held by the physician's left hand in a vertical position, while the spreader slide is held by the right hand, perpendicular and over the other slide. The spreader slide is held at an angle so that its superior edge is poised above the specimen droplet. Then, smoothly, the spreader slide touches the lower slide, homogenizes the specimen droplet, and, with a constant and gentle pressure, makes the specimen drawn along the length of the lower slide. The surface of the spreader slide must always be parallel to the surface of the specimen slide, and the smear should finish before the end of the specimen slide. It is also important that the smear occupies only a small area of the slide.

The two-step method is used for liquid or hemorrhagic specimens within cells, and tissue particles are suspended. The fluid sample is placed from the middle to the labeled edge of the slide. The spreader slide is held at a 45° angle to the specimen-bearing slide, and its end is brought into contact with the fluid. Then, the spreader slide advances toward the specimen slide's label, carrying the fluid and suspended particles. The surface tension causes the fluid to spread out in a line behind the edge of the spreader slide. The spreader slide, thus, returns in the opposite direction, stopping in the middle of the specimen slide, where the tissue particles remain concentrated. The spreader slide is quickly pulled away from the specimen slide, which is turned to one side in order to drain the fluid excess. After that, the spreader slide is turned perpendicular to the specimen slide, and the line of sediment tissue particles is smeared as it is in the one-step method. This technique is more complex, and the smears are not that good looking as in the one-step method, but it allows a better smear quality to fluid or hemorrhagic samples: the tissue particles are concentrated in the middle of the slide, which makes microscopic observation easier, and the excess of fluid or blood is removed, allowing rapid drying and a better fixation of the slide.

For different reasons, sometimes it may be necessary to prepare more than one smear from a single droplet of specimen, for example, when performing aspirations from tumor masses, which usually yield very cellular material. If too much sample is placed on one slide, the sample may be too thick for microscopic examination. Alternatively, sometimes even though a small amount of specimen is obtained by fine needle aspiration, but for some reason, it may not be feasible to perform another aspiration, yet the physician knows that immunohistochemistry or another special staining is needed, it may be of interest to split the specimen droplet in additional slides. In such cases, the entire sample is expelled onto a glass slide, and the slides are held as described in the maneuver in the one-step method. The end of the spreader slide is placed in the sample droplet at a 45° angle. The spreader slide quickly touches the sample and carries off its edge. This can be done at least four times with the same specimen slide. Each portion of the original sample in the spreader slide is smeared in a new slide, while the original sample is smeared with the spreader slide, following the one-step method.

Fluid or bloody specimen may be expelled directly to a liquid-based vial: in the former the aim is to concentrate the amount of cells in a smaller portion of the slide, and in the later the aim is to reduce the number of erythrocytes, allowing a better microscopic examination. Liquid-based specimen may also be used to perform additional techniques, such as immunohistochemistry and flow cytometry. Cell blocks, which are very useful for ancillary techniques, also can be prepared from cells suspended in liquid. In our practice, when it is possible to do on-site assessment, we suspended the cells in saline, and according to the morphological assessment, we can send directly fresh cells to flow cytometry or transfer for vials containing methanol for liquid-based cytology or fixed the cells in formalin for cell blocks.

Although Papanicolaou stain is the most widely used staining method in cytology, most cytopathologists prefer to use a combination of two complementary stains in FNAC: Papanicolaou and Romanowsky-based stain. Papanicolaou staining includes the hematoxylin nuclear stain and two cytoplasmic counterstains, orange G and EA. There are several types of stains under the designation of the Romanowsky method, such as Giemsa, May-Grunwald-Giemsa, and Diff-Quik, but all present the same staining pattern that characterizes the Romanowsky method. Some cytopathologists prefer the classical histologic hematoxylin and eosin (H&E) method, mainly due to easier and quicker comparison between the histologic and cytologic characteristics of nuclei, cytoplasm, and stroma.

The smears prepared after the aspiration procedure can be either intentionally air-dried for further Romanowsky staining or immediately fixed in 97 or 70% ethanol for Papanicolaou or H&E stains. The air-dried smears are submitted to post-fixation with methanol.

The best staining is obviously the staining that the cytopathologist is most familiar, but each staining method has its

advantages and works better together. The air-drying required for Romanowsky method results in cell swelling, and the cells tend to look larger than in Papanicolaou or H&E methods. Romanowsky stains have the ability to react with several tissue components in a metachromatic way, giving them a reddish-purple color. This can be observed in nucleic acids, mucins, and extracellular matrix components, such as in mixed tumors of salivary glands. Colloid is also easily recognized using Romanowsky stains.

On the other hand, nuclear details are not the strength of the Romanowsky method. As already mentioned, the nuclei usually look larger in Romanowsky stains, but they also lack chromatin and membrane contour details. For this reason, Papanicolaou stain may be performed whenever the nuclear detail is important for the diagnosis or the subclassification of tumors. Squamous differentiation, a frequent finding in head and neck aspirates (cleft branchial cysts, metastatic squamous cell carcinoma, among others), is also more easily observed using Papanicolaou or H&E stains.

16.2.4 Ancillary Techniques in Cytological Material

In head and neck pathology, special stains, immunocytochemistry, flow cytometry, and molecular techniques are the most common ancillary techniques used as adjuncts to morphology for diagnosis and prognosis. In some specific cases, the study of markers of therapeutic response has been emerging in the last few years in this field. All these techniques can be applied not only in histological biopsies but also in cytological material. There are many advantages in the use of cytological material over histology to perform ancillary studies: ease of obtaining fresh material, ability to check the quality of the material immediately after harvest, and better preservation of DNA and RNA [8]. The use of immunological techniques in cytology has expanded rapidly over the last 20 years. Akin to the situation in histopathology, it has led to an increased diagnostic accuracy. In head and neck, the study of markers to define primary sites in metastatic lymph nodes is one of the fields with largest application in FNA aspirates. In many institutions, FNAC is used as a first-line approach to evaluate lymphadenopathies, including primary neoplasia. The combined use of morphology, flow cytometry for immunophenotyping, and genetic analysis has increased the accuracy of diagnosis and correct categorization of lymphomas on FNAC samples [9]. The possibility of using genomic and proteomic studies in small amounts of material obtained by FNAC can minimize invasive procedures and allow the monitoring of cancer, including therapeutic response, with repeated testing. The introduction of LBC offered the possibility of preservation of cells in an environment of excellent quality when compared with formalin-fixed paraffin-embedded tissues.

The molecular techniques most commonly used on cytology include polymerase chain reaction (PCR) and in situ hybridization (ISH). However, other techniques such as in situ PCR, microarrays, and proteomic and sequencing (including next-generation sequencing) methodologies are now being validated [10]. PCR methods are ideal for cytology material, and some applications are detection of gross chromosomal alterations as deletions and translocations or even point mutations in individual genes. RT-PCR uses cDNA as a template and primer exon sequences to flank rupture points of translocations. PCR applications are centered in the detection of infectious as well as diagnosis of solid tumors detecting gene mutations or clone gene rearrangement. PCR analysis can be performed directly with fresh collected material from FNA, in liquid-based cytology samples, or even with cells scraped from FNA slides. The amount and quality of DNA obtained by FNA for PCR assay do not seem to be a problem, and 50–100 cells are adequate to obtain good PCR results. FNA-obtained cells provide excellent representative samples, with poor contamination of stroma or local structures. In head and neck squamous cell carcinoma (HNSCC), studies have been published on the detection of HPV on cytological material with relevant role in the diagnosis and prognosis [11]. These will be discussed in the lymph node section on this chapter. ISH can also be applied to cytology, permitting, with either fluorescent or chromogenic markers, to detect high-risk HPV and numerical or structural aberrations of chromosomes. This technique is a reliable technique, particularly useful in cytology as it can be applied directly in smears. Monolayer smears are ideal for ISH techniques, and slides with ethanol or air-dried fixed preparations, as well as cell blocks, are equally suitable.

Proper specimen processing is of utmost importance for any ancillary technique. The most commonly used preparations are direct smears, cytospin centrifugations, cell blocks, and liquid-based cytology (LBC) preparations. Direct smears are prepared from FNAC material or brushings. After being air-dried, they should be fixed in formalin followed by alcohol and perform well in immunostaining for nuclear antigens such as Ki-67, TTF-1, and ER; they are, however, less suitable for staining of membrane or cytoplasmic antigens because of high background staining due to cell damage caused by the smearing. The cytomorphology of direct smears is excellent, but the number of slides, in particular from FNAC biopsies, is usually limited and will prevent the use of an extensive marker panel. Although the cost of preparation of direct smears is low, the amount of antibodies needed to cover the entire slide is relatively high. Direct smear can be used for molecular studies. After removal of the coverslip with xylene, it is possible to scrape the cells previously stained to an Eppendorf tube, to perform lysis and extract DNA of good quality for PCR or DNA sequencing [12]. Cytospins are prepared from cell suspensions in non-

fixative solutions such as PBS and RPMI of FNAC material. Cytospin offers an excellent source of staining for most antibodies although various fixatives have to be used. The technique is less suitable for specimens with a rich admixture of blood or mucous. In most cases a large number of cytospin slides can be prepared, which allows the use of panels with many antibodies. At present cytospin material is one of the choices for evaluation of lymphoid lesions. However, in our daily practice, flow cytometry from cell suspensions had been the preferential option in the workup of lymphoproliferative lesions. The cytomorphology is somewhat different from direct smears. The technique is relatively labor intensive but uses low amounts of reagents, including antibodies. Air-dried cytospin slides can be stored for many years at −70 L^{a}C without loss of antigenicity and excellent DNA preservation. LBC preparation systems are today available in most cytology laboratories. They can be used for almost all types of cytological specimens. LBC preparations can be stained with various antibodies to nuclear, cytoplasmic, and membrane-bound antigens in a reproducible way. Unfortunately the material is not optimal for evaluation of lymphoid neoplasms. Several slides can be produced from LBC material, which allows a complete immunological workup in most cases. The cytomorphology of LBC material is different to that of direct smears, which can initially be a restraining factor. The cost for LBC preparations is considerably higher than for other types of material. LBC slides, post-fixed in 95 % ethanol, can be stored for months at −70 °C without change in immunoreactivity. LBC preparations are suitable for preserving cell samples and DNA with sufficient quality to be used in several molecular analyses such as PCR, RFLP, and even sequencing [9]. Cell blocks can be prepared from all types of cytological specimens, except preparations with low cellularity. There are several techniques to produce cell blocks, such as cytocentrifugation, either with direct formalin fixation or fixation after addition of plasma thromboplastin. Cell blocks perform in a highly reproducible way when stained with most antibodies, except for some used in the workup of lymphoid lesions. One distinct advantage of cell blocks is that many slides can be prepared for extensive panels of immunostains. In addition, the quality control of cell block staining is identical to that of histopathology. The morphology of cell blocks is identical to that seen in histological specimens and therefore familiar to most pathologists. The technique, which is available in most laboratories, is relatively time consuming, and the cost is comparable to that of the cytospin technique. Since most of molecular techniques are now standardized for paraffin-embedded tissues, they can apply directly to cell block preparations with excellent results.

Cytology is playing an important role in the management of head and neck lesions. The application of ancillary techniques on cytology is increasingly the importance of the technique. The most important applications on head and neck are detection of infectious disease (e.g., PCR detection of tuberculosis, HPV, among others), workup of lymphoproliferative diseases detected on head and neck lymph nodes, detection of primary tumors in head and neck metastasis, and definition of undetermined lesions on thyroid FNAC. There are more applications coming, for example, in the evaluation of HER2 in high-grade salivary tumors and possible therapeutic response. All these different applications will be discussed in the respective sections along this book.

16.3 Cytology of the Oral Cavity and Oropharynx

The lesions of the oral cavity which may be cytologically investigated are represented by benign masses, among which cysts account for the majority of these lesions and tumors. It is important, when the cytopathologist endures the interpretation of a cytologic picture of a lesion of the oral cavity and the oropharynx, to emphasize the importance of an appropriate clinical history and the knowledge of the instrumental findings (especially ultrasonography, x-rays, and CT scans) in achieving a correct diagnosis [13, 14].

16.3.1 Benign Lesions

Cystic lesions of the oral cavity usually occur in the minor salivary glands sited in the lips and in the floor of the mouth and are caused by retention of their mucous secretion. These nodules, which appear as small- to medium-sized granules with a gray translucent surface, often undergo FNAC for both diagnostic and therapeutic purposes: in fact the aspiration yields clear or mucinous material which can sometimes be completely removed with the final disappearance of the lesion. The ranula, in respect to other retention cysts, has a distinctive clinical appearance as its color is bluish (its name, ranula, in Latin is frog-like indicating the color of the belly of a frog), and it is mostly located in the floor of the mouth. The cytologic picture of a ranula may yield only vacuolated histiocytes (plunging ranula) or a few pavimentous or cylindrical cells (simple ranula). Oral lymphoepithelial cysts (OLEC) arise in the Waldeyer's ring area, usually in the floor of the mouth, but the palatine pillars and the ventral or posterolateral tongue are frequent sites of occurrence [15]. OLEC are less common than retention cysts of the oropharynx, but they may share a similar clinical appearance, and their cytologic picture may mimic either a squamous carcinoma or a malignant lymphoma since both squamous and lymphoid cells with atypical features may be identified in the smear [16].

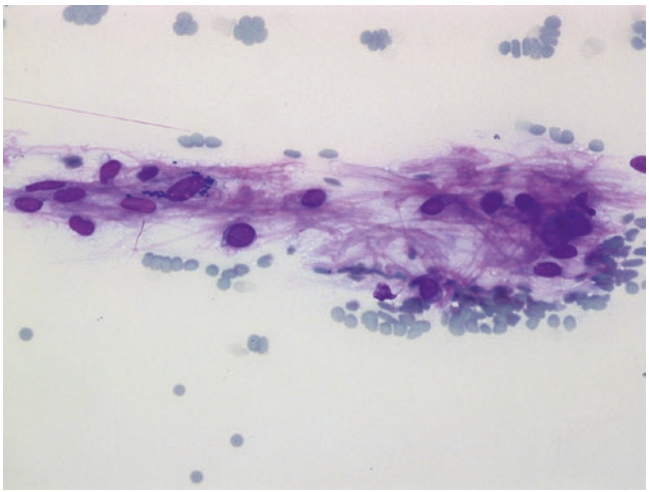

Fig. 16.2 Neurilemmoma of the oral region. Note the characteristic fibrillar stroma in the background

Benign tumors of the oral cavity which are likely to undergo fine needle aspiration mostly belong to the group of mesenchymal neoplasms (see below). Vascular tumors which occur in the tongue, in the floor of the mouth, and in the lips (hemangiomas and lymphangiomas) are seldom sampled because of their distinctive clinical appearance and the limited amount of cells which is usually extruded [17–21]. Schwannomas and neurofibromas (Fig. 16.2) may occur in patients with neurofibromatosis type 1 [22, 23] and type 2 (neurofibromas), whereas mucosal neuromas are distinctive features of the MEN (multiple endocrine neoplasia) type 2B [15]. Other mesenchymal tumors as lipomas [24] and their variants, fibromas and leiomyomas, are uncommon findings in the oral cavity, and when they are sampled, the diagnostic efficacy of the cytology is controversial [25]. A mesenchymal tumor which may distinctively arise as a firm mass located either in the base of the tongue or in the parapharyngeal space is rhabdomyoma, especially the adult-type and the juvenile-intermediate-type variants [26]. The cytology of the adult-type rhabdomyoma shows large polygonal cells with sometimes eccentrically placed nuclei and dense eosinophilic or vacuolated cytoplasms, only occasionally showing the distinctive cross-striations. These tumors arising in the tongue may be misinterpreted as granular cell tumors [27].

Granular cell tumor (GCT or Abrikossoff myoblastoma) is a neural-derived tumor which usually pursues a benign course and may arise as a firm nodule in any site of the oral cavity, but especially in the tongue. In the neck region a district where GCT has been also described with interesting frequency is the thyroid gland. Its cytologic picture is quite distinctive: large cells usually isolated or in small sheets, with round centrally placed nuclei showing a prominent nucleolus and granular cytoplasms [20, 28–30]. These cells are characteristically positive for both S-100 and CD68 and are regarded as of schwannian origin [20, 28–30]. Mitotic figures and marked atypical figures should raise the suspicion of the malignant variant of this tumor which may recur after the surgical removal and metastasize to the regional nodes [31, 32]. Epulides and congenital granular cell epulis (CGCE), which are regarded as variants of GCT [15], may occasionally undergo fine needle aspiration since they can be misdiagnosed as cysts of the gum or as lymphangiomas [33]. The cytologic picture of the CGCE in the newborn is similar to the GCT, but unlike cysts or lymphangiomas, they do not give rise to a liquid sample. The cytology is similar to GCT although, unlike this tumor, cells of CGCE do not express S-100. Other tumors that may occasionally present as protruding mass in the oropharyngeal region are meningioma and neurilemmoma [34].

16.3.2 Malignant Neoplasms

Malignant epithelial tumors of the oral cavity are usually preceded by precancerous lesions that may present as either relevant plaques or ulcers in areas suffering from traumatism (e.g., caries, metal devices) and do not regress after anti-inflammatory treatment. For a systemic description of the malignant neoplasms of the oral cavity and the oropharynx, refer to Chaps. 4 and 6.

The cytology of squamous cell carcinoma (Fig. 16.3a, b) and precancerous lesions of the oral cavity has been described by some authors [35–37] using different sampling systems (e.g., curettage and needle aspiration). Navone et al., using liquid-based preparations, achieved interesting results in terms of diagnostic accuracy and possibility of application of special techniques, even if the sampling method is difficult to apply to routine practice, and the cytological processing should be used only in centers with specific experience [38]. The material obtained from a biopsy of an oral neoplasm may be also used for the assessment of the predictive markers of treatment such as the HPV-associated protein p16 and other tumor parameters [11, 39]. Fine needle aspiration cytology also plays a pivotal role in the identification and characterization of cervical metastases from primary SCC of the oral cavity. In particular, FNAC is the keystone of the algorithm for the identification of the primary tumor in cervical nodal metastases from unknown origin. The evaluation of the cellular details (squamous, glandular, undifferentiated, pigmented cells) and the application of molecular techniques (immunocytochemistry and mutational analysis) allow a correct diagnosis in the majority of cases [39–43]. Less frequent carcinomas of the oral cavity are tumors derived from minor salivary glands (e.g., mucoepidermoid carcinoma, adenoid cystic carcinoma, and carcino-

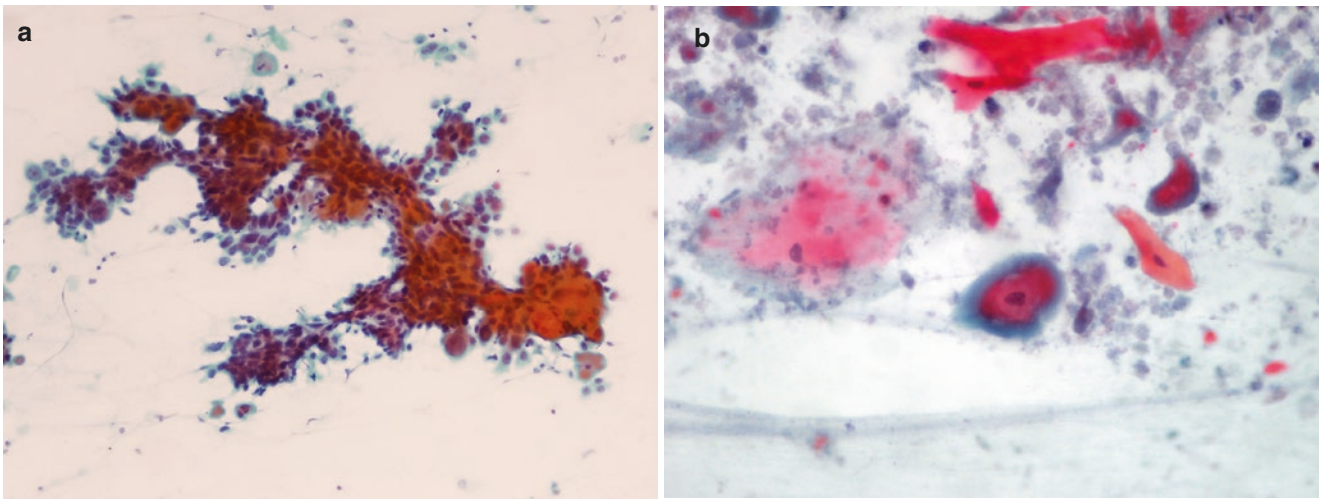

Fig. 16.3 (**a**, **b**) Details of nuclear and cytoplasmic features in a squamous cell carcinoma of the oral cavity (**a**) on conventional cytology). (**b**) With liquid-based cytology

sarcoma of the palate [44–46] which may arise in the floor of the mouth and in the palate and be misdiagnosed as benign cysts or Fordyce granules (remnants of the sebaceous oral glands).

The oral cavity may be site for other nonepithelial malignant tumors such as non-Hodgkin lymphoma [47], primary and metastatic melanoma [48], and various soft tissue malignancies [49–52]. Among them, rhabdomyosarcomas need a short mention. Alveolar and embryonal rhabdomyosarcomas represent a significant clinicopathologic entity which is characterized by a cytologic picture of a round blue cell tumor showing multiple binucleated and plurinucleated cells [53, 54]. The differential diagnosis with other blue cell tumors may be sometimes puzzling, but the presence of multinucleated cells, the immunocytochemical positivity for myogenin and desmin, and negativity for leukocyte-common antigen (LCA) are the hallmark of this important neoplasm which may show a good response to chemotherapy. The identification of the specific translocation t [2; 13][q35; q14] on the cytologic sample may help in the definitive diagnosis of the alveolar variant of rhabdomyosarcoma.

16.4 Cytology of Nasal Cavity and Paranasal Sinuses and Nasopharynx

Anatomically the respiratory system includes the nasal cavity, the paranasal sinus and nasopharynx, the oropharynx, and the lower respiratory tract (larynx, trachea, bronchi and bronchioles, and the air spaces beyond) which are extensively described in other chapters of this book. Due to the roles of the upper respiratory tract, it can be affected by several conditions including both benign and malignant entities. Although the histological evaluation represents the main diagnostic approach, the application of cytology has gained popularity and interest in several of these conditions. The main method used is a direct scraping which can be done with instruments or a washing of the cavity. In this regard, spontaneous nasal secretions might be collected on cytological samples, fixed in alcohol, and stained by Papanicolaou method. The May-Grunwald-Giemsa (MGG) on air-dried smears might be used especially in cases of flogistic or allergic diseases. The nasopharyngeal sampling has been proposed of a screening method in areas with high incidence of nasopharyngeal carcinomas. In this tract, the use of FNAC is less frequently applied, while the cytological analysis of nasal secretions is the best available material. Regarding the methods, conventional cytology, LBC, or cell block can be successfully performed with good results.

Before evaluating specific benign and malignant lesions, a short outline may be addressed to the two different types of epithelium lining the upper respiratory tract which include both stratified squamous epithelium which are numerically predominant and the presence of layer of glandular cells. The evidence of extracellular material and extraneous elements needs to be identified in order to avoid misdiagnoses. In some of these cytological specimens, the presence of damage effects by environmental agents is typically found. Adequate clinical details and patient history and use of special stains may enable the cytopathologist to confirm the diagnosis [55]. In this regard, we might mention reactive squamous cells with enlarged hyperchromatic nuclei, anucleate keratinized squamous cells, or some hyperplastic features.

16.4.1 Benign Lesions

The majority of benign conditions in the upper respiratory tract are represented by inflammatory and infective diseases. Unfortunately cytology has little to offer in the identification of disease mainly ascribed to the low specificity and scant material. Some diagnoses might not fall short on cytology such as fungal infection, when the fungal microorganism (e.g., *Rhinosporidium seeberi*) is identified or some viral infections with their typical cytopathic nuclear-cytoplasm effects. The main role of cytology in these cases is to rule out a malignant lesion [55].

Some of these conditions might be isolated to the nasal cavity (rhinitis), isolated to the paranasal sinuses (sinusitis), or involve both nasal cavity and paranasal sinuses (rhinosinusitis). Usually they do not require samples, but when it occurs, samples are obtained in order to establish a diagnosis or to exclude other possibilities which may be associated with rhinosinusitis. The cytological findings are characterized by a nonspecific inflammatory infiltrate including lymphocytes, plasma cells, eosinophils, histiocytes, and neutrophils. Focal squamous metaplasia and fibrotic debris may be reported. In cases of asthma, the major evidence is a preponderant component of eosinophilic cells. The specific agent might be recognized based on the identical parameters used for the histological diagnosis.

The cytological sample of a nasal polyp is rarely performed mainly because of the surgical excision and the sequential histological evaluation. Few papers reported series of polypoid nasal cytology. The main feature is a normal edematous mucosa with respiratory epithelial cells, some squamous metaplasia, and scant bland-appearing fibroblast and endothelial cells.

A more frequent lesion is defined by the paranasal sinus mucocele in which a cystic lesion of the paranasal sinus is seen. This diagnosis is made by the correlation between clinical, radiological, and cyto- or histological parameters mainly because the cytohistological features may mimic a normal epithelium with flattened pseudostratified ciliated columnar cells and variable amount of inflammatory cells.

16.4.2 Malignant Lesions

Malignant sinonasal tumors comprise less than 1% of all human cancers and 3% of all head and neck carcinomas [55]. A great association with Epstein-Barr virus (EBV) has been reported mainly in the undifferentiated nasopharyngeal carcinoma (Fig. 16.4a, b). Despite the low rate of malignancy, a great variety of histological cancers may be found, and the use of ancillary techniques might represent a valid aid.

The most frequent histological types are keratinizing squamous cell carcinoma, non-keratinizing squamous (cylindrical) cell carcinoma, undifferentiated carcinoma (Fig. 16.5a–c), malignant lymphoma, malignant melanoma, intestinal-type adenocarcinoma, adenoid cystic carcinoma, low-grade adenocarcinoma, and olfactory neuroblastoma [56–59]. Although all these histological types present their specific and distinctive morphological features, a cytological detailed diagnosis is not always possible so that the main role of cytology is likely to identify with the highest diagnostic accuracy and the correct classification of the malignant lesion into their histotype. The identification of malignancy involves a grading according to the degree of differentiation, cellular pleomorphism, and mitotic activity. Well-differentiated carcinomas are uncommon in this district, and they might be misdiagnosed as pseudoepitheliomatous types of hyperplasia and verrucous carcinoma. Most conventional carcinomas are moderate to poorly differentiated tumors with special histotypes only occasional found. The paradigmatic features of a sinonasal undifferentiated carcinoma are composed of small- to medium-sized cells, lacking evidence of squamous or glandular differentiation as well as of rosette formation [57–59].

In the field of poorly differentiated carcinomas, the main challenge is the distinction with an epithelial, mesenchymal, or lymphoid origin mainly due to the undifferentiated cells [57–60]. In this perspective, the application of immunocytochemistry for cytokeratins, neuroendocrine markers (low-molecular-weight cytokeratins, EMA, neuron-specific enolase, Leu-7, CD56, synaptophysin, chromogranin), mesenchymal markers, and lymphoid markers might correctly discriminate. The neuroendocrine tumors of the sinonasal tract are uncommon and not precisely characterized, but mainly before placing a tumor in this category, a primary neuroendocrine lung neoplasm must be ruled out. The typical features include nests, cords, sheets of small cells with different grades of differentiations, and positivity for two neuroendocrine markers [60].

The diagnosis of sinonasal adenocarcinoma includes several differential entities [61–63]. The first is the intestinal-type adenocarcinoma which originates through intestinal metaplasia of the ciliated respiratory cells lining the Schneiderian membrane. This is the most common sinonasal adenocarcinoma mainly associated with a history of exposure to different type of dust. The patterns, which might be found also in cytological samples, are papillary, glandular, mucinous, and mixed [61–63]. In presence of a cytological sample with sinonasal adenocarcinoma, a metastatic gastrointestinal origin must be always ruled out. The absence of epidermoid and squamous differentiation separates this entity from mucoepidermoid and adenosquamous carcinomas. The enteric origin of these cells is confirmed by positivity for cytokeratin 20, CDX2, and frequently cytokeratin 7.

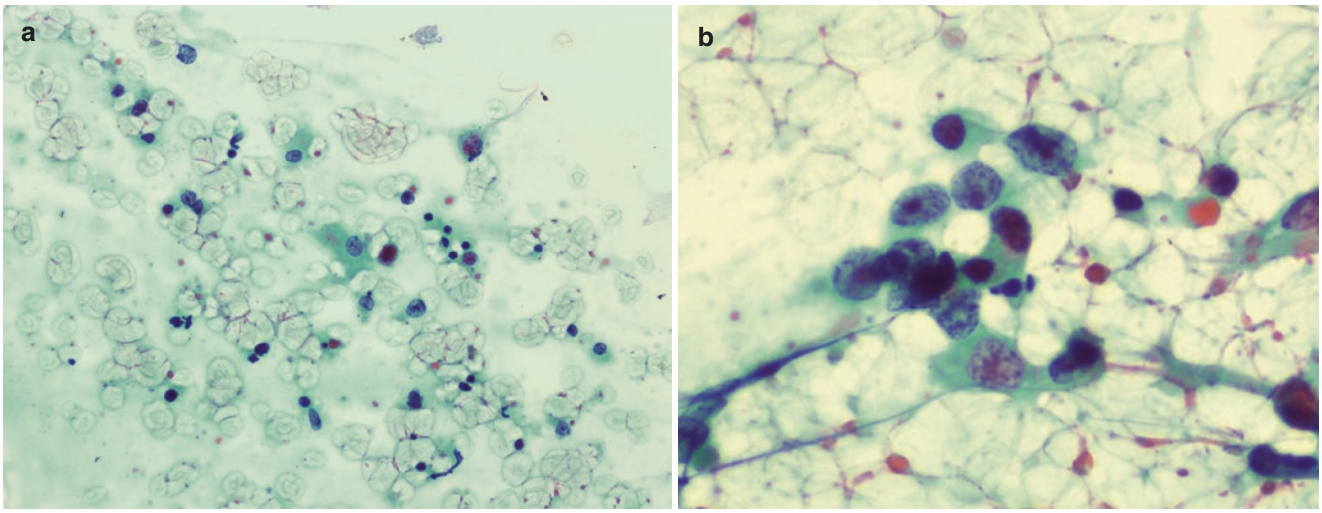

Fig. 16.4 (**a**, **b**) Undifferentiated nasopharyngeal carcinoma metastatic to bone marrow. (**a**) Mixture of mature lymphocytes and large neoplastic cells. (**b**) Detail of the neoplastic cells showing vesicular nuclei with prominent nucleoli

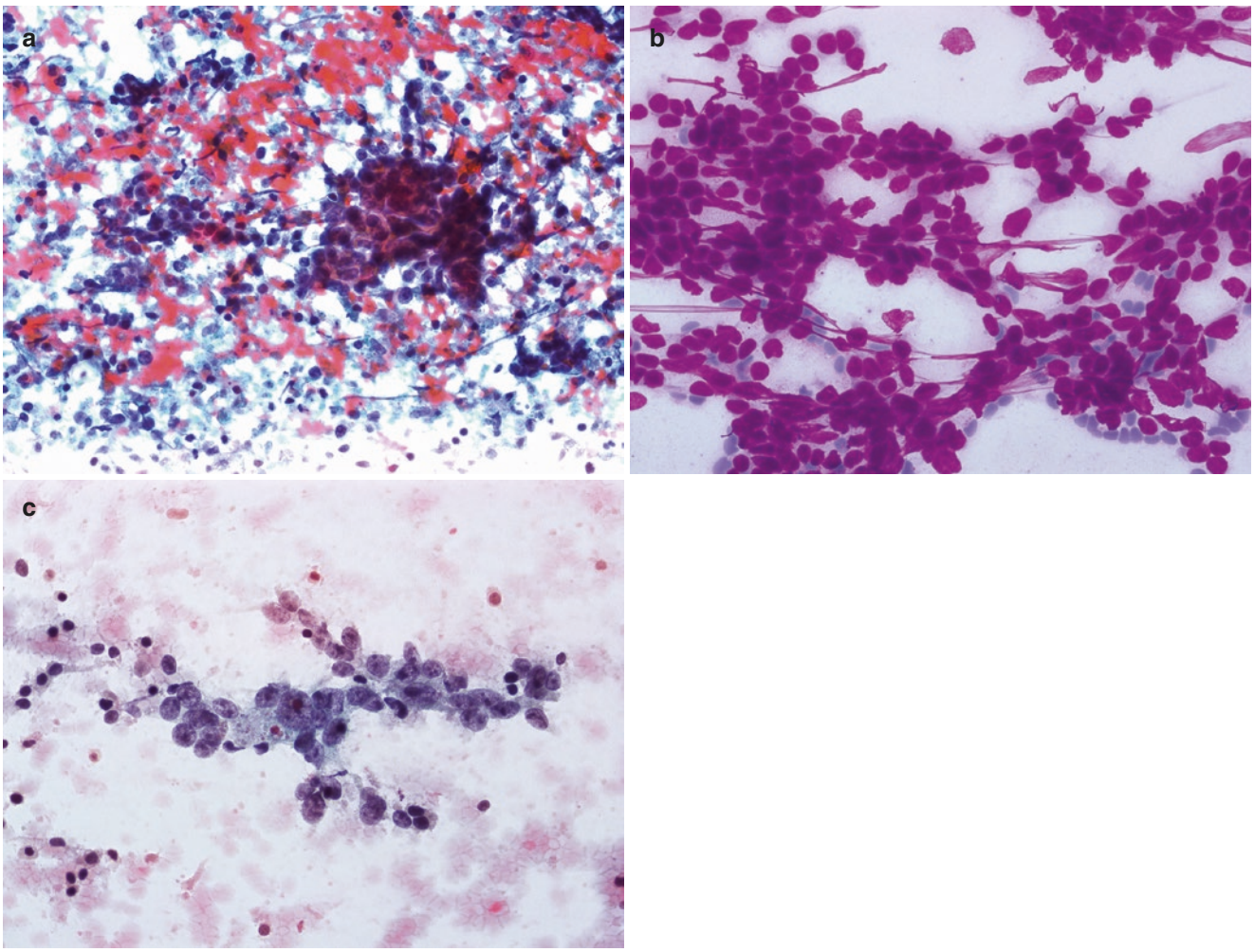

Fig. 16.5 (**a**–**c**) Sinonasal undifferentiated carcinoma. (**a**) Cluster of medium sized, highly undifferentiated cells on a necrotic background. (**b**, **c**) Details of tumor cells with high nuclear-cytoplasmic ratio, irregular chromatin, prominent nucleoli, and delicate cytoplasms

Other adenocarcinomas include the salivary-type high-grade adenocarcinomas which are quite rare in the sinonasal region with the exception of adenoid cystic carcinoma. This latter entity is usually cytologically diagnosed as adenocarcinoma not otherwise specified (NOS) due to the absence of convincing diagnostic features on cytology.

An important differential diagnosis, which might be ruled out, is a malignant melanoma which represents between 0.5 and 1.5 % of all melanomas and between 20 and 30 % of sinonasal malignant neoplasm [64, 65]. They are characterized by a polypoid lesion arising in the anterior part of the septum with the typical feature of skin melanoma, and a possible metastatic sinonasal site must be ruled out before a diagnosis of primary melanoma. In suspected cases, the application of immunocytochemistry for S100, Melan-A, and HMB45 confirm the diagnosis. The use of immunocytochemistry might help also in amelanocitic melanomas which are quite frequent in the sinonasal tract.

Olfactory neuroblastoma is defined as a malignant tumor composed of neuroblasts derived from the olfactory membrane mainly the olfactory mucosa lining the upper part of nasal cavity [66, 67]. Usually they are solitary polypoid lesions with microscopic features of small neuroblasts showing round to oval nuclei with stippled chromatin, absent or small nucleoli, and minimal cytoplasm (Fig. 16.6a, b). These lobular structures are typically separated by a neurofibrillary matrix formed by neuronal cell processes in which axons might be demonstrated by conventional silver stains. This background, present in almost 85 % of olfactory neuroblastoma, might help in a suggestion of the correct diagnosis. The classical Homer Wright rosettes are quite difficult to recognize on cytological samples. The application of immunocytochemistry shows diffuse positivity for NSE and synaptophysin with negativity of cytokeratins and EMA. Neurofilament protein and other markers of neural differentiation are often expressed.

Another entity is the extraosseous Ewing's sarcoma/PNET, an exceedingly rare sinonasal sarcoma composed of poorly differentiated small round cells that shows varying degrees of neuroectodermal differentiation and originates from a pluripotential neuroectodermal cell progenitor, for which more specific details are reported in the soft tissue cytological pathologies (Fig. 16.7a, b).

Malignant lymphomas of the sinonasal region account 6 % of all sinonasal malignancies [68]. In Western countries, about 50 % are of B-cell type, whereas the remaining 50 % present NK/T-cell lineage. The typical cytological samples include diffuse proliferation of large lymphoid cells or a diffuse mixed pattern of small and large cells. The immunocytochemical positivity for B markers (including CD20 and CD79a) and λ light-chain restriction are typically found. The NK/T-cell lymphoma is composed of cells which may be small, medium, or large sized or anaplastic with mixture of inflammatory cells. This lymphoma shows positivity for CD2, CD56, and cytoplasmic CD3 epsilon +. Sinonasal lymphoma might be differentiated from small round cell tumors and extramedullary plasmacytoma.

The evidence of mesenchymal malignant tumor includes fibrosarcoma, leiomyosarcoma, malignant hystiocytoma, and rhabdomyosarcoma which might be recognized as mesenchymal malignant neoplasms and not always characterized on cytology even when morphology and immunocytochemistry are combined [69, 70].

Regional lymph node involvement is seen in about 20 % of sinonasal squamous carcinomas so that the cytological aspiration of these lymph nodes might imply the consideration of this primary sinonasal site.

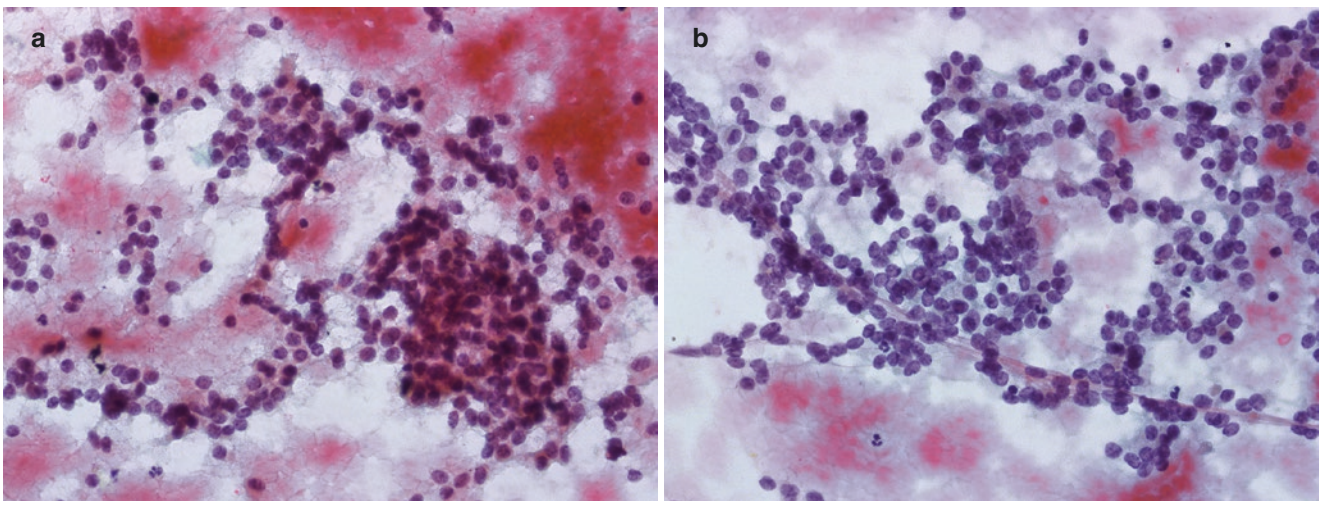

Fig. 16.6 (**a**, **b**) Olfactory neuroblastoma composed of uniform round cells, isolated or in aggregates (**a**), with conspicuous rosette formation (**b**)

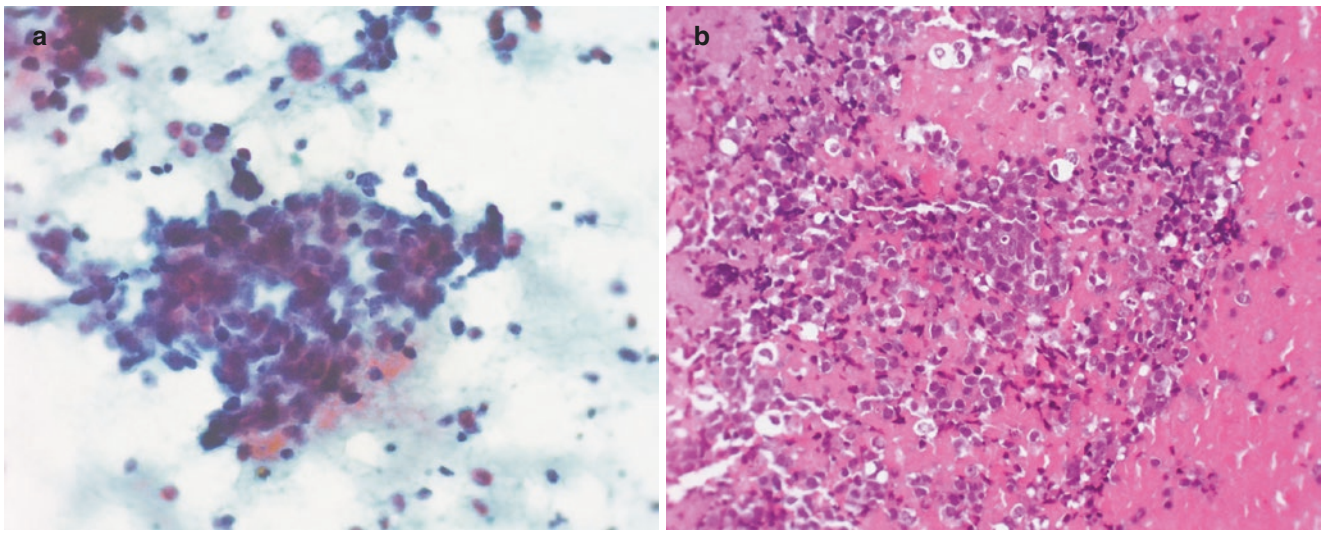

Fig. 16.7 (**a**, **b**) Ewing's sarcoma/PNET, small blue cells, isolated and in irregular clusters. On conventional cytology, a biphasic pattern with light and dark stained nuclei is characteristic of this tumor (**a**). Cell block (**b**)

16.5 Cytology of Cysts in the Neck and Jaws

The chapter of cystic lesions of the head and neck district includes several conditions and sites which are commonly linked by the evidence of a cystic wall with different lining surfaces. The intent of our evaluation is to overview the most frequently encountered in organs with different origins and cellular component. Apart from the benign cystic condition, we report malignant cystic lesions and also the evidence of metastatic cystic lesions.

16.5.1 Branchial Cleft Cysts, Sinus, and Fistulae

These cystic lesions are more frequent in children and frequently associated with a position in the anterolateral region of the neck. The cytological findings reveal squamous, columnar, and ciliated cells or also presence of mucinous cells. Usually these lesions show a clean background with an inflammatory pattern characterized by lymphocytes, macrophages, and plasmocytes which depends on the intensity of the flogistic event (Fig. 16.8a, b).

16.5.2 Thyroglossal Duct Cyst

Thyroglossal duct cyst (TDC) represents a rare congenital entity more frequent in childhood, whereas it is accounted in 7 % of adult population as well as no predisposing factors or clinical examination can lead to their preoperative diagnosis [71].

Its origin is supposed to be linked with the incomplete atrophy of the thyroglossal tract or with the retained epithelial cysts which can predispose to the resulting structural cystic organization [71].

As reported by literature, the detection of this benign congenital abnormality is quite frequent mainly in childhood, whereas the likelihood of cancer rate is assessed at 1 % of all TDC as numbered approximately in 215 malignant thyroglossal cases since the first description by Brentano in 1911 [72]. The aspirates yield a mucoid yellow fluid with inflammatory cells and single or clustered squamous and cylindrical cells, but they may contain thyroid tissue. Areas of colloid goiter and thyroiditis can be found in the wall surface of this tissue. Rarely do these lesions present a malignant papillary carcinoma. In these cases, physical examination and evaluation of the thyroid gland is essential to rule out a possible metastatic thyroglossal carcinoma [73]. To the best of our knowledge, the data provided about thyroglossal carcinomas are scant and mainly organized as single case report. Only recently Choi provided a meta-analytical evaluation of 163 patients distributed in 23 series with a number of patients ranging from 3 to 18 [74].

16.5.3 Cervical Thymic Cyst

This entity is often diagnosed by its well-demarcated margins on ultrasound guide, chest x-ray, or computed tomography in which size usually reduced after fine needle aspiration [75]. Fine needle aspiration of cervical thymic cyst shows a straw-colored fluid. The cytological features reveal foamy macro-

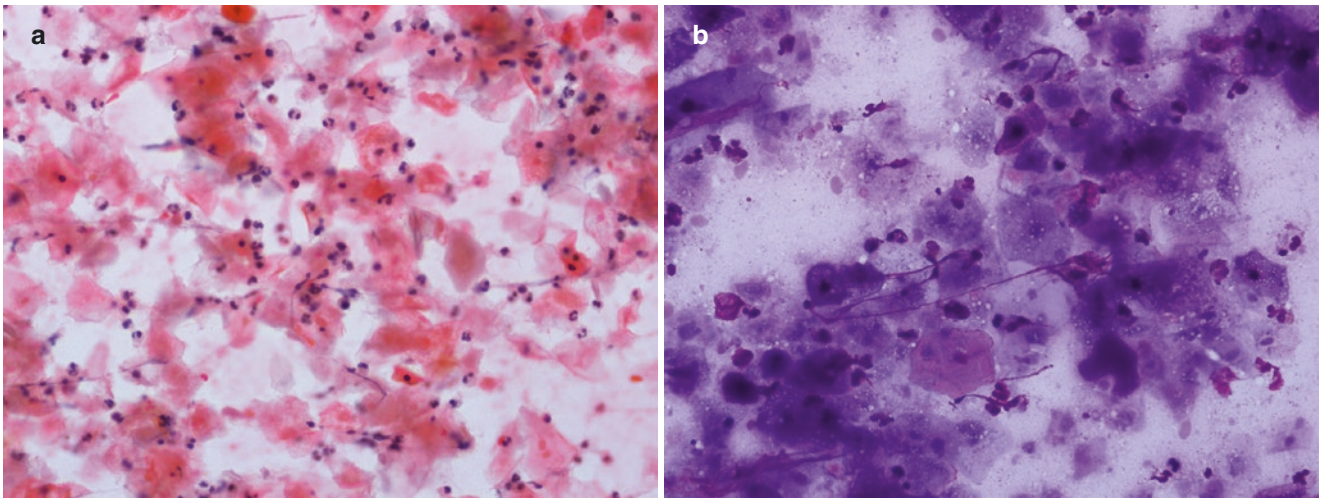

Fig. 16.8 (**a**, **b**) Branchial cleft cyst: Mixture of anucleated squamous and keratinized cells with some neutrophilic infiltrate

phages and benign lining cells. Thymic cysts are composed of lymphocytes, macrophages, and benign epithelial cells which can be squamous or ciliated, cuboid, and columnar with a proteinaceous debris. The main differential diagnosis is with cystic thymoma which is based on radiologic and histologic evaluation.

Some malignant lesions such as thymic carcinomas, seminomas, mature teratomas, Hodgkin lymphoma, and extranodal marginal zone B-cell lymphoma may present focal cystic features. In the presence of a cystic component, fine needle aspiration on both solid and cystic parts is essential for a correct diagnosis.

16.5.4 Cervical Parathyroid Cyst

Parathyroid cysts are a very rare entity as reported by just over 300 cases with an incidence ranging between 3 and 0.075 % as stated by McCoy or by Cappelli in their unselected series [76, 77].

Large cysts of the parathyroid gland may be detected as palpable lesions in the neck. Usually they are located on the posterior surface of thyroid gland but also as high as in the submandibular region. Cystic lesions show a thin wall with watery fluid without cells in many instances which might induce a suspect for parathyroid cyst (Fig. 16.9). Layfield stated that even a colored cystic fluid might be associated with a nonfunctioning parathyroid cyst [78]. The analysis of parathormone on the watery fluid is highly diagnostic of its nature. A critical point is the evidence that nonfunctioning parathyroid cysts encompass 90 % of all as opposite to the less frequent symptomatic functional cysts which are associated with primary hyperparathyroidism and elevated calcium level.

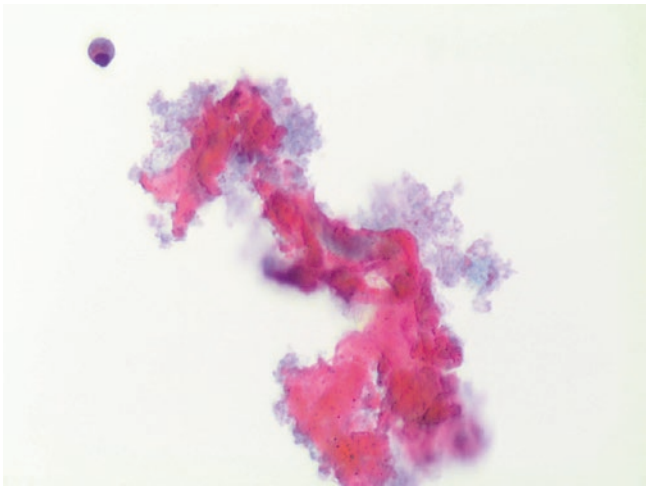

Fig. 16.9 Features of amorphous material from a parathyroid cystic lesion on liquid-based cytology

16.5.5 Cervical Bronchogenic Cyst

Bronchogenic cysts are a common cystic finding in the cervical and mediastinum regions. They belong to the group of foregut cysts including bronchogenic, esophageal, and gastroenteric cysts. They usually present as an anterior mass in young adulthood [79]. The combining evaluation of fine needle aspiration, radiologic finding, and location might be highly informative. The cytological specimen shows foamy macrophages, tufts of cilia, and some inflammatory cells. Benign ciliated columnar and some squamous cells may be present [79].

16.5.6 Dermoid and Teratoid Cysts

The term dermoid cyst refers to the cystic lesions in which skin and adnexal structures are identified [80]. Cytological aspiration is characterized by moderate-high cellular sample with squamous cells and keratinaceous cellular material. The presence of other differentiated types of cells including sebaceous cells, hair fat cells, and enteric and/or respiratory epithelium leads to the definition of teratoid cyst.

16.5.7 Cysts of the Jaws

These cysts derived from the dental-jaw apparatus. They are composed of fluid between the layers of epithelium or between epithelium and the crown of the tooth. They can be single or multiple cysts. Cytological smears are composed of nests of squamous cells with inflammatory components and fluid amorphous material. Rarely some goblet or ciliated cells are seen [81].

16.5.8 Cystic Hygroma and Lymphangioma

Lymphangioma is a rare cystic lesion, which is typically encountered in childhood. Very often these lesions composed of dilated lymphatic channels are associated with Turner syndrome. The most common sites are the neck, axilla, and groin, while the cavernous types are most common in the mouth, upper trunk, and limbs. Cytological specimen is characterized by red blood cells and lymphocytes with histiocytes [82, 83]. The presence of a proteinaceous material is often detected.

16.5.9 Teratomas

Cervical teratomas are relatively frequent in young population and usually with congenital origin [80]. They are usually characterized by large soft cystic lesions in the anterior neck region. Mature teratomas are composed of ectodermic mature components, whereas immature teratomas show immature neuroepithelium and neuroblastic features [84]. The cytological finding shows sebum, corneous, and keratin debris, squamous epithelial component, epithelial cells of sebaceous glands, hair fragments, and inflammatory cells in the background [84]. Some respiratory-type ciliated columnar cells or mature intestinal elements such as goblet cells with vacuolated cytoplasms and hyaline cartilage might be present in the smears. Giemsa and Diff-Quik stains may demonstrate the characteristic metachromatic staining of chondromyxoid component.

In cases with immature component, the cytological smear shows loose groups of small round cells with fibrillary matrix which are paradigmatic of neuroectodermal component. The use of immunocytochemistry seems to be limited to the evaluation of the immature neuroectodermal component. The most important differential diagnoses include a bronchogenic cyst and in cases with abundant squamous cell component the possibility of a squamous carcinoma.

16.5.10 Cervical Salivary Gland Cystic Neoplasms

The evidence of a cystic component is reported in both benign and malignant salivary gland lesions. This component is a further difficult point in the evaluation of salivary gland mainly because the fluid component does not lead to a conclusive diagnosis. In the field of cystic lesion, the most common is a ductal retention cystic lesion resulting from an obstruction (sialolithiasis) with a watery fluid. These cysts might seem as solid and firm mass inducing a clinical diagnosis of solid neoplasm. The cytological findings show clear watery or viscous fluid which might become yellowish in presence of flogistic components. The cellular component is characterized by a few histiocytes and degenerative epithelial cells which are cuboid and squamous. In several cases, FNAC is diagnostic and therapeutic, whereas if a residual mass persists, the aspiration should be repeated because some benign and malignant salivary gland tumors may have both solid and cystic components.

Small superficial cysts are frequent in minor salivary glands of the lips and the oral mucosa with a clear mucous component.

16.5.11 Cystic Lesions of the Thyroid

Cystic lesions of the thyroid gland account for 15–25 % of all thyroid lesions, and several of them are a consequence of ischemic disorders in a nodular goiter or follicular neoplasm (including both benign and malignant conditions) [85]. Although up to 50 % of surgically resected thyroid cysts are neoplastic, a malignant diagnosis is signed out in 1–15 % of cases [86, 87]. Cystic lesions are the most common responsible for nondiagnostic/nonrepresentative cases [88]. A thyroid cyst is seldom a true pseudocystic lesion (without a wall of follicular cells): more frequently it represents a cystic or hemorrhagic regression of a nodule which might result benign or malignant after repeated samplings. Conventional cytology shows hemorrhagic smears with

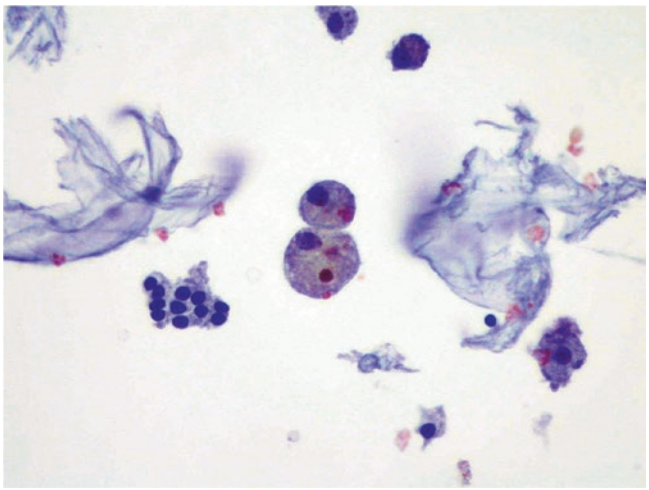

Fig. 16.10 Features of a thyroid cystic lesion with scant regular follicular cells and some macrophages on liquid-based cytology

scattered well-preserved follicular cells or few cells with degenerative features (Fig. 16.10). Some elongated cells with large nuclei and distinctive nucleoli may correspond to either reparative cells or stromal component. Some authors reported evidence of epithelioid cells in the lining cystic wall [89].

On LBC preparations we observed many hemosiderin-laden histiocytes with very few clusters of thyrocytes and colloid droplets [90, 91]. A conclusive diagnosis is based on the morphologic features of the thyrocytes, if their amount meets the adequacy criteria, and on the number of colloid droplets. Otherwise, the final diagnosis is nonrepresentative. In our studies the cystic/hemorrhagic lesions accounted for about 7 % of all cases.

When a FNAC of a cystic nodule with a solid component is repeatedly nonrepresentative, the surgical excision of the nodule may be considered to avoid the possibility of a malignant lesion (between 8 and 19 % of cases according to literature) [88, 92].

16.5.12 Metastatic Cystic Lesions on Head and Neck

A specific group of lesions is characterized by the cystic metastatic lesions of head and neck district. Cystic metastases of head and neck districts may mimic primary malignancies although some of them might be the result of cystic degeneration of metastatic lymph nodes. Cytological findings show a dense fluid which might be diagnosed as negative. The evidence of few neoplastic cells is not a common finding so that some authors suggest considering this lesion as malignant. Some papers report that in 90 % of cases, squamous carcinomas (mainly primary carcinoma from Waldeyer's ring of the tonsilla) are likely to show these cystic metastases. The remaining 10 % is composed of adenocarcinoma, melanoma, and other rarer histological variants [93]. In these cases, patients should be checked for a primary carcinoma which is often located in the tonsillae or oropharyngeal site [93–95].

This is extremely important because the patient will be treated with a site-specific therapy. All patients with an unknown cystic carcinoma require a thorough head and neck history and clinical-instrument evaluation. Recently the use of both panendoscopy with tonsillar biopsies and positron emission tomography has been advocated as a useful instrument [94, 95].

16.6 Cytology of Odontogenic Cysts and Tumors

Cystic lesions and tumors of odontogenic origin are uncommon finding in routine cytology since the sampling of these tumors often requires the use of devices able to penetrate the bone. However, since 2000 the group of August et al. [96] have demonstrated the possibility to obtain cytologic smears from intraosseous odontogenic tumors achieving interesting results in terms of diagnostic accuracy, even with the use of immunocytochemical stainings. The goal of needle aspiration of odontogenic cysts is to distinguish tumors, which may cause destruction of the osseous tissue of the maxillary bones and may recur after the excision, from benign cysts, which usually pursue a less destructive course and present a slow growth rate. The correct diagnosis is almost always achieved through a multidisciplinary consultation between the cytopathologist and the radiologist. The cytological sample of benign cysts, which include dentigerous, radicular, and inflammatory cysts, is characterized by the presence of isolated large typical squamous cells with small monomorphic nuclei mixed with keratinic amorphous material and histiocytes; some bundles of fibrocalcific tissue may at times be seen in the background. Odontogenic benign tumors include calcifying epithelial odontogenic tumor (CEOT or Pindborg's tumor), odontogenic keratocyst (OKC), calcifying cystic odontogenic tumor (CCOT), and ameloblastoma (for a more detailed description, see Chap. 4). These tumors exhibit unilocular or multilocular cystic areas, and only infrequently their radiologic picture features a completely solid texture. The cytologic picture of CEOT shows pleomorphic epithelial squamoid cells admixed with amyloid-like material and concentric calcifications which, interpreted by an unexperienced cytopathologist, may be misdiagnosed as metastases from a squamous cell carcinoma [97, 98] CCOT shows a similar picture with the additional presence of cylindrical cells but without ghost elements which are the hallmark of

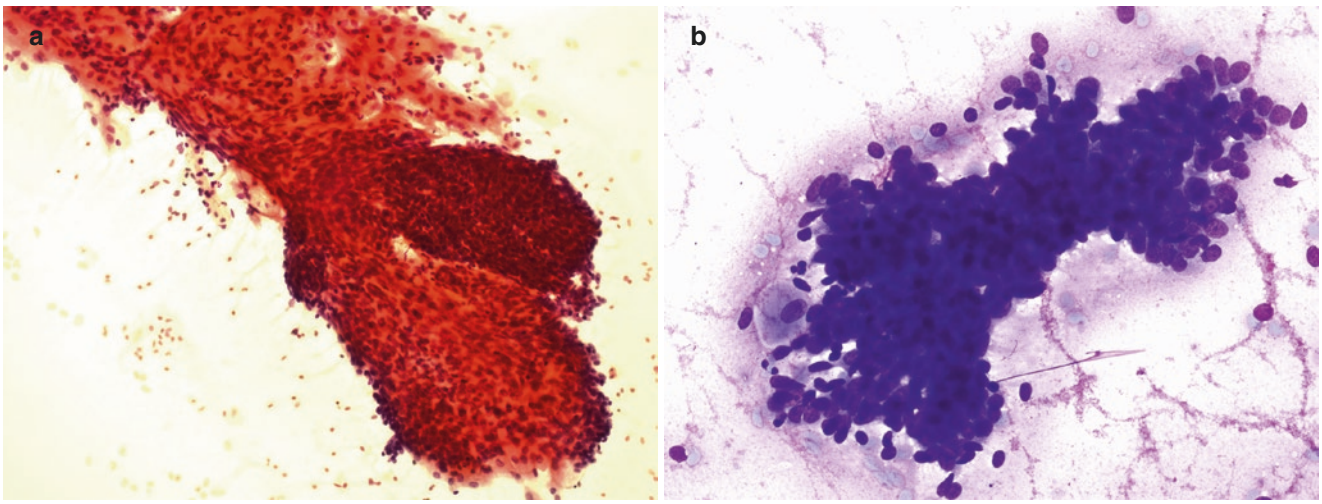

Fig. 16.11 (**a**, **b**) Ameloblastoma. (**a**) Cluster of small basaloid cells, mature squamous component, and spindle cell stroma. (**b**) Detail of the basaloid component with some peripheral palisading

the ghost cell odontogenic carcinoma (GCOC) which often pursues a malignant course [99]. Ameloblastoma (AMB) of the jaw represents the most common odontogenic tumor. It exhibits a destructive expansion into the osseous structure of the jaw and a distinctive tendency to recur, even if metastatic spread is exceedingly rare [96]. Its radiologic picture shows different patterns of cystic (uni- or multilocular) and solid patterns which correspond, at cytology, to clusters of non-keratinizing epithelial cells with slightly pleomorphic nuclei and scant cytoplasms, sometimes exhibiting squamous metaplasia, admixed with fragments of fibrous tissue (Fig. 16.11a, b) [100, 101]. Malignant AMB shows a marked predominance of the epithelial component which presents atypical features and occasional mitotic figures [102]. The cytology of OKC is characterized by squamous cells at different stage of keratinization without atypical features. Parakeratotic OKC show more keratinic material in the background and a higher amount of isolated squamoid cells than non-parakeratotic tumors, and this distinction is important since parakeratotic KCOT have a higher tendency to recur.

16.7 Cytology of Salivary Gland

Several conditions including cystic lesion, inflammatory conditions, degenerative process, and benign or malignant neoplasm may be encountered in the salivary glands (major and minor glands) [103, 104]. The first experience with salivary glands cytology started in 1933 when Stewart reported that salivary gland is particularly suitable with aspiration [105]. Probably this is the tissue mostly characterized by various and heterogenic pathologies [106]. The attempts of obtaining material for an adequate diagnosis through incisional or cutting-needle biopsies have not been accepted because of many complications. For these reasons, FNAC is suitable and applicable not only to parotid and submandibular gland but also to the sublingual and minor salivary gland. This cytological approach spared one-third of patients to surgery, and its clinical impact has been demonstrated in several papers [107–109]. The cytological interpretation of an aspirate from salivary gland must be evaluated in two phases. Firstly to define the salivary gland origin is confirmed and secondly to diagnose the possible pathological lesion. The most frequent problem is the distinction between primary salivary gland neoplasms and other entities which can mimic them. For instance, a critical point is the distinction between enlarged lymph node and sialomegaly due to the presence of lymph node enclosed into the salivary gland which is difficult on cytological smears [110]. Similarly a metastatic malignancy in the intraparotid lymph node can mimic a primary salivary gland neoplasia. But other entities can mimic sialomegaly such as branchial cyst, carotid body tumor, parathyroid lesions, pilomatrixoma, or other skin and soft tissue lesions [110, 111].

FNAC of salivary glands is a difficult field for cytopathologists but with experience and good clinical correlation, sensitivity and specificity are high ranging from 94 to 100 % [107, 111, 112]. When we consider the type-specific diagnostic accuracy, there is an 80 % correlation among the benign than the malignant neoplasms. For instance, the diagnosis of pleomorphic adenoma is quite simple, while mucoepidermoid carcinoma and carcinoma ex pleomorphic adenocarcinoma are rarer and can lead to misdiagnoses [113]. The diagnostic accuracy of FNAC is comparable with the results obtained with frozen sections as assessed by Auclair in his revision of the frozen section from salivary

gland neoplasms [104]. As discussed in some papers, the causes of false-negative and false-positive errors and diagnostic problems are related to subrepresentative sampling and difficulties in appreciating some specific morphological details especially for basal cell adenoma or some difficult malignancies [113]. As assessed by the paper of Eneroth, the major difficulties were in the diagnoses of adenoid cystic and acinic cell carcinomas which benefited from the work of Zajicek with a high increase in sensitivity up to 98 % [114]. The accuracy of benign diagnoses varies between 96 and 99 %.

16.7.1 Nonneoplastic Lesions

Several nonneoplastic conditions of known and unknown etiology may affect the salivary tissue of both major and minor glands. Some of them are uncommon, but it is essential to recognize in order to avoid misdiagnoses as some of them are included in the heading of tumorlike lesion in the WHO classification. In this contest, there are developmental disorders including some ectopic site which might show benign and malignant nature based on the evidence of normal or benign acinar cells or even malignant neoplasms [115].

Other developmental disorders are adenomatoid hyperplasia which is frequently misdiagnosed as a neoplasm and polycystic (dysgenetic) disease due to malformation of the ductal system which is misdiagnosed as cystic lesion [115].

In this section, we do not consider the cystic salivary conditions because they have been previously discussed in the cystic lesion section.

Sialoadenosis is a benign non-inflammatory condition of one or both parotid glands. The major histological feature is due to hypertrophy of the acinar cell. The origin is unknown although diabetes, cirrhosis, and nutritional and hormonal disorders might be involved. The cytological smear is composed of abundant normal or slightly enlarged acinar cells [116] (Fig. 16.12). The main confusing diagnosis is with a well-differentiated acinic cell carcinoma.

Sialolithiasis is a consequence of a mineralization of debris with the production of calculi which might induce acute or chronic infections (sialoadenitis). The most common site is the submandibular gland. The cytological smear of obstructive chronic sialoadenitis is usually scanty and composed of ductal cells with squamous metaplasia, paucity of atrophic acinar cells, inflammatory cells, and mucous in the background. When in presence of a longstanding obstruction, cytological smears show atrophy and fibrosis. In a few cases cystic changes might be found [117]. Another possible cause of chronic sialoadenitis is radiotherapy of the oral cavity. In this latter case, the cytological smear reveals sparse ductal cells in clusters or sheets and scanty acinic groups with atrophy and occasional fibroblasts, lymphocytes, neutrophils, and macrophages.

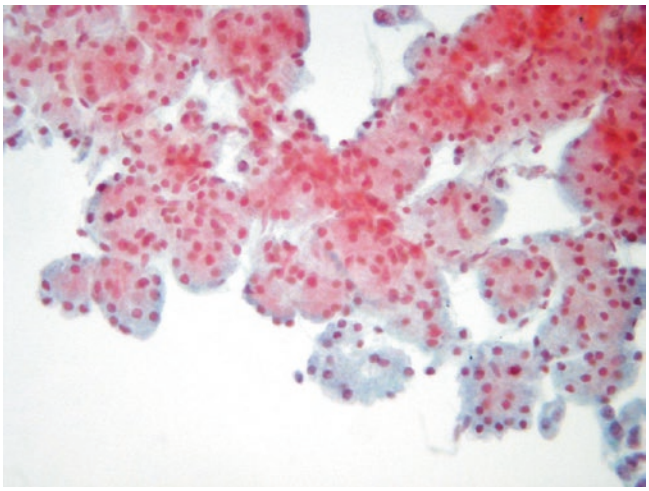

Fig. 16.12 Normal acinic cells on FNA of sialadenosis

The presence of an acute inflammatory disease is more often detected in young individuals because viral infections have a predilection for parotid gland. The diagnosis is mainly based on a clinical evaluation with rarely cytological confirmation. Acute bacterial sialoadenitis occurs with equal frequency in both the parotid and submandibular salivary glands. Cytological smears are characterized by a background with fibrin, cellular debris, neutrophils, lymphocytes, and histiocytes.

Granulomatous sialoadenitis might be the result of several causes. Sarcoidosis might involve salivary glands in 6 % of cases. The aspirates show multinucleated giant cells and cluster of epithelioid cells in a background of lymphocytes [118, 119]. Tuberculosis, fungal infections, brucellosis, cat scratch disease, and toxoplasmosis might affect the salivary glands, but FNAC is not the first tool of evaluation, and the clinical history is likely to lead to an infectious disease.

Another nonneoplastic condition is the cystic lymphoid hyperplasia of the salivary glands which is one of the manifestations of HIV infection, and it is cytologically defined by a proliferation of the epithelium, cyst formation, and lymphocytosis with changes similar to that of Sjogren's syndrome [120].

The presence of small lymph nodes inside the parotid gland is well known. Their enlargement presence can be difficult to differentiate from a salivary neoplasm. In this type of lesions, FNAC might be useful in identifying the lymphoid nature of the lesions and also differentiate reactive lymph node hyperplasia, nonspecific lymphadenitis, granulomatous lymphadenitis, and lymphoma. A further help is

the application of ancillary techniques in order to achieve a conclusive diagnosis.

Another disease which might involve salivary glands is the benign lymphoepithelial lesion which is an autoimmune disease known as Sjogren's syndrome. The cytological findings include lymphoid cells composed of small lymphocytes admixed with follicle center cells, plasma cells, histiocytes, and epithelial cells including clusters of ductal and myoepithelial cells. The differential diagnosis with a malignant lymphoma (low or high grade) is required and based on the monoclonal lymphoid cell population. Other conditions with a florid lymphocytosis are chronic sialadenitis, Warthin's tumor, mucoepidermoid carcinoma, and acinic cell carcinoma.

In the field of nonneoplastic lesion, lipomatosis is defined by the presence of fibrofatty connective tissue in the salivary gland. This condition is evident in older patients with glandular atrophy and may induce a fatty replacement, or it is associated with obesity and diabetes. We need to remember that pleomorphic adenoma might contain adipocytic differentiation so that the presence of fat cells needs to be evaluated with an attention to the adequacy of the smear and presence of other components [121].

A rare condition is necrotizing sialometaplasia causing ulceration of the palate mainly based on an ischemic origin. It may be due to irradiation, and it is characterized by necrosis of acinar cells, bizarre squamous metaplasia, and pseudoepitheliomatous hyperplasia. These features might be suspected and misdiagnosed for a mucoepidermoid or squamous cell carcinoma [122].

16.7.2 Benign Neoplasms

Several studies report that the incidence of salivary gland neoplasm is of 2 per 100.00 persons annually with a percentage of malignant tumors varying from 22 to 37 %. Both benign and malignant tumors are more frequent in the parotid gland than in the other minor salivary glands. Due to the heterogenic and diverse nature of the salivary glands, several classifications have evolved and changed in these last years [123].

Pleomorphic adenomas represent about 75 % of all neoplastic lesions. The typical feature of this tumor is a well-capsulated, circumscribed, solitary, painless, and slow-growing nodule. The majority of them are in the superficial portion of the parotid gland. The variability of the histological pattern, which is composed of epithelial and myoepithelial cells intermingled in an abundant fibrillary mucomyxomatous stroma, is reflected on FNAC [123]. The cytological diagnosis of the great majority of pleomorphic adenomas is quite straightforward. Cytological aspirates from a pleomorphic adenoma show thick and gelatinous consistency. While in MGG-stained smears the mesenchymal-fibrillar and chondromyxoid substance appears as red to dark purple, in Papanicolaou smears, it is gray to pale pink. Spindled and plasmacytoid myoepithelial cells with elongated or round nuclei and homogenous cytoplasm are abundant. The epithelial component is organized in tubules, ducts, or sheet of cells with uniform chromasia which are dispersed or aggregates in the described mesenchymal background (Fig. 16.13a, b). Some large myoepithelial cells which might create problems with a malignant differential diagnosis have been reported. Some squamous cells with atypia and mucous-producing cells might lead to a suspicion of malignancy [124]. The differential diagnosis with adenoid cystic carcinoma is difficult and may be overcome with an extensive sampling of different areas of the lesion and immunophenotyping panels.

Malignant transformation of a pleomorphic adenoma might be encountered. It is characterized by an aggressive, undifferentiated carcinoma which does not cause diagnostic difficulties. The cytological problems correspond to two reasons: (1) the predominance of one element hiding the other component or (2) the presence of atypical cytomorphological features [106, 111, 112, 125]. If the mucomyxomatous component is very abundant overwhelming the few epithelial cells, the lesion may be misdiagnosed as a retention cyst especially with MGG. More serious is a false-positive diagnosis of malignancy mainly based on epithelial cells which show loss of cohesion, nuclear enlargement, and hyperchromasia.

Myoepithelioma (myoepithelial adenoma) is a benign neoplasm composed exclusively of myoepithelial cells which is rare in its pure form. It may affect both major and minor salivary glands [123–126]. Cytological specimens are characterized by monomorphic myoepithelial cells of either plasmacytoid or spindle cell type (Fig. 16.14). Some authors reported evidence of sparse collagenous stroma and/or mucoid ground substance, binucleated cells, and cells with pseudonucleoli. The most important differential diagnosis is with pleomorphic adenoma which is based on the absence of chondromyxoid stroma which favors myoepithelioma. We need to remember that treatment is exactly identical.

Adenolymphoma (Warthin's tumor) usually occurs in the lower portion of the parotid glands and accounts for 5 % of all parotid tumors [123]. It is more common in males and in the middle age or elderly patients. It is characterized by a soft, painless, swelling, fluctuant mass, with poorly defined borders and which is bilateral or monolateral. Cytological samples show mucoid and watery material composed of granular amorphous substance with sheet of cells presenting oncocytic

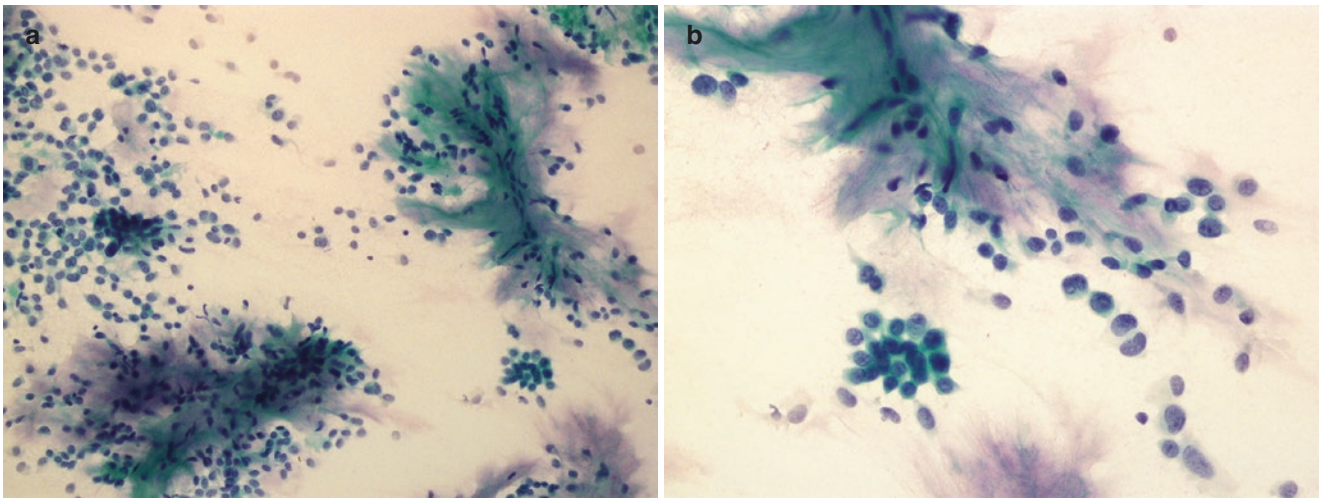

Fig. 16.13 (**a**, **b**) Pleomorphic adenoma composed of epithelial and myoepithelial cells intermingled in an abundant fibrillary mucomyxomatous stroma on conventional cytology

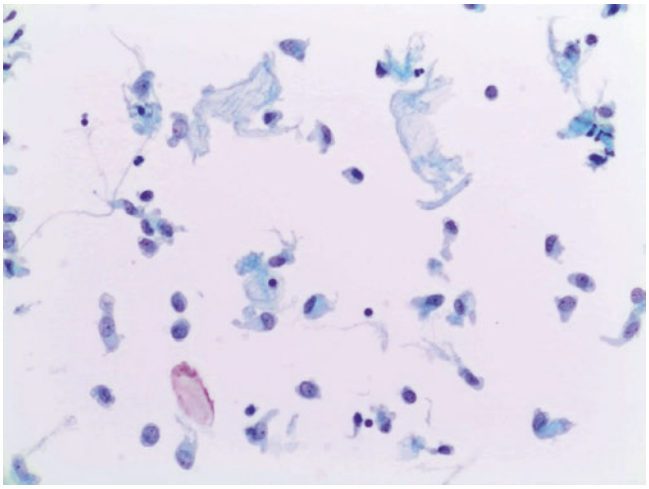

Fig. 16.14 Myoepithelioma of salivary gland characterized by monomorphic myoepithelial cells of either plasmacytoid or spindle cell type. Note plasmacytoid cells

features and macrophages. The lymphoid component is always present but with varying percentage [127, 128]. The typical features of this lesion are the presence of sheet of finely granular oncocytic cells which might be organized in a honeycomb pattern. Evidence of papillary fragment with lymphoid component and oncocytic cells might be present (Fig. 16.15a). The oncocytic cells show the paradigmatic features with round nuclei, small nucleoli, and granular cytoplasm (Fig. 16.15b). Mast cell might be a frequent finding in MGG whereas they are difficultly recognized in Papanicolaou stains. Although the presence of fluid and mucoid material might induce in the most obvious diagnosis of a nondiagnostic smear, the cytopathologist should remember the possibility of an adenolymphoma [128].

The evidence of abundant mucoid material with small clusters of epithelial cells with minimal atypia in a lymphoid background may lead to exclude a differential diagnosis with a well-differential mucoepidermoid carcinoma. Even if in the latter diagnosis, the cells are not organized in monolayers and they produce mucous. Another differential diagnosis is with a squamous cell carcinoma based on the metaplastic epidermoid cells with sometimes true keratinization [128]. This latter condition should be diagnosed only after an exclusion of a primary squamous carcinoma in the districts of oral and upper respiratory tract.

The last condition which might create confusion in an aspirate with a predominance of oncocytic cells and only few lymphocytes is the diagnosis of oncocytoma even though they are treated with the same management [128].

Oncocytoma represents a rare benign neoplasm composed of oncocytic cell [123]. The differential diagnosis with Warthin's tumor is based on the unicentricity and absence of lymphoid component. Cytological smears are characterized by clusters of uniform large oncocytic cells with abundant granular cytoplasm, round central nuclei, distinct nucleoli, and absence of debris, fluid, and lymphoid cells (Fig. 16.16a, b). It is well known that the oncocytic cells show pleomorphic feature with different degrees of atypia. It may be misdiagnosed as nodular hyperplasia, Warthin's tumor, and acinic cell carcinoma. The distinction with other benign conditions presenting oncocytic component is not so important because they are treated with the same surgical excisional approach. Nevertheless, the distinction with malignant oncocytic condition is essential for the different types of treatment. These differential diagnoses are based on the evaluation of mitotic figures and different cytoplasms with granulation and necrosis which are typically encoun-

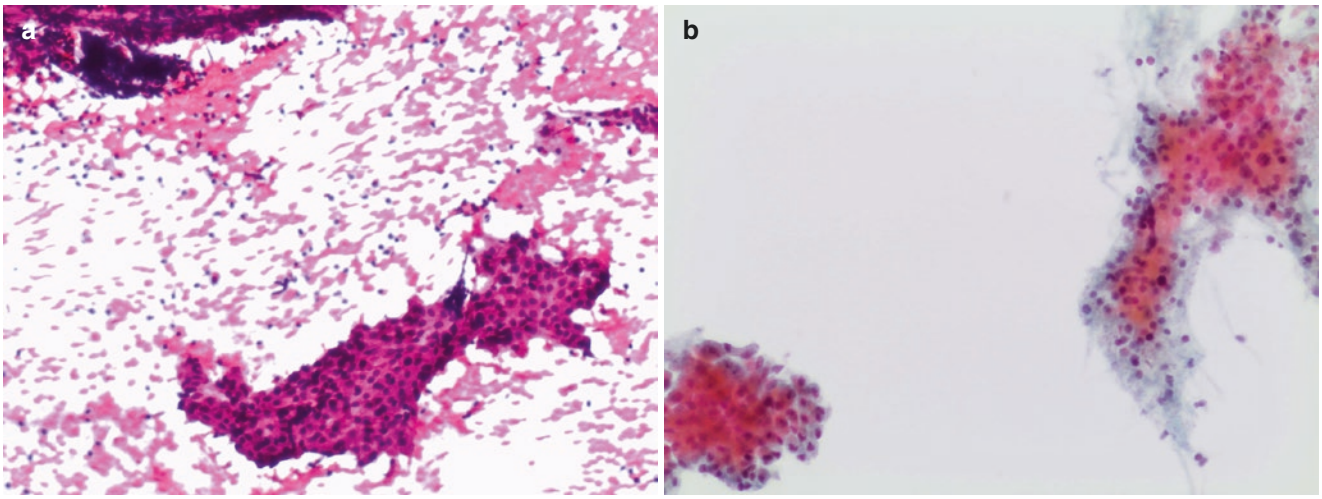

Fig. 16.15 (**a**, **b**) Warthin's tumor on conventional cytology composed of sheets of cells presenting oncocytic features and few macrophages and lymphocytes in the background (**a**). Features of Warthin's tumor on LBC smears. Note oncocytic cells and lymphocytes (**b**)

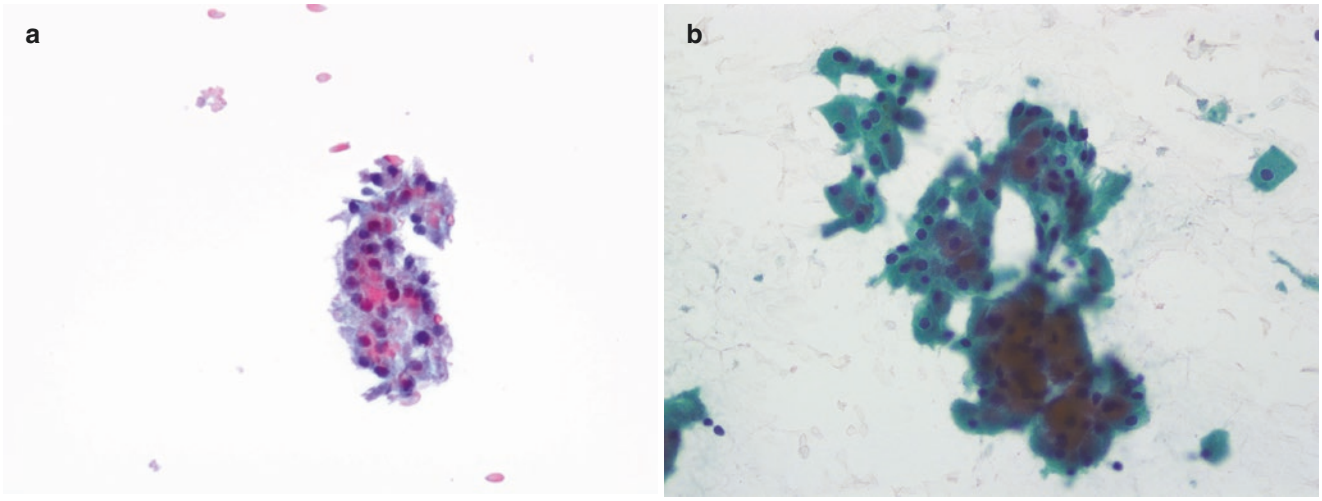

Fig. 16.16 (**a**, **b**) Oncocytoma cells with abundant granular cytoplasm, round central nuclei, and absence of debris and lymphoid cells (**a** LBC, **b** conventional cytology)

tered in a malignant lesion. A metastatic renal, thyroid, and apocrine breast carcinoma should never be forgotten [123]. Taken together all these drawbacks, sometimes the best diagnosis is to sign out the cases as an "oncocytic neoplasm" relaying the differential diagnoses with the caveat that malignancy cannot be excluded.

Basal cell adenomas represent 2 % of all primary tumors of the major salivary glands and more often encountered in elderly patients [129]. This entity is defined by a well-defined basaloid pattern with trabecular, tubular, papillary, and solid structures. Cytological smears are cellular with cohesive solid groups with branching cords (Fig. 16.17). The single cells are small, uniform, with scanty cytoplasm, oval nuclei with finely granular chromatin. Single cells appear as naked nuclei with small amounts of fibrous and amorphous material at the edges of cell clusters. The typical matrix might be recognized as bright red with MGG and translucent in Papanicolaou stains. This hyaline stroma is not uncommonly seen in FNAC as metachromatic globules or droplets surrounded by epithelial cell, which might resemble the adenoid cystic carcinoma. This type of pseudo-adenoid cystic appearance, which is difficult to properly diagnose, may also occur in pleomorphic adenoma, basal cell adenocarcinoma, and epithelial-myoepithelial carcinoma.

Sebaceous cell adenoma is a very rare solid or cystic neoplasm made up of flat polygonal cells similar to the sebaceous cells. The cytological smear shows tumor cells in

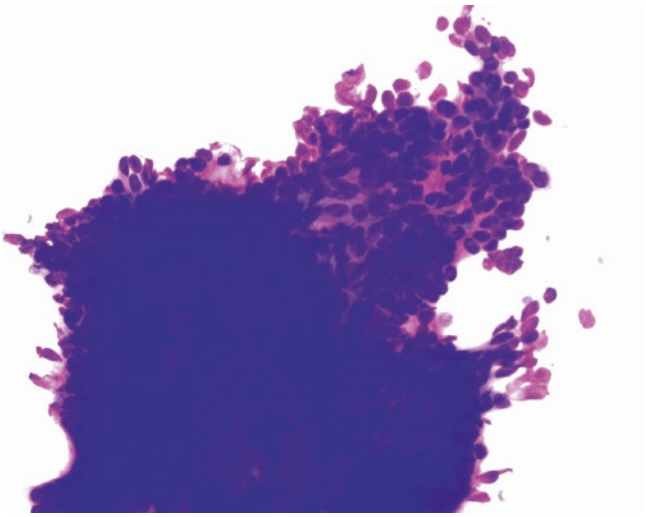

Fig. 16.17 Basal cell adenoma characterized by cluster of basaloid cells with densely packed nuclei

dense clusters with few dispersed cells and monomorphic cells with round nuclei and vacuolated cytoplasm [130].

Clear cell adenoma is a very rare entity which is composed of prominent clear cells that form tubular structures [114].

16.7.3 Malignant Neoplasms

Acinic cell carcinoma is a rare malignant entity of both major and minor salivary glands and accounts for 1 % of all malignant tumors [123]. It is more frequently found in the parotid gland as a solitary, asymptomatic solid neoplasm with a slow growth. Cytological smears have a bland monomorphic finding composed of regular acinic cells loosely cohesive with fragile cytoplasm and small dark nuclei. The cytoplasm is finely vacuolated or dense, and the nuclei are small round, uniform oval with small central nucleoli (Fig. 16.18a–c). Numerous naked nuclei are present. Some cases may present a pseudopapillary appearance with laminated calcifications resembling psammoma bodies and also rich admixture of lymphoid cells [131, 132]. In poorly differentiated acinic cell carcinoma, the cells are large with dark pleomorphic nuclei and prominent nucleoli. The evidence of acinic structure is often described in well-differentiated carcinomas. They might resemble oncocytic cells so that the main differential diagnoses are oncocytoma, nodular hyperplasia, and Warthin's tumor which might represent difficult diagnostic challenges on cytology. A wise attitude with this type of cytological report is to be rather descriptive and only underline the presence of acinic cells.

Adenoid cystic carcinoma represents 3–5 % of all salivary tumors including both major and minor salivary glands [123]. It is the most important malignant lesion in minor salivary glands characterized by a slow growth with early neural invasion. This malignancy often recurs after surgical excision due to the infiltrative pattern of growth. The first cytological description was provided by Eneroth and Zajicek and then studied by Klajienieko and Vielh and Nagel [133–135]. Cytological smears show different patterns such as abundant clusters of cohesive small uniform epithelial cells organized around hyaline globules, tubular structures with uniform cells, and solid fragments of tumor cells. The evidence of hyaline mucoid globules and tubular structures represents the paradigmatic features for a diagnosis of adenoid cystic carcinoma (Fig. 16.19a–c). The key features for a cytological diagnosis of adenoid cystic carcinoma are spherical aggregates, rosette-like group, papillary structure or solid fragments with dispersed naked nuclei, and presence of magenta-stained hyaline mucoid globules in MGG with small uniform cohesive cancer cells with distinct nucleoli and minimal cytoplasm.

In the absence or in the presence of only few paradigmatic features, the cytological diagnosis may be difficult, and the major differential diagnoses are with pleomorphic adenoma and basal cell adenoma, low-grade adenocarcinoma, and epithelial-myoepithelial carcinoma [134, 135]. Some of these differential diagnoses and mainly pleomorphic adenomas might show some similar globules, and this is important because pleomorphic adenoma is one of the most frequent lesions of the salivary glands. By the way in presence of nerve damage and a clear smear with features of adenoid cystic carcinoma, a conclusive diagnosis can be signed out. In absence of clear symptoms, few hyaline globule and not sure features of an adenoid cystic carcinoma, the cytological diagnosis must not be rendered considering the effect of this diagnosis which might lead to a radical surgery with facial nerve excision.

Mucoepidermoid carcinoma represents the most frequent malignancy of the salivary glands accounting for 20 % of all carcinoma originating in the salivary gland [123]. This tumor derives from ductal cells with variable history either as a slow-growing, painless, non-symptomatic low-grade carcinoma or as a rapidly growing, symptomatic, high-grade malignancy. Frequently the low-grade form is cystic, whereas the high-grade is predominantly solid. The first cytologic evaluation was provided and defined by Zajicek with further studies by Cohen and Klijanienko and Vielh who have revised 50 cases and highlighted an excellent description of the cellular details [136, 137].

The malignancy is characterized by a biphasic pattern with glandular and squamous structures characterized by three types of cells: mucous-producing cells, squamous cells, and intermediate cells. The low-grade cytological smears are composed of extracellular mucoid material from

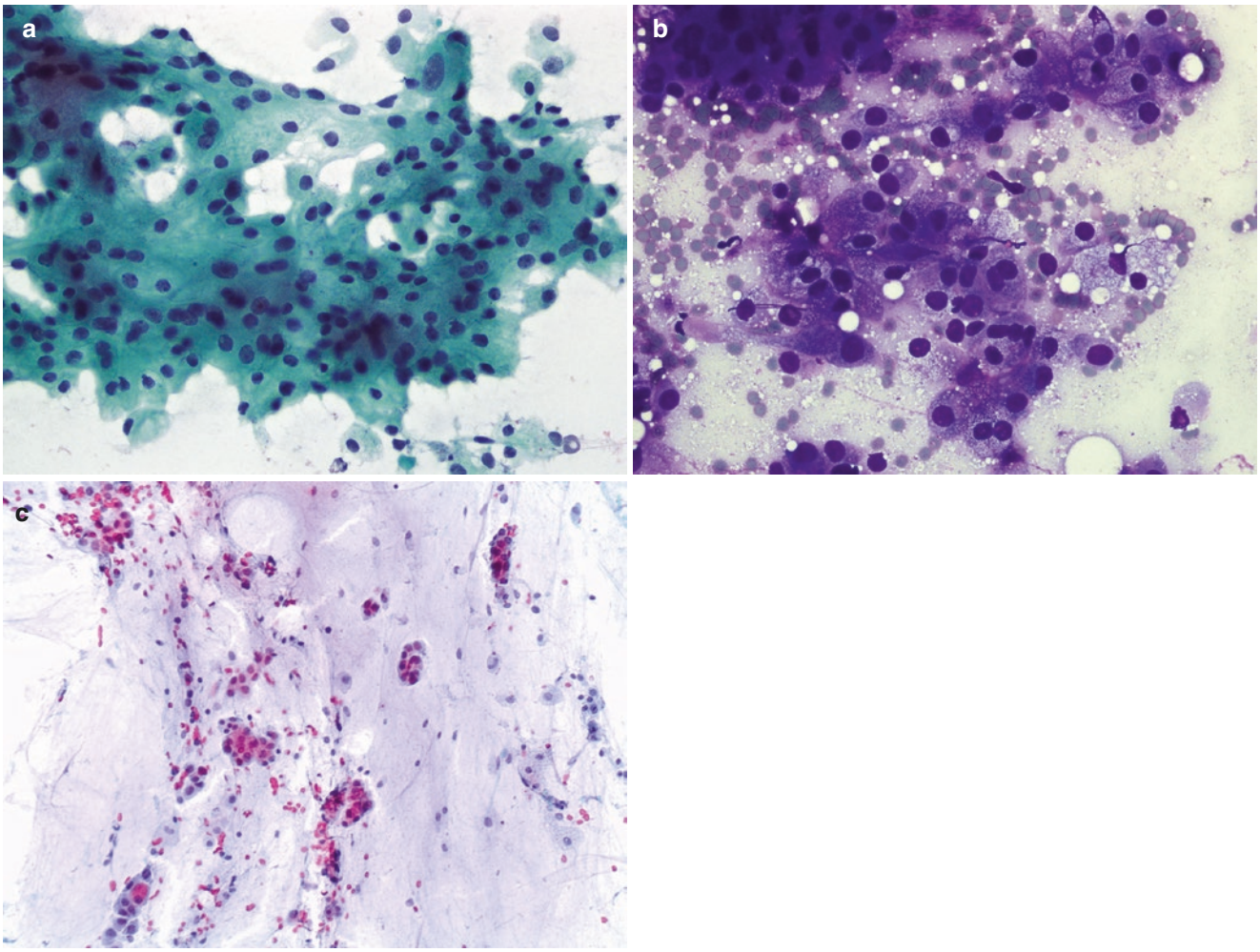

Fig. 16.18 (**a**–**c**) Features of acinic cell carcinoma with bland monomorphic features composed of regular acinic cells loosely cohesive with fragile cytoplasm and small dark nuclei (**a**, **b** conventional cytology, **c** LBC)

the cystic component, large pale vacuolated glandular tumor cells, intermediate cells with dark nuclei and sparse cytoplasm, and inconspicuous malignant squamous cells [136, 137].

Aspirates from high-grade subtype show numerous malignant squamous cells, with some intermediate cells and scant or absence of the mucus glandular cells (Fig. 16.20a, b). The presence of necrosis is reported in these high-grade lesions. The squamous component shows pleomorphic, pyknotic, hyperchromatic nuclei. Both low-grade and high-grade subtypes of mucoepidermoid carcinoma present some diagnostic problems. The presence of only extracellular mucoid material may lead to a diagnosis of a cystic lesion. But also chronic sialoadenitis with squamous metaplasia, necrotizing sialometaplasia, and adenomatoid hyperplasia are other nonneoplastic conditions which may be considered in the differential diagnoses mainly in a low-grade mucoepidermoid carcinoma [136, 137]. Considering the high-grade type, a diagnosis of malignancy is quite straightforward although both primary malignant squamous carcinoma and metastatic squamous carcinoma to the parotid gland may mimic a mucoepidermoid carcinoma and should be ruled out. In cases with less characteristic features, it is advisable that a cytopathologist renders a descriptive report with some of the alternative diagnoses [136, 137].

Polymorphous low-grade adenocarcinoma is the second most common malignancy in the minor salivary gland after adenoid cystic carcinoma [123, 138]. It is frequently seen in the oral cavity and palate. Cytological smears are composed of pseudopapillary and dense fragments of cells with eosinophilic stroma often organized in globular, cribriform, and tubuloductal structures. The neoplastic cells are round to oval with little pleomorphisms, and the cytoplasm is frequently scant and poorly defined. The main difficult differential diagnosis is from adenoid cystic carcinoma but even pleomorphic adenoma might create some problems (Fig. 16.21).

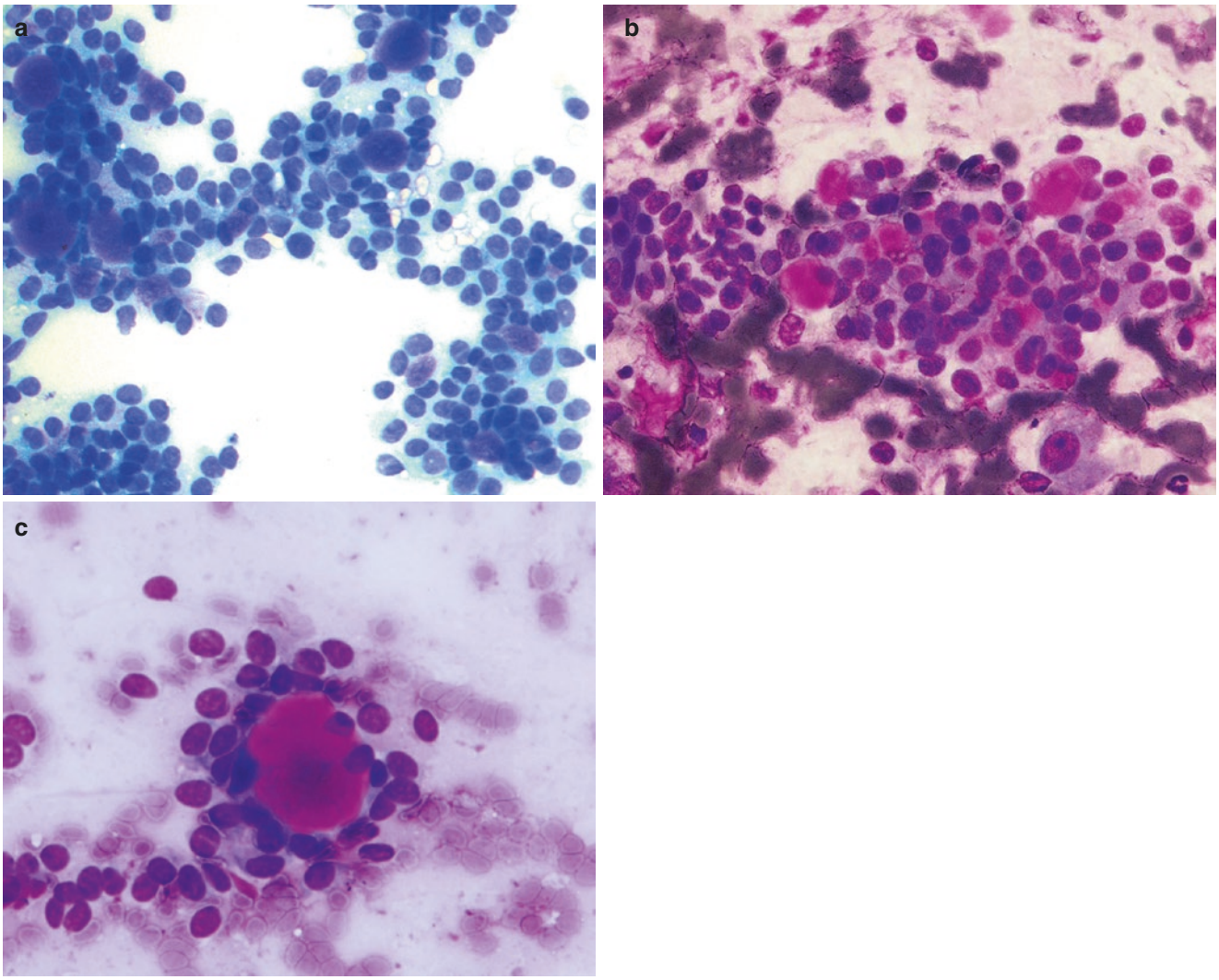

Fig. 16.19 (**a**–**c**) Adenoid cystic carcinoma on conventional cytology with clusters of cohesive small uniform epithelial cells organized around hyaline globules, as seen with Papanicolaou (**a**) and Giemsa stains (**b**). Note the hyaline globule in detail on Giemsa stain (**c**)

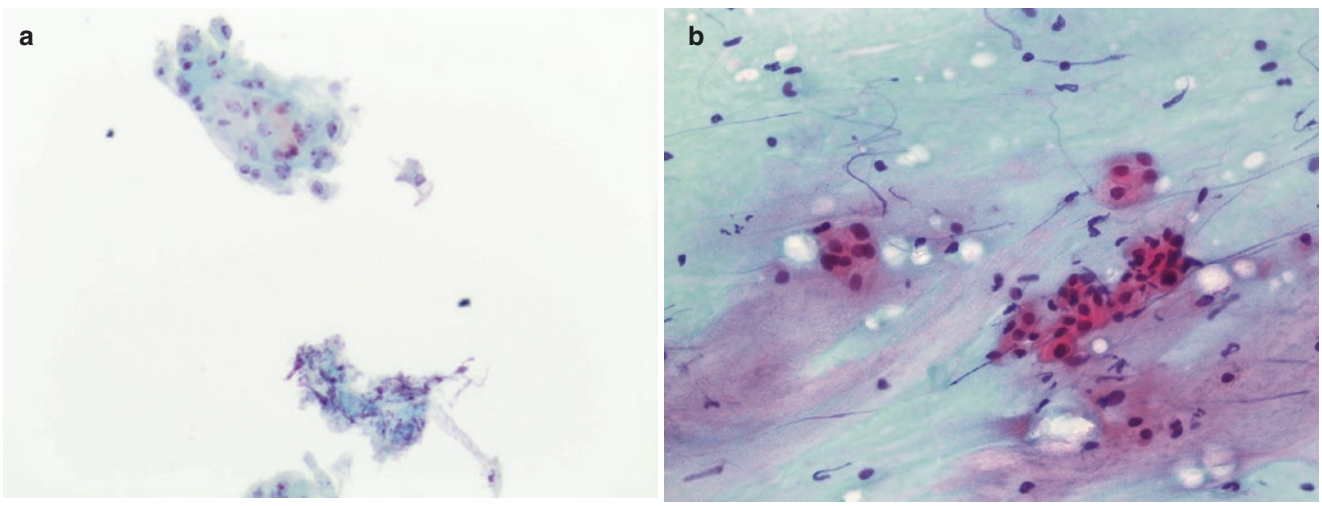

Fig. 16.20 (**a**, **b**) High-grade mucoepidermoid carcinoma in liquid-based cytology; it shows clusters of malignant squamoid cells with nuclear atypia (**a**). In (**b**), a low-grade mucoepidermoid carcinoma with glandular and intermediate cells in a mucinous background on conventional cytology

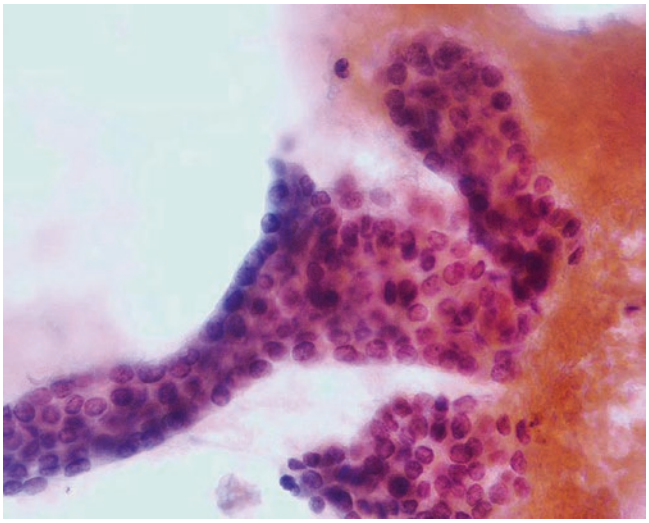

Fig. 16.21 Details of cells from a polymorphous low-grade adenocarcinoma. Cells are round to oval with little pleomorphism, and the cytoplasm is frequently scant and poorly defined

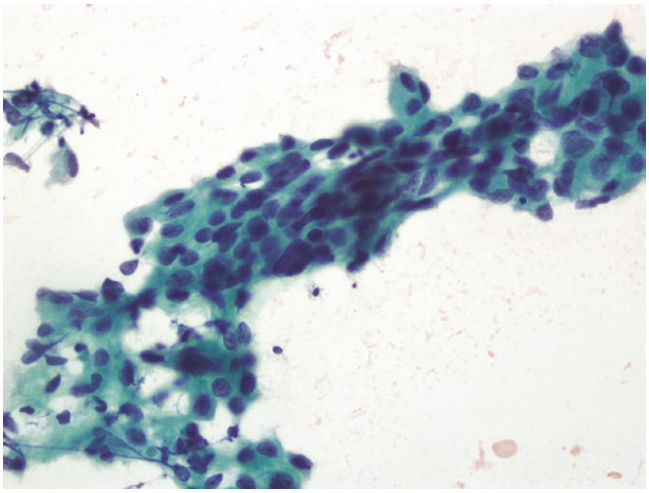

Fig. 16.22 Salivary duct carcinoma. Cytological smears are composed by clusters of cells with ductal formation and nuclear atypia

Myoepithelial carcinomas can originate in both major and minor salivary glands with a predilection for the parotid gland [123, 139, 140]. This is the malignant counterpart of a myoepithelioma. Smear from myoepithelial carcinoma shows clusters of cells with a pseudopapillary organization, eosinophilic stroma, spindle-plasmacytoid cells with irregular nuclei and intranuclear inclusions, and basophilic and vacuolated cytoplasm. Mitoses might be present [140]. The immunopositivity for myoepithelial cells (including calponin, myosin, smooth muscle actin, GFAP) is positive.

Salivary duct carcinoma is a rare entity which usually involves the parotid gland. It is a rapidly growing malignancy with aggressive features including lymph node metastases [123, 141].

Cytological smears are composed of single cells or clusters of solid cells organized in papillary, cribriform, solid, or comedo structures (Fig. 16.22). The cells show moderate to severe atypia. Necrosis is frequently present. This lesion is usually diagnosed as adenocarcinoma not otherwise specified (NOS). Immunocytochemistry may help in a conclusive diagnosis showing positivity for CEA, EMA, PSA, and androgen receptor [142, 143].

Carcinoma ex pleomorphic adenoma (adenocarcinoma arising in a mixed tumor) entity includes carcinoma ex pleomorphic adenoma, carcinosarcoma ex pleomorphic adenoma, and metastasizing pleomorphic adenoma [123, 144]. Non-invasive carcinoma (intracapsular carcinoma) is also considered as part of this category from the WHO classification. Carcinoma ex pleomorphic adenoma is one of the most common types of carcinoma of the parotid and sublingual. It accounts 5 % of all epithelial neoplasms and 20 % of all salivary carcinomas. The reliable separation of non-invasive (intracapsular) and true invasive carcinoma is not always achievable on FNAC even if cellularity, atypia, and necrosis may be helpful. The majority of high-grade invasive carcinomas are diagnosable on FNAC, but false-negative results might be common with low-grade carcinoma [144].

Carcinosarcoma ex pleomorphic adenoma is a less common type of this malignancy and quite rare to encounter even if the detection of the biphasic pattern is paradigmatic. The diagnosis of metastasizing pleomorphic adenoma is defined by its clinical behavior rather than cytological differences with the benign counterpart.

Squamous cell carcinoma shows the same diagnostic criteria of other sites. This is mainly a diagnosis of exclusions of a metastatic squamous carcinoma, mucoepidermoid carcinoma, and carcinoma ex pleomorphic adenoma [145]. History and clinical findings may help in the correct determination and treatment. Cytological pattern shows cells with abundant demarcated cytoplasm, keratin formation, epithelial pearls, and fragments of keratinized cytoplasm and intercellular bridges.

Oncocytic carcinoma is a rare malignancy representing the malignant counterpart of benign oncocytoma. It is a high-grade carcinoma, but caution should be advised in reaching this diagnosis on FNAC due to the degree of atypia which are typical of oncocytic cells [146]. The cytological pattern is very similar to that of salivary duct carci-

noma, but an absolute distinction between these two aggressive tumors is not of prime clinical importance because of the treatment.

Basal cell carcinoma represents the malignant counterpart of basal adenoma showing high neurotropism and angioinvasion. Cytological smears show solid, trabecular, tubular, and membranous subtypes with hyaline stroma and focal squamous differentiation. The most common differential diagnosis is with adenoid cystic carcinoma which was clearly defined by Klijanienko [147].

Undifferentiated and neuroendocrine carcinomas include both large cells and small cell variants which might be difficult to separate from high-grade carcinomas and lymphomas. Cytological findings are identical of those described in the specific section of neuroendocrine tumors. In these cases, if the material is sufficient, an immunopanel might help in subclassifying this group of tumors.

Adenocarcinoma not otherwise specified (NOS) is reported in both major and minor salivary glands. Aspirates are composed of loose clusters of malignant (Fig. 16.23) cells and isolated cells which may not be further classified and diagnosed in a specific subtype of salivary gland carcinoma [123]. Necrosis, mitosis, and hemorrhages might be seen.

Nonepithelial tumors include benign and malignant conditions such as lipoma, neurofibroma, neurofibrosarcoma, and other benign mesenchymal conditions but also sarcomas. Benign lesions and sarcoma display the same morphological features of similar tumors in other anatomical sites which have been described in other sections of this and the other histological chapters of this book [104, 148].

Malignant lymphomas include two entities: extranodal lymphoma of the salivary gland (MALT lymphoma) and lymphoma arisen in the intrasalivary lymph nodes. The most common lymphomas are follicular lymphoma and diffuse large B-cell lymphoma. The use of cytology associated with immunocytochemistry and flow cytometric may help in refining the morphological diagnosis (Fig. 16.24).

Metastatic tumors are also possible malignant lesions which are impossible to distinguish from a primary neoplasm. Malignant lesions which might metastasize to the parotid glands are squamous cell carcinoma, malignant melanoma, and hematolymphoid. Less frequent metastases are from the kidney, thyroid, and breast [149]. In cases of patients with parotid mass and with a previous history of cancer, the possible metastatic nature of the parotid lesion must be considered. This is important because metastatic tumors to the salivary glands have a poor prognosis with a survival rate of only 12 % after 5 years.

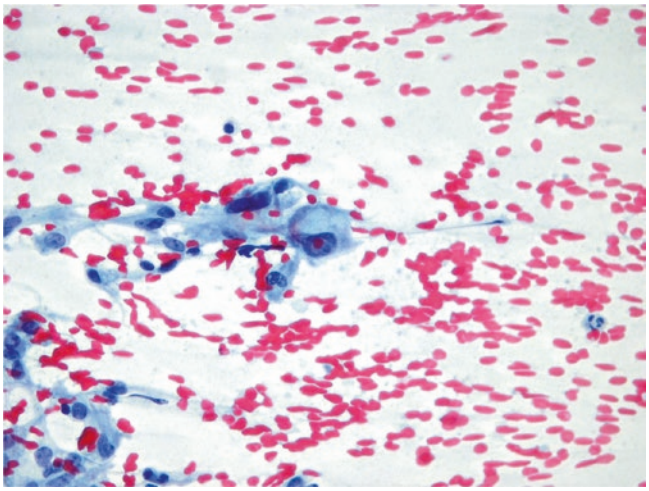

Fig. 16.23 Salivary gland adenocarcinoma not otherwise specified on conventional cytology. Aspirate is composed of loose malignant cells with prominent nucleoli which may not be further classified

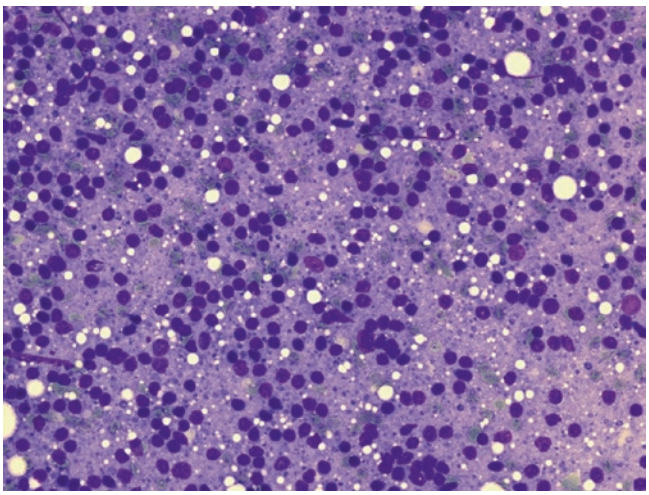

Fig. 16.24 Cellular features of low-grade non-Hodgkin lymphoma arisen in a salivary gland. Note heterogeneous population with predominance of small lymphocytes

16.8 Cytology of Paragangliomas, Neuroendocrine Tumors, and Neuroblastomas

Paragangliomas are neuroendocrine tumors derived from the neural cells of the autonomic system which, in the neck, is physically organized in the carotid body and along the jugular veins. These neural structures are deputed to regulate the feedback system of the blood pressure and are composed by ganglion and glial cells and nervous fibers. The carotid body

and jugulotympanic and vagal paragangliomas are rare tumors which are usually underdiagnosed until they present as slowly growing firm cervical masses showing a rich vascular network at Doppler examination. These tumors are seldom biopsied because their vascular component may contraindicate the operation, but sometimes a differential diagnosis with either a metastatic carcinoma or lymphomatous localization in a cervical node may require a sampling of these lesions. The background of the smear distinctively shows a delicate vascular network, but the hallmark of the diagnosis of paraganglioma is the identification of large cells with round pleomorphic nuclei, often showing prominent nucleoli, and large granular eosinophilic cytoplasms resembling those of hepatocytes (Fig. 16.25a, b). Admixed with these neuroendocrine cells, isolated elements with elongated nuclei, corresponding to the sustentacular cells, may be occasionally identified [14, 150]. The paraganglioma cells express synaptophysin and chromogranin A, whereas the sustentacular cells are positive for S-100 because of their derivation from Schwann cells [14]. Cervical paragangliomas of the carotid body, which are the most common paraganglionic tumors of the head and neck district, and jugulotympanic may be uncommonly (less than 10% of cases) multiple [151], bilateral [152], and familial [153]; these tumors may pursue a malignant course in 3–6% of cases [15] and may represent a clinical problem when they arise in a cranial foramen.

Another neuroendocrine tumor which may occur in the head and neck region is Merkel cell carcinoma (MCC). This cutaneous malignant tumor usually arises in the face, in the neck, and in the extremities and usually grows rapidly, undergoing ulceration in its surface and giving early metastases to regional nodes and distant sites. MCC may also present as nodal metastasis before the detection of the primary site and shows a challenging cytologic picture. In fact, the small uniform tumor cells may mimic an undifferentiated small cell carcinoma (pulmonary type), and only their round nuclei and the fragile cytoplasms may suggest a diagnosis different than carcinoma or high-grade lymphoma. The immunocytochemical stainings exhibit a distinctive pattern of cytokeratins expression (dot-like in the cytoplasm) which is a very helpful clue for the diagnosis [14, 154]. Neuroblastomas are primitive malignant neoplasms of neuroectodermal derivation that arise in along the chain of the sympathetic nervous system. In the neck they are exceedingly rare. Cytologically, the presence of true rosettes and/or pseudorosettes is the most characteristic feature. Nuclei are round to oval, of variable size, with salt and pepper distribution of the chromatin. Cells may depict an irregular cytoplasmic rim. Immunohistochemically, neuroendocrine markers show strong positivity (Fig. 16.26a–c).

16.9 Cytology of Mesenchymal Tumors

16.9.1 Benign Lesions and Tumors

Mesenchymal benign lesions of the head and neck district are a relatively common finding in the clinical practice, but only occasionally they are submitted to aspiration biopsy because of both the possibility of getting a poor cellular sample and the limited diagnostic accuracy of the technique. The diagnosis of mesenchymal neoplasms requires a multidisciplinary approach in which the radiological and ultrasound findings are compared with the cytopathological and clinical pictures [155]. Nonetheless, there are a few lesions which deserve an individual description since their cytologic picture is discriminant for the diagnosis and treatment.

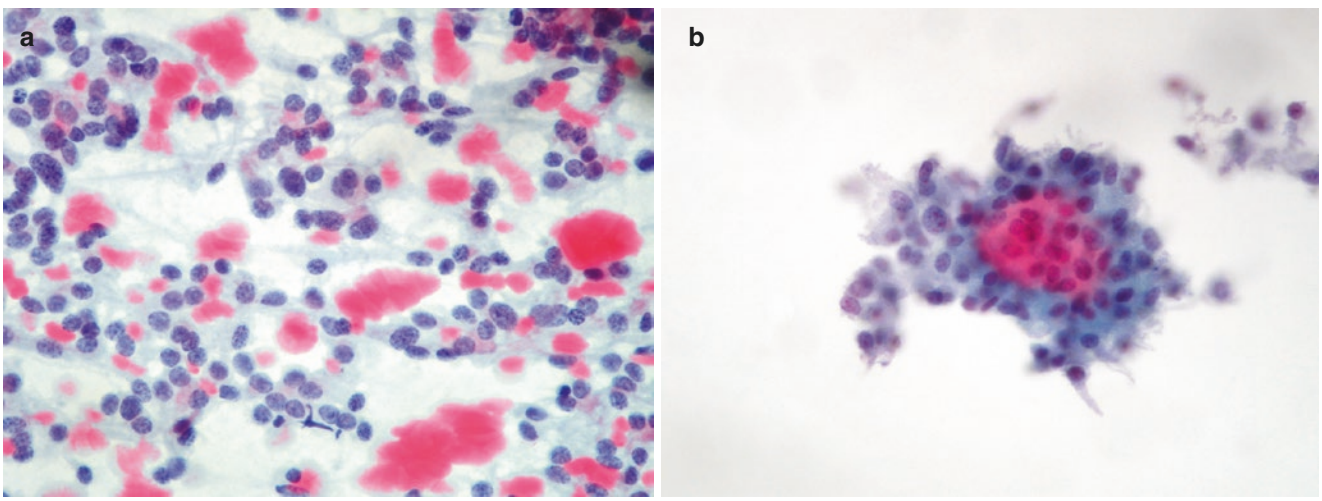

Fig. 16.25 (**a**) Paraganglioma on conventional cytology. It shows isolated cells with salt and pepper chromatin distribution on a bloody background. (**b**) Liquid-based cytology. A cluster of cells with similar nuclear features and granular cytoplasm

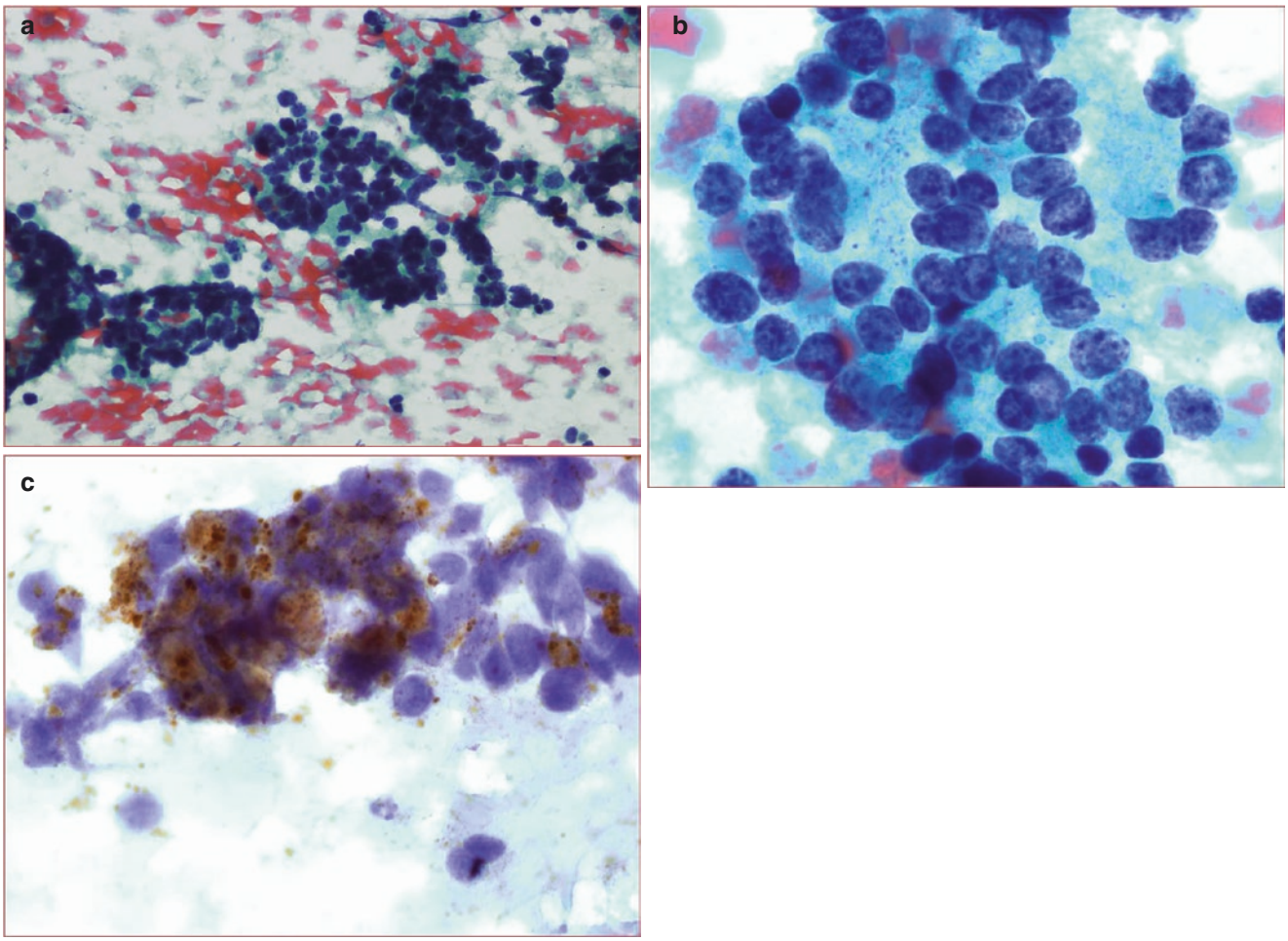

Fig. 16.26 (**a**–**c**) Neuroblastoma of the neck: Small blue cells arranged in rosettes (**a**). Detail of the tumoral cells with salt and pepper chromatin and scant cytoplasm (**b**). Strong cytoplasmic reaction with synaptophysin (**c**)

Nodular fasciitis (NF) is the most common form of the group of pseudosarcomatous lesions of soft tissues which include proliferative myositis, proliferative fasciitis, and myositis ossificans [156]. NF is an infrequent though worrisome lesion which may arise in the subcutaneous layer of the cervical region, sometimes in a traumatized area. The superficial nodule may present a hard consistency and may grow rapidly like a sarcoma or mimic a tumor of the parotid gland [157]. The aspiration biopsy of NF may show, in a background of inflammatory cells and tiny fragments of cellular connective tissue, scattered ganglion-like cells with large pleomorphic nuclei and prominent nucleoli (Fig. 16.27) giving the appearance of a high-grade mesenchymal tumor [158]. The absence of necrosis and the limited amount of ganglion-like elements may suggest the diagnosis of NF which is a reactive lesion regressing in a few months without any residual disease.

Lipomas are the most common benign soft tissue tumors in the cervical region. They can occur either in the subcutis or within the parotid gland and are usually slow-growing lesions that can be clinically misdiagnosed as epidermal cysts, sharing with the latter the sonographic finding of an isoechoic lesion [155, 159]. The classic cytologic picture of lipoma, characterized by a poor cellular sample showing scattered flakes of fibroadipose tissue showing cells with small eccentric dark nuclei and wide empty cytoplasms, may sometimes show focal but important changes. Lipoblasts (cells with cytoplasmic vacuoles indenting the nucleus) and floret cells (large cells with multiple hyperchromatic nuclei arranged in circle or semicircle) are the distinctive features, respectively, of lipoblastoma [160] and pleomorphic lipoma [161] which are benign lipomatous tumors mimicking liposarcoma. A bland spindle cell component may be present in the setting of an otherwise classic lipoma raising the suspicion of a sarcomatous transformation of the original tumor. Benign vascular and neural tumors have been already described in the chapter on tumors of the oral cavity and oropharynx (see above). The cytologic pictures of neural tumors (schwannomas and neurofibromas) may often overlap show-

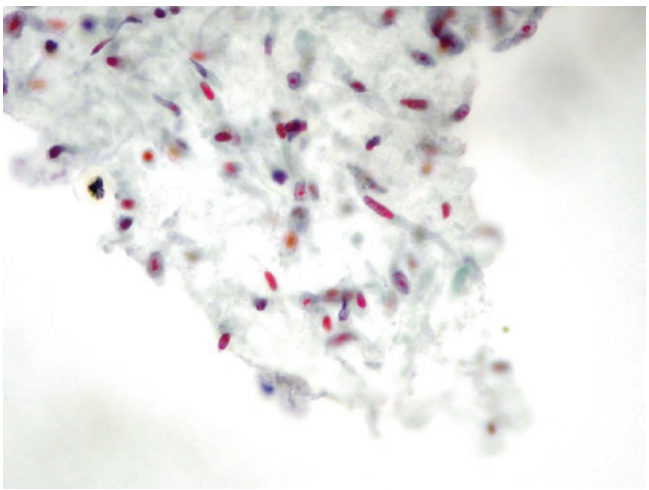

Fig. 16.27 Details from a nodular fasciitis of the neck region on conventional cytology. Fragments of loose connective tissue, containing spindle cells with prominent nucleoli

ing a hypocellular smear exhibiting bundles of fibrous tissue composed by elongated cells with hook-shaped extremities (comma-shaped cells) with dark monomorphic nuclei [155]. Focal nuclear pleomorphism can be occasionally seen, whereas fragments of necrotic tissue and prominent nucleoli must raise the suspicion of a malignant nerve sheath tumor. Schwannomas may rarely occur as multiple bead-like nodules along the sympathetic chain, raising the suspicion of a NF-1 or a metastatic spread from an unknown primary [162].

Benign tumors of smooth and striated muscle are uncommon findings in the head and neck region. The distinctive cytologic picture of rhabdomyoma has been described in the chapter of tumors of the oral cavity and oropharynx (see above).

Benign fibrous histiocytoma is also a tumor which might be submitted to aspiration cytology and yields bundles of spindle cells with ovoid monomorphic nuclei and large vacuolated cytoplasms. The presence of myxoid stroma may lead to an erroneous diagnosis of sarcoma, but the lack of obvious pleomorphic nuclei and necrotic material together with the circumscription of the lesion are the hallmark for a diagnosis of benign tumor [163].

16.9.2 Malignant Tumors

There are two morphologic parameters which bear a clinical significance and must be taken into account in a cytological sample from a soft tissue tumor: the predominant pattern and the cytological grade. In malignant soft tissue tumors, one of the following patterns is often predominant: myxoid, spindle cell, pleomorphic, round cell, and polygonal/epithelioid cell pattern [155]. The tumor grade is also very important and is strongly correlated with the prognosis and the treatment [164]. There are some published grading systems, but the most reproducible seems to be a two-tier grading method which identifies low- and high-grade tumors [165, 166]. According to Palmer et al. [167], the grading system for soft tissue sarcomas is not perfectly applicable to spindle cell and myxoid tumors, while it is effective in correctly identifying high-grade sarcomas belonging to round cell, pleomorphic, and epithelioid/polygonal cell patterns [165].

The abovementioned patterns may be shared by several tumors of different cell lineages, but the identification of the predominant pattern helps in achieving the correct subtyping in more than 60 % of all sarcoma cases, with an overall inadequacy lower than 10 % and in more than 90 % of pediatric cases [4, 164].

For the classification and the histologic patterns of the individual sarcomatous types, we refer to the chapter of soft tissue tumors (Chap. 13). The myxoid pattern, which is one of the most difficult to identify since the myxoid substance can be misinterpreted as mucin or the presence of isolated bland nuclei can be underdiagnosed as benign tumor, includes the majority of low-grade sarcomas such as liposarcoma, leiomyosarcoma, myxofibrosarcoma (Fig. 16.28a, b), and extraskeletal myxoid chondrosarcoma [168, 169]. Myxoid liposarcoma is characterized by the presence of scattered lipoblasts (see above) in a myxoid substance, but the hallmark of the differential diagnosis with lipoblastoma is the presence of a plexiform vascular network [155, 170]. The round cell pattern mostly encompasses high-grade blue cell infantile sarcomas such as rhabdomyosarcoma, Ewing sarcoma, mesenchymal condrosarcoma, desmoplastic small round cell tumor, and the round cell liposarcoma, which is regarded as the high-grade counterpart of myxoid liposarcoma [164]. The cytologic picture of the most representative tumor of this pattern (rhabdomyosarcoma) has been described in the paragraph on malignant tumors of the oral cavity. The polygonal/epithelioid pattern embraces low- and high-grade sarcomas showing clusters of epithelioid cells which usually need to be distinguished from poorly differentiated carcinomas and melanoma: this group includes epithelioid hemangioendothelioma and angiosarcoma, epithelioid sarcoma, clear cell sarcoma, alveolar soft part sarcoma, hemangiopericytoma, and malignant granular cell tumor [171, 172]. Spindle cell pattern is shared by several low-grade sarcomas of the connective tissue (malignant nerve sheath tumors, conventional and infantile fibrosarcoma, leiomyosarcoma [173] and some special types such as gastrointestinal stromal tumors, synovial sarcoma, malignant fibrous solitary tumor, and Kaposi's sarcoma) [174]. In the latter cases, the distinction between low- and high-grade tumors relies upon the identification of markedly atypical nuclei, mitotic figures, and cellular necrosis [164, 166, 167]. The last group includes adult pleomorphic high-grade sarcomas of various origins (Fig. 16.29) [175, 176]. On cytologic specimens, it is also possible to

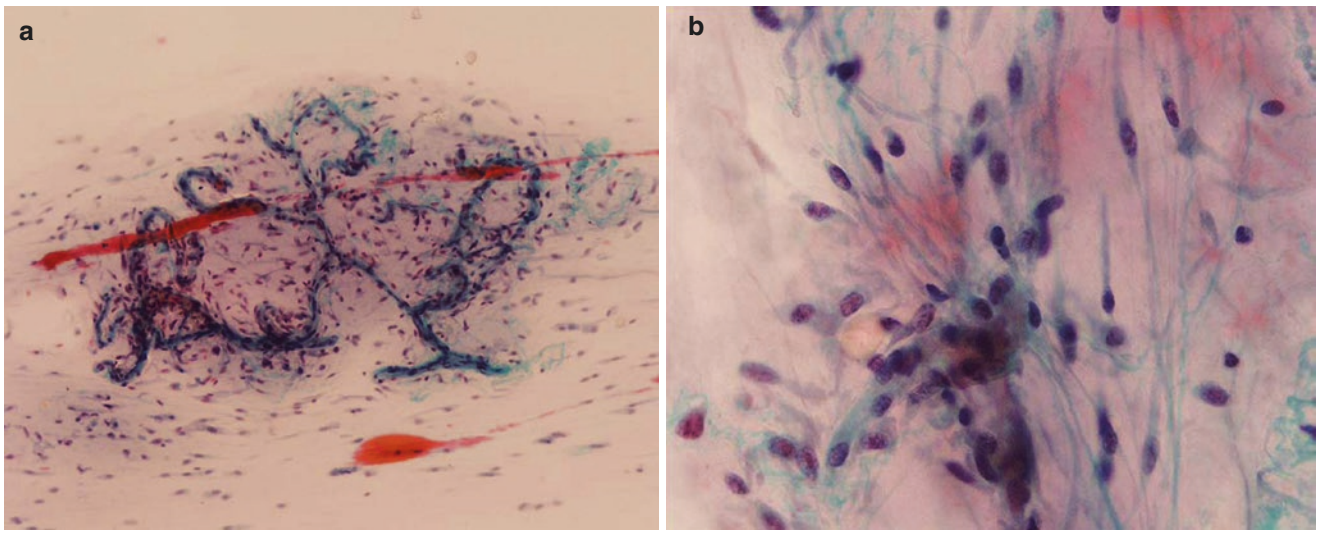

Fig. 16.28 (**a**, **b**) Characteristic features of a cervical myxofibrosarcoma with the prominent curvilinear vessels (**a**). Spindle cells, with nuclei showing low-grade atypia, appear interspersed in a myxoid background (**b**)

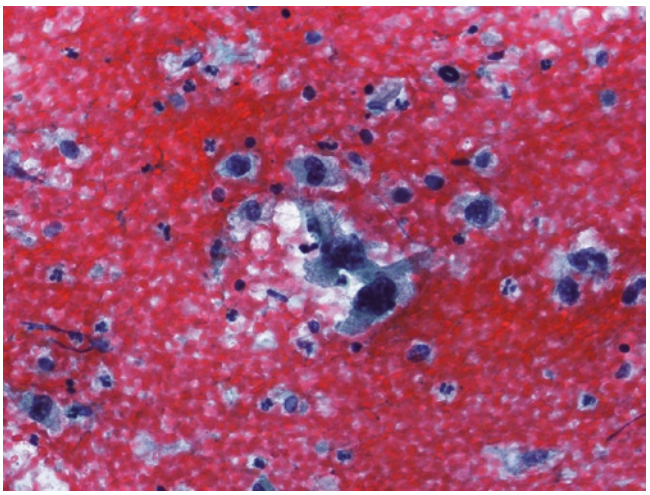

Fig. 16.29 High-grade sarcoma of the neck showing dispersed pleomorphic cells with highly atypical nuclei

carry out molecular studies, among which FISH is particularly useful since it can be easily applied to cytological material. Cytogenetic analysis is becoming decisive for the correct diagnosis of some malignant soft tissue tumors, such as Ewing's sarcoma, synovial sarcoma, liposarcoma, and rhabdomyosarcoma [170, 174, 177].

16.10 Cytology of Cervical Lymph Nodes

FNAC is the first diagnostic approach to investigate lymphadenopathies, and enlarged lymph nodes are the most common aspirate site on head and neck clinics, especially in the pediatric age group patients [1, 4, 5]. The majority of patients do not have significant disease, and in these circumstances, it is important to assess this correctly and prevent unnecessary anxiety [1]. To avoid waste resources, it is important to know that there is no role of cytology in evaluating lymph nodes which are not enlarged and have a normal ultrasound appearance despite the fact that these may be easily palpable if superficial [1]. The most common cytological diagnoses from cervical lymph nodes are reactive lymphadenopathy, infectious diseases, metastatic carcinoma, and lymphoma. FNAC of cervical lymph nodes had a sensitivity of 94.2 % and specificity of 96.9 % [5]. Although there is a large body of evidence supporting the use of cytology as a primary method of diagnosis in reactive, infectious, and metastatic lymphadenopathy, the diagnosis of malignant lymphoma by FNAC has been much more controversial. However, several studies and the personal experience of the authors have shown conclusively that a combined cytological and immunological evaluation of aspirated lymphoid cells results in distinctly improved diagnostic accuracy in cases of lymphoma [9, 178, 179]. We reinforce the opinion of other authors [179] that laboratories involved in the diagnosis of patients with lymphadenopathy should use FNAC in conjunction with immunological characterization. This diagnostic approach will have a substantial impact on the diagnostic accuracy and consequently the clinical management of the patients.

16.10.1 Reactive Lymphadenopathy

Lymph nodes respond to different agents by enlarging and becoming more active. This response may take form of enlargement of the lymphoid follicles which develop active germinal centers. These are characterized by numerous follicle center cells, macrophages containing tingible bodies, and a surrounding cuff of small lymphocytes. Alternatively,

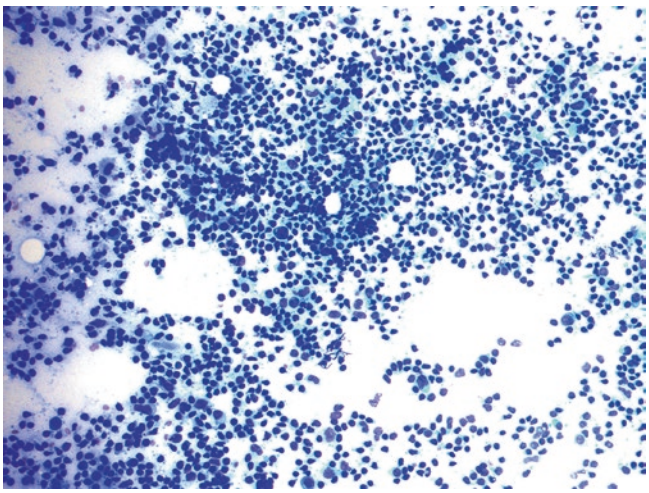

Fig. 16.30 Reactive lymphadenitis on conventional cytology. Smear contains some centroblasts and centrocytes mixed with mature lymphocytes

there may be expansion of the interfollicular tissue by numerous mature lymphocytes, lymphoplasmacytoid cells, plasma cells, and varying number of immunoblasts. In some cases, there is a mixture of both patterns. This is reflecting on cytological smears from reactive lymphadenopathy. If the first pattern is sampled, the smears contain many centroblasts and centrocytes mixed with tingible body macrophages and mature lymphocytes (Fig. 16.30). In extreme cases this pattern may mimic follicular cell lymphoma, and flow cytometry is needed to resolve this diagnostic problem. If the second pattern of reaction is predominant in the sample, we observe smears rich in mature lymphocytes, plasma cells, and lymphoplasmacytoid cells. Such smears can be difficult to differentiate from a low-grade lymphoma, and again, analysis of light-chain immunoglobulin restriction is quite useful for a definitive diagnosis.

Some specific conditions can be suspected when we have a predominant cytological pattern of reactive lymphoid hyperplasia in head and neck lymph nodes. For example, an exuberant follicular hyperplasia with many immunoblasts rises as suspicious of HIV infection. Presence of large and some atypical immunoblasts can be related to infectious mononucleosis or viral and post-vaccinal lymphadenitis. The presence of interdigitating dendritic cells, with pale indistinct cytoplasm and macrophages containing brown melanin pigment, is suggestive of dermatopathic lymphadenopathy, especially if the patient has a history of chronic skin disorder. The presence of many plasma cells, with Russell bodies, can be related with autoimmune diseases such as rheumatoid arthritis or lupus erythematosus.

A rare condition that causes massive enlargement of cervical lymph nodes, especially in black children and adolescents, is the sinus histiocytosis with massive lymphadenopathy. The cytological findings of this pathology are characterized by numerous lymphocytes and large pale histiocytes which have vesicular nuclei with small nucleoli and an abundant vacuolated cytoplasm. These cells often contain on the cytoplasm and well-preserved lymphocytes (emperipolesis) and are S100 positive at immunocytochemistry [180].

16.10.2 Infectious Lymphadenitis

According to the morphological pattern, sometimes it is possible to categorize the type of infection affecting the lymph nodes. Suppurative lymphadenitis and granulomatous lymphadenitis are the most common infections described on head and neck lymph nodes. In many of occasions, microbiological culture or PCR technique is very useful to precisely identify the infectious agent. Lymph nodes draining or adjacent to a focus of bacterial infection may be directly invaded by the organisms, causing acute lymphadenitis followed in some cases by suppuration. The cytological findings of acute suppurative lymphadenitis are at initial phase, presence of a proteinaceous background with cell debris, mature lymphocytes, and sparse granulocytes. Later the aspirate becomes purulent with many degenerative neutrophils in a background rich in cell debris.

Although the most common cause of granulomatous lymphadenitis in European countries is sarcoidosis, in many other areas, in patients with immunodeficiency, other etiologies, especially tuberculosis, are the main causes of granulomatous lymphadenitis in the head and neck region [2, 4]. The general cytology aspect in aspirates from granulomatous lymphadenitis is characterized by clusters of epithelioid cells which have elongated nuclei, arranged in a syncytial fashion with abundant ill-defined cytoplasm. A variable number of multinucleated Langhans giant cells may be present, with the nuclei polarized at one part of the cell border. The presence or absence of necrosis at background can help in the diagnosis. Sarcoidosis aspirates are free of necrosis, while in tuberculosis this is a frequent finding. Necrosis appears as a pale stained amorphous material in the background of the smears. It is important to emphasize that sometimes smears from tuberculosis can be predominantly suppurative. For definitive diagnosis, acid fast bacilli can be identified using the Ziehl-Neelsen (ZN) stain in cell blocks. Nowadays, this stain is being replaced by PCR techniques to identify the mycobacteria. Atypical mycobacterial infection can cause head and neck lymph node enlargement in immunodeficient patients, including those with AIDS. The smears are characterized by many histiocytes with abundant pale cytoplasm. In Giemsa-stained preparations, the mycobacteria present as cylindrical "negative images" at cytoplasm of the cells and are strongly positive for ZN.

Other infections can affect head and neck lymph nodes, but they are less common. Fungal infections such as histo-

plasmosis, cryptococcosis, actinomycosis, and paracoccidioidomycosis among others can be diagnosed by FNAC when we are able to detect the respective agents at the smears.

16.10.3 Malignant Lymphomas

Malignant lymphomas (ML) are divided into two major categories: Hodgkin lymphoma and non-Hodgkin lymphomas. They can be further divided into several subgroups, which are important to identify because of their different clinical behaviors. Much effort has been spent on the diagnosis of ML by FNAC, attempts which have until recently been only partially successful. One major reason for this is that most neoplastic lymphoid cells lack the traditional cytological features of malignancy. Such cells are close replicas of their normal counterparts [179]. If the cytology sample is composed of only one cell type, a confident diagnosis of non-Hodgkin lymphoma (NHL) can usually be made. However, some lymphomas are composed of several types of neoplastic cells, while others contain an admixture of benign lymphoid cells with neoplastic elements, which obviously obscures the picture. The complexity of such samples may be an overwhelming task even for the most experienced cytopathologist. In the case of Hodgkin lymphoma (HL), the finding of cells with large atypical nuclei and multilobated nuclei has been considered diagnostic. At present no system of classification has been constructed for FNA cytology material. Older classifications relied partly on architectural features, which meant that cytology was suboptimal for primary diagnosis. As the immunological characterization of lymphoid neoplasms has evolved, the importance of architectural features has diminished, and distinction from reactive conditions is more reliable. As a consequence, FNAC can be used for primary diagnosis provided a full workup including immunological studies and flow cytometry (FCM) is performed [1]. In fact, cytology specimens seem ideal for immunological evaluation; recent studies show a high diagnostic accuracy if the cytological findings are combined with results from immunophenotyping and clonal restriction analysis. Patients who are known to have lymphoma may present with new or progressive disease. In this scenario, FNAC is the method of choice for disease monitoring, instead to subject the patient to surgical biopsy every time recurrence or progression is suspected. As well as diagnosing recurrence or progression, the possibility of transformation to high-grade disease or concurrent infection should be considered.

16.10.3.1 Non-Hodgkin Lymphomas

Non-Hodgkin lymphomas (NHL) comprise 2–3 % of all malignancies in developed countries. They represent a spectrum of neoplasms ranging from indolent to aggressive tumors, the latter having a rapidly fatal course. The age-specific incidence increases throughout life. The clinical presentation of NHL shows an extremely variable pattern. It is not infrequent that enlargement of head and neck lymph nodes be the first clinical presentation of a NHL and FNAC the first diagnostic method used. The histological diagnosis and method of classification have long been a matter of debate and is extensively discussed at Chap. 13 of this book. It is out of the scope of this chapter to describe all the cytological aspects of the NHLs. The reader can find this in specific cytological books. Here we will discuss the general cytological aspects of NHL in smears as well as the most frequent entities recognized on cytology.

The cytological evaluation of NHL smears from FNAC should take into account the identification of the different cell types present, the estimation of proportion of these cell types, and the evaluation of the individual cell characteristics. A monotonous pattern is present when one cell type predominates, but even so, additional cell types can be found (Fig. 16.31). This monotonous composition indicates abnormal expansion of one or at the most two subtypes of lymphoid population and can be seen in most follicular lymphomas and some large cell lymphomas. A mixture of lymphocytes of all types indicates stimulation of the entire lymphoid population and suggests reactive lymphadenitis, although some B- and T-cell neoplasms can present a mixed population of cells.

As previously mentioned, the morphological assessment of a lymph node aspirate should be accompanied by an immunological workup, and this led to an improvement in the rate of conclusive diagnosis. Cytospin preparations or

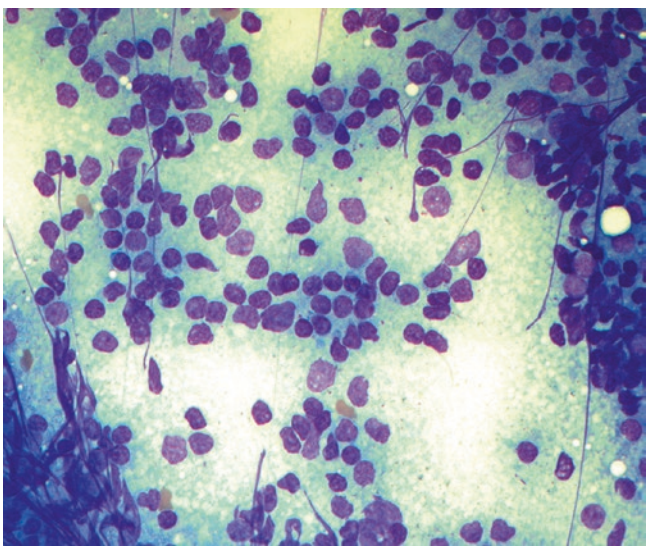

Fig. 16.31 High-grade non-Hodgkin lymphoma on conventional cytology. Note the monotonous aspect of the, except for the pleomorphism of the cells

suspensions for FCM are used for assessment of phenotype to establish whether the cells are T or B in origin and whether clonal restriction of light-chain production is present. In Western countries, most lymphomas are of B-cell lineage with immunoglobulin light-chain expression restricted to either kappa or lambda. Clonal expansion in T-cell lymphomas is more difficult to demonstrate. Loss of pan-T-cell subtype antigen or anomalous expression of T-cell subset antigens can be taken as evidence of clonality. The initial immunological workup should be based on a preliminary cytological evaluation. A diagnosis of reactive hyperplasia or B-cell lymphoma should entail a limited panel of antibodies to include pan-T and pan-B antibodies as well as antibodies to the light chains, Bcl-2 and CD10 (cALLa). A kappa-to-lambda ratio below 5:1 or lambda-to-kappa ratio not exceeding 3:1 strongly favors a polyclonal reactive B-cell population. Values exceeding these figures suggest a monoclonal malignant expansion of B cells. The subclassification of the B-cell lymphomas sometimes requires the additional staining with antibodies to CD5, CD23, and CD43. The common acute lymphoblastic leukemia antigen (cALLa or CD10) is expressed in some follicular lymphomas and large cell tumors. A majority of high-grade lymphomas are readily diagnosed as large cell neoplasms in cytological preparations, and in these cases immunocytochemistry is needed only to phenotype the neoplastic cells. In contrast, a conclusive cytological diagnosis of many low-grade T-cell lymphomas is difficult. The lack of strict immunological criteria for monoclonality further compounds this diagnostic dilemma. As in histopathology, in many cases it will require T-cell receptor (*TCR*) gene rearrangement analysis by PCR to prove that the process is neoplastic. In addition several subtypes of non-Hodgkin lymphomas are characterized by specific translocations which can be identified by FISH or PCR. The fraction of proliferating cells in NHL is related to prognosis and response to chemotherapy. Several methods are available to estimate the proliferation rate. Mitotic counting is time consuming and inaccurate. Flow cytometry is a rapid and an accurate technique but is not available in all laboratories. Staining with Ki-67 antibody or antibodies to proliferating cell nuclear antigen (PCNA) offers a highly sensitive procedure which can be performed in most laboratories.

In the head and neck, malignant lymphoma (ML) is the most common nonepithelial malignancy, accounting for 12.4 % of all head and neck malignancies [181]. In the head and neck, extranodal involvements by ML are more common than nodal involvement, and the oropharynx is the anatomic site more frequently involved. In a recent review [182], it was demonstrated that among the NHL, the most common that affect head and neck lymph nodes are diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), peripheral T-cell lymphoma NOS (PTCL), anaplastic large cell lymphoma (ALCL), node marginal zone lymphoma (NMZL), Burkitt lymphoma (BL), precursor T-lymphoblastic, and leukemia/lymphoblastic lymphoma (TLBL). We provide a brief cytological description of these subtypes in this chapter [179].

Diffuse large B-cell lymphoma (DLBCL) is mostly seen in elderly patients. The most common type of DLBCL is composed of large round centroblasts presenting nuclei with several small nucleoli, often at the nuclear membrane. The cytoplasm is scanty and may contain a few vacuoles. In addition, a number of other cells such as centrocytes, small mature lymphocytes, and macrophages may be found. Some centrocytes and small mature cells are regularly observed. In some variants of DLBCL, the tumor cells are immunoblasts, characterized by the presence of an eccentric nucleus with unevenly distributed chromatin and a central distinct nucleolus. The cytoplasm is abundant with a grayish blue staining in Giemsa staining. Rarely, poorly differentiated carcinomas or melanomas may on cytomorphology be misdiagnosed as this variant of lymphoma. The cells in DLBCL often express several B antigens such as CD19, CD20, and CD22. Kappa or lambda light chains are expressed in most cases, and CD10 positivity is common in the centroblastic variants. A population of mature T cells is regularly present. The fraction of proliferating cells is usually around 50 % or more, which predicts relatively aggressive clinical behavior. The immunoblasts are CD38 and CD138 negative, which differentiates them from a myeloma. Approximately one-third of the cases show t[14;18] translocation.

Follicular lymphoma (FL) comprises one-third of all NHL. It usually affects middle-aged patients. The predominating cell is the medium-sized centrocyte which has scanty cytoplasm and an irregular cleaved or angulated nucleus. These cells may seem to form aggregates. Centroblasts are present, but the proportion varies. FL are subdivided into Grade I, Grade II, and Grade III based on the proportion of large cells present. Thus, Grade I has 0–5, Grade II 6–15, and Grade III >15 centroblast/high-power field, respectively. Other cell types present are small mature lymphoid cells, macrophages, and epithelioid cells. A smear with a high number of nonneoplastic cells may be impossible to differentiate from reactive lymphadenopathy unless cytomorphology is complemented by immunological evaluation. The B-cell lineage of these tumor cells is identified by expression of CD19, CD20, and CD79a. Light-chain restriction can usually be demonstrated as well as positive staining for CD10. Expression of BCL-2 is seen in a majority of cases. Mature reactive T cells are present and may constitute up to 50 % of the lymphoid population. The proliferation fraction in the neoplastic B cells varies con-

siderably from case to case. Figures below 5 % are seldom seen, but in occasional cases, up to 75 % of the neoplastic population may react positively to proliferation markers. Such cases show aggressive behavior and should be treated as high-grade lymphomas irrespective of their cytological grading. A majority of these lymphomas show a t[14;18] translocation.

Peripheral T-cell lymphoma NOS (PTCL) is most common in adults but can affect all ages. The prognosis is dismal, and most patients die after 5 years of diagnosis. Cytology shows atypical cells ranging from small to large in size. The nuclei are irregular, with prominent nucleoli and coarse chromatin. Occasional large cells with multilobated or multiple nuclei are often seen. The cytoplasm of the atypical cells is also variable, but the medium-sized and large cells have a rich cytoplasm which commonly stains pale gray in MGG preparations. Epithelioid cells, plasma cells, and eosinophils are present in varying proportions. Fragments of vessels are often found. A majority of the cases are CD4 positive. Aberrant T-cell antigen expression and deletion of pan-T-antigens strongly indicate a neoplastic lymphoid population. The TCR genes are rearranged in most cases.

Anaplastic large cell lymphoma (ALCL) is most frequent among children and young adults, but it can occur at all ages. Two main morphological variants of ALCL exist: a large pleomorphic cell type and a small cell variant. The large cell type is characterized by pleomorphic tumor cells with multilobated and horseshoe- or ring-shaped nuclei. The ample cytoplasm is gray-blue (MGG) and often vacuolated. The small cell variant has small- to medium-sized cells with irregular nuclei and a moderate amount of cytoplasm. Often some large cells with distinct nuclear atypia can be found. Strong membrane positivity for CD30 is present in all large tumor cells. The cells are often EMA positive and express T-cell antigens such as CD2 and CD4. ALK expression is seen in a majority of cases. The rate of proliferation is over 50 %. The T-cell receptor genes are rearranged in most cases of ALCL. Translocation t[2;5] and t[1;2] can be detected in 70 % and 20 %, respectively.

Nodal marginal zone lymphoma (NMZL) affects patients over 50 years old. Cytology shows small- to medium-sized cells, the marginal zone cell which is centrocyte-like but with indistinct nucleoli and a more distinct cytoplasm. Some cases will have monocytoid-like cells due to an abundant cytoplasm. Plasma cells, centroblasts, and some monocytoid B cells are often present. The tumor cells are of B-phenotype and show light-chain restriction but no expression of CD5, CD10, CD23, or CD43. The plasma cells are often monoclonal. The proliferation rate is usually low. The extranodal variant shows t[11;18] in half of the cases. The nodal type only rarely shows this abnormality.

Burkitt lymphoma (BL) is most common in children. The Burkitt cells are medium sized with a low nuclear-cytoplasmic ratio. They have deep blue cytoplasm on MGG staining which contains many punched out vacuoles. The nuclei are round with clumped chromatin and central nucleoli. Mitotic figures are frequent as well as macrophages with apoptotic bodies. The neoplastic cells are CD19 and CD20 positive, and light-chain restriction is demonstrated in all cases. The CD10 is usually expressed, but TdT cannot be detected. The fraction of Ki-67-positive cells is above 90 %. Most cases have a t[8;14] translocation, but t[2;8] and t[8;22] translocations also occur.

Precursor T-lymphoblastic leukemia/lymphoblastic lymphoma (TLBL) is most frequent among adolescent males. The cells are medium sized with irregular nuclei and basophilic sparse cytoplasm often with vacuoles. Macrophages with apoptotic bodies are frequent. Most cells are CD3 and CD7 positive, while other T-cell markers are variably expressed. TdT is positive in all cases and CD10 may be expressed. Several translocations have been described, the most common involving the T-cell receptor loci.

16.10.3.2 Hodgkin Lymphomas

Hodgkin lymphoma (HL) accounts for approximately 1 % of all malignancies in the Western world. It shows a bimodal age incidence curve with the first peak between 15 and 30 years of age, followed by a second peak in elderly people. Most patients present with localized lymphadenopathy which affects cervical or mediastinal nodes in approximately 70 % of cases. Systemic symptoms such as weight loss, fever, itching, and night sweats are relatively frequent.

The morphological diagnosis of HL rests on the identification of mononuclear Hodgkin cells and giant cells with lobated nuclei, the so-called Reed-Sternberg cells (Fig. 16.32). These two cell types can occur in different background settings, which form the basis for subtyping. The following subtypes are included in the WHO classification: classical HL (nodular sclerosis, mixed cellularity, lymphocyte depletion, and lymphocyte rich) and nodular lymphocyte predominant HL. These subgroups can in most cases be identified in smears of aspirates by evaluation of the proportion of large atypical cells and reactive cells. The subtyping of Hodgkin lymphoma has clinical relevance with respect to prognosis. The nodular variant of the lymphocyte predominant Hodgkin lymphoma has an excellent prognosis sometimes even when untreated. Of the classical Hodgkin lymphoma subtypes, nodular sclerosis has been reported to have the best and lymphocyte depletion the worst prognosis.

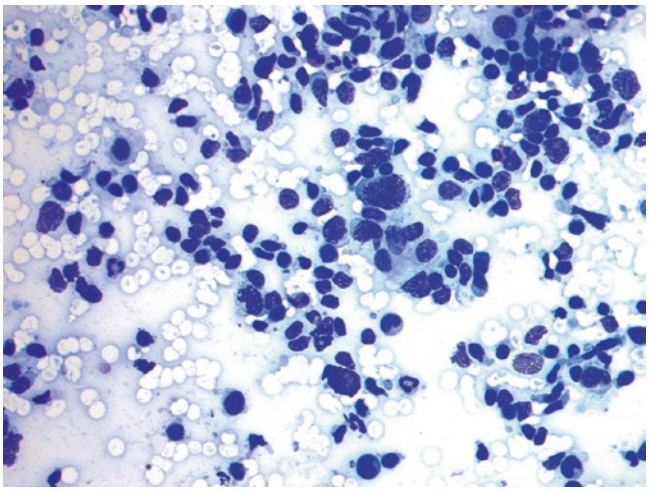

Fig. 16.32 Hodgkin lymphoma on smears. Note Reed-Sternberg cells mixed with a polymorphic background of lymphocytes

The main cytological findings of HL are the presence of Hodgkin cells that are large mononuclear cells with a prominent nucleolus and abundant cytoplasm and the presence of Reed-Sternberg (RS) cells that has a bilobed or multilobated nucleus with distinct nucleoli and an abundant pale gray cytoplasm at Giemsa stain. In nodular sclerosis variant, the smears are often poorly cellular and contain stroma cells and eosinophils in addition to the diagnostic Hodgkin and RS cells. Mixed cellularity variant has a mixed of cell population with lymphocytes, eosinophils, histiocytes, and plasma cells along with the diagnostic cells. The lymphocyte-depleted subtype shows a predominance of large atypical cells with rare lymphocytes. The nodular lymphocyte predominant form can be identified by the large number of lymphocytes with few diagnostic cells. True RS are rarely seen, but we can observe tumor cells with high multilobulated nuclei (popcorn cell). The rare suppurative variant of Hodgkin disease may pose diagnostic problems on FNA smears due to the paucity of tumor cells in a heavy background of granulocytes and cell debris.

In classical Hodgkin lymphoma both Hodgkin and RS cells are CD30 (Ki-1) and often CD 15 (Leu-M1) positive. Both cells are negative for CD45 as well as to pan-T and pan-B markers. It can sometimes be difficult to demonstrate the antigenic profile of the large atypical cells in cytospin preparations, because the Hodgkin and RS cells are fragile and are often present in low numbers. HL can be also a cause of false-negative diagnosis when analyzed by FCM without appropriate morphological workup, because the cell population at background is polyclonal. In the nodular lymphocyte predominant HL, the tumor cells are positive for CD20, CD79a, CD45, and BCL6. EMA can be detected in 50 % of cases, while CD30 and CD15 are not expressed.

16.10.4 Metastasis in Cervical Lymph Nodes

Lymph nodes enlarged by metastatic tumor spread often show diffuse involvement; therefore, an FNA from an involved node will almost invariably result in diagnostic cells. Such "metastatic" cells are in most instances readily identified in a background of lymphoid cells. The diagnostic accuracy of FNA cytology in detecting lymph node metastasis is high, and figures above 90 % are usually quoted [179].

In previously healthy patients, the cytological identification of a lymph node metastasis results in a search for the primary tumor. This investigation will be focused on various organs depending on factors such as age, sex, clinical history, site of metastatic node, and the cytological features. The most frequent metastatic sites for lymph nodes in the neck are the oral cavity, pharynx, larynx, salivary glands, thyroid, lung, and breast. If we considered lymph nodes in the supraclavicular fossa, the breast, lung, gastrointestinal tract, ovary, and prostate should be also considered as likely sites of the primary tumor. The search for a primary tumor can be facilitated by immunological characterization of the aspirated cells. In metastases from epithelial tumors, the CK7 and CK20 profile can often be helpful to focus on possible sites of the primary tumor. In head and neck metastasis, the profile CK7+/CK20− suggests the lung, thyroid, or breast as primary sites, while CK7−/CK20+ suggests the gastrointestinal tract. Using some additional panel of antibodies (see along the text), it is often possible to obtain correct information about the primary site. Unfortunately, some metastases challenge all diagnostic efforts and their origin remains obscure. The cytological presentation of different tumor is relatively independent of metastatic site, so here we describe the different metastasis focusing on the identification of the of tumor cell type.

Squamous cell carcinomas (SCC) account for the vast majority of non-thyroid head and neck cancers, being one of the most frequent cancers that appear as a metastasis in a cervical lymph node. The classical cytological appearance of SCC is readily recognizable: keratinized cells, with hyperchromatic nuclei and sometimes showing squamous pearl formation (Fig. 16.33). Although the incidence of most SCC of the head and neck (HNSCC) is decreasing, SCC from the oropharynx is becoming more frequently diagnosed, especially in young patients, and most of them are HPV related. This is relevant because many of these tumors are clinically occult and the first manifestation is an enlargement of a neck lymph node. Knowing the HPV status of the tumor can direct the clinical evaluation to specifically focus on the oropharynx where the primary tumor may be small and occult. The possibility of an HPV-associated tumor should be considered in any neck FNA-diagnosed SCC but is particularly impor-

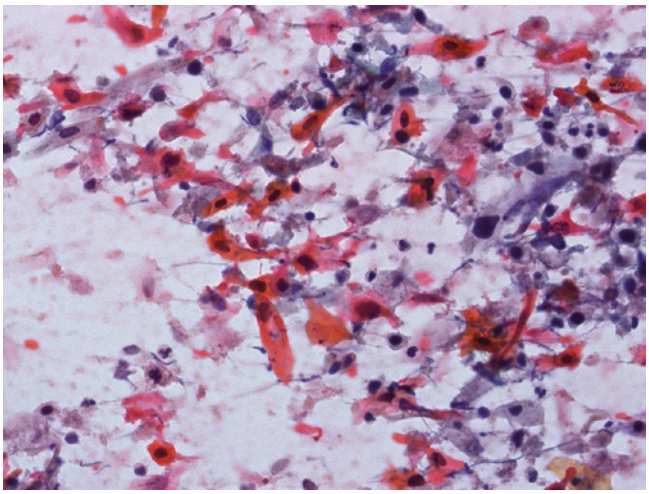

Fig. 16.33 Metastatic squamous cell carcinoma to cervical lymph node. Necrotic background and highly atypical keratinized cells with abnormal shapes

tant in patients with no known primary. There are three cytological patterns of HNSCC that should prompt consideration of an HPV-associated tumor [11]:

1. Basaloid pattern: cellular smears with cohesive sheets and individual cells with high nuclear: cytoplasm ratios and hyperchromatic nuclei usually without prominent nucleoli. Mitoses and single cell necrosis are frequent, making the aspirate easy to recognize as a high-grade tumor. Metastatic small cell carcinoma and high-grade lymphomas are the main differential diagnosis. Focal keratinization can be present, and markers of squamous differentiation (p63 and CK5) can be helpful for the diagnosis;
2. Cystic pattern: in these cases a yellow turbid thick material is aspirated from metastatic nodes. The smears consist largely of inflammatory cells and debris, and malignant cells may be sparse, requiring careful search preferably in Papanicolaou-stained smears. If such material is aspirated from a neck tumor the possibility of a branchial cleft cyst should be considered. In an inflamed branchial cyst, the epithelium can show some degree of atypia and thus mimic squamous cell carcinoma. In cases with slightly atypical squamous cells a repeat FNA from the periphery of the lesion may yield diagnostic cells. The clinical impression of a branchial cleft cyst should be viewed with caution with increasing patient age as well as with a smoking history;
3. Undifferentiated pattern: the smears are cellular having either clusters or single tumor cells in close association with lymphocytes. The tumor cells have indistinct cell borders, often growing in a syncytial manner. Nuclei are large, often with pale vesicular chromatin and single prominent nucleoli. This pattern is identical to the "lymphoepithelial" carcinoma pattern that one sees with non-keratinizing undifferentiated nasopharyngeal carcinomas. These tumors are essentially associated with Epstein-Barr virus (EBV) infection and are endemic in East Asia. In non-endemic areas, tumors with this appearance are now more likely to represent metastasis from an HPV-associated oropharyngeal tumor than an EBV-associated nasopharyngeal primary. Special stains for EBV and HPV are needed to make the distinction and lead the clinician to the likely primary site.

All SCC of unknown primary metastatic to neck lymph node should be tested for high-risk HPV. However, it remains unclear what methodology is best for testing for HPV. P16 immunostaining is easy to perform and is positive in essentially all HPV-associated tumors but lack specificity since 20 % of non-HPV-related HNSCC can be positive for P16. In situ hybridization for high-risk HPV is high specific but is less sensitive. Polymerase chain reaction test and methods such as Hybrid Capture II are also acceptable alternatives with good sensitivity. All can be performed on LBC or cell blocks.

Diagnosis of metastatic **adenocarcinoma** by FNAC does not present significant problems, but their site of origin may be difficult to determine. Papillary thyroid carcinomas (PTC) are a frequent primary site of metastatic neck lymph nodes. The smears show the same findings of PTC described on the thyroid section. PTCs may present as an occult primary with lymph node involvement. Metastatic deposits of this tumor are frequently cystic with evidence of previous hemorrhage (hemosiderin-laden macrophages). The aspirated fluid may be of low cellularity and the epithelial component elusive, prompting an erroneous diagnosis of a cyst. It is advisable to suggest careful assessment of the patient, including thyroid US, in patients with unexplained hemorrhagic cystic lesion on the neck. Additional features can be helpful to identify the primary site of the metastatic adenocarcinoma in a lymph node. Mucin production is often seen in gastrointestinal and lung carcinomas. In metastases from lobular breast carcinomas, some cells may have cytoplasmic lumina with pinkish purple inclusions and magenta bodies on Giemsa staining. Cells with pale gray vacuolated large cytoplasm and a nucleus with a central nucleolus are suggestive of a renal cell carcinoma. Smears from FNA of metastatic colon carcinoma usually show fragments of palisading atypical cells in a necrotic background. In most cases this presentation, as well as CDX2 positivity, is enough to allow a conclusive diagnosis on cytology alone. Smears of aspirates from poorly differentiated adenocarcinomas can be impossible to differenti-

ate from other poorly differentiated tumors, and subtyping can only be made after immunocytochemistry. Evaluation of CK7 and CK20 expression can substantially reduce the number of possible primary sites. Positive staining for prostate-specific antigen (PSA), thyroglobulin, or calcitonin will conclusively identify the primary site in appropriate cases. The presence of ER and PR strongly favors metastatic breast carcinoma. Expression of CDX-2 and villin favors gastrointestinal tract and TTF-1 lung cancer or thyroid.

Metastases from **small cell carcinoma** of the lung yield crowded clusters of tumor cells showing molding, with scanty cytoplasm, coarse chromatin, frequent mitoses, and a background of necrosis (Fig. 16.34). They may resemble lymphoma cells, but the presence of molding in cohesive clumps of tumor cells and positivity for cytokeratin is strong evidence against a diagnosis of lymphoma. Paranuclear dots are commonly seen in cytokeratin-stained smears from small cell carcinoma. Positivity for CD56, chromogranin, and synaptophysin is also reported.

FNAC from **metastatic melanoma** often have typical features, with polymorphic dissociated cells which may rarely contain fine pigment granules staining darkly on Giemsa staining. However, the cytoplasm often shows vacuoles only, and this is referred to as "negative pigmentation." The nuclei have large nucleoli which occasionally may be replaced by cytoplasmic invaginations into the nucleus. It is not infrequently to find binucleated cells with each nucleus apart from the other ["divorced nuclei"] (Fig. 16.35). The cytology of metastatic melanoma can mimic either carcinoma or sarcoma, or even sometimes lymphoma. Even if the patient has a history of melanoma, it can be virtually impossible in some cases to arrive at a conclusive diagnosis based on cytomorphology alone. Positivity for vimentin, S-100, and HMB-45 will conclusively identify a metastatic melanoma. However, it is important to remember that positivity for HMB-45 is absent in approximately 20 % of melanomas.

Sarcomas rarely spread to lymph nodes. In head and neck, knowledge about the clinical history will allow a correct identification of a metastasis from a previously diagnosed sarcoma. The possibility of a primary lymph node malignancy such as a histiocytic or dendritic cells sarcoma should be born in mind, since they mimic "true" sarcomas. The importance lies in the fact that they are potentially curable in early stages. Markers to epithelial, melanocytic, and lymphoid cells give negative staining reactions in sarcomatous metastases. Vimentin and markers for neural, vascular, and myogenic differentiation will confirm the diagnosis of metastatic sarcoma.

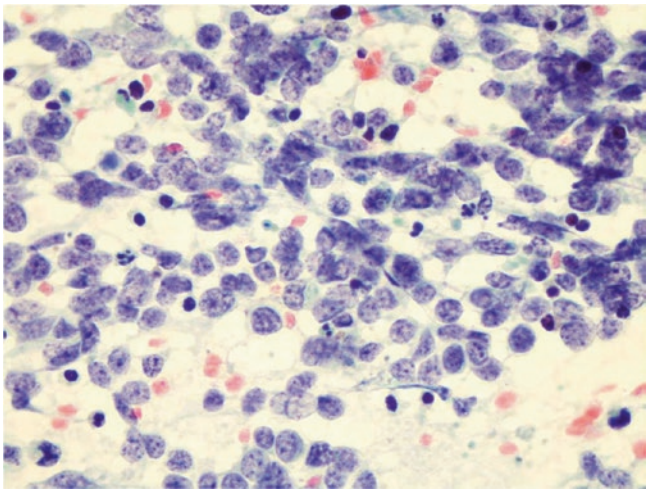

Fig. 16.34 Features of a metastatic small cell lung carcinoma in a neck lymph node on conventional cytology. Smear includes crowded clusters of tumor cells showing molding, with scanty cytoplasm, coarse chromatin, frequent mitoses, and a background of apoptotic cells

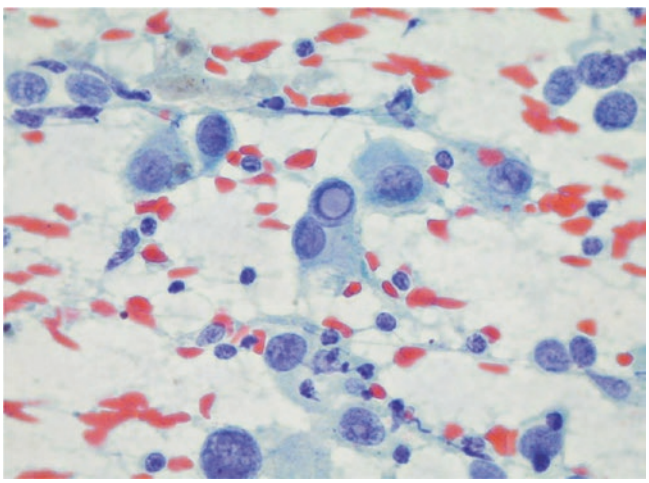

Fig. 16.35 Features of a metastatic melanoma in a neck lymph node on conventional cytology. Typical features, with polymorphic dissociated cells which may rarely contain fine pigment granules and typical nuclear pseudoinclusions

16.11 Cytology of Thyroid and Parathyroid

FNAC represents the most important and, generally, primary diagnostic tool for evaluating thyroid lesions. It has a worldwide application because of its simplicity, safety, and cost-effectiveness, leading to a correct diagnosis in more than 70 % of cases and to a correct clinical approach in significantly more than 90 % of cases [183, 184].

Numerous studies demonstrated that more than 70 % of all thyroid FNAC are benign, 5–10 % are reported as "malig-

nant," and the remaining 20 % represents the so-called "gray zone" in which different benign and malignant entities are included and resulted in a high number of unnecessary thyroidectomy with additional morbidity and higher health-care costs [183].

16.11.1 Nonneoplastic/Benign Thyroid Lesions

This chapter includes both inadequate smears and benign diseases. The final cytological specimen must be adequate in terms of amount of cellularity and satisfactory in terms of quality (thickness, fixation, and staining). The definition of unsatisfactory smears implies difficulties in fixation, smearing, or staining artifacts which might impair the interpretation of the final slide. A slide is nonrepresentative when cellularity does not represent the true components of the lesion (e.g., insufficient amount of follicular cells) [185]. In the first instance, the inadequacy of the sampling could be attributed to an incorrect technique, and in the latter, the characteristics of the lesion do not allow a definitive cytological diagnosis [186–189]. In both CS and LBC the adequacy criteria are met when at least six clusters of 10–20 well-preserved cells are observed [183, 187–189]. The further advantage of using LBC preparation is that a second slide can be prepared with the residual preservative cells in order to meet the adequacy criteria. In this perspective up to 18 % of cases diagnosed as inadequate with the first slide can be reclassified as adequate with the second slide. The LBC ThinPrep-processed slides may present scant ill-preserved cells at the periphery due to the positive pressure which may be responsible of cellular artifacts. On the other hand, SurePath, according to Geers and Bourgain, showed 25 % inadequacy rate on thyroid FNAC which can probably be an effect of the low sedimentation rate of the colloid droplets which might obscure their inclusion in the final slide [190].

In the category, we need to include also cystic lesions which are most commonly responsible for nonrepresentative cases [191, 192]. We need to distinguish a true pseudocystic lesion in absence of a wall of follicular cells or a more frequent cystic or hemorrhagic regression of a nodule which might turn out to be benign or malignant after repeated samplings. LBC slides show many hemosiderin-laden histiocytes with very few clusters of follicular cells and colloid droplets. In our studies cystic/hemorrhagic lesions accounted for about 7 % of all cases of our series which is in agreement with the general acceptance [193, 194].

A benign diagnosis is the most common cytological finding accounting for 65 % of all cases in the majority of series. The term benign thyroid lesion should be preferred to "negative for malignancy" and "nonneoplastic." Considering all the benign conditions, we need to subclassify them into (1) benign nodule of goiter, (2) thyroiditis, and (3) other less common entities.

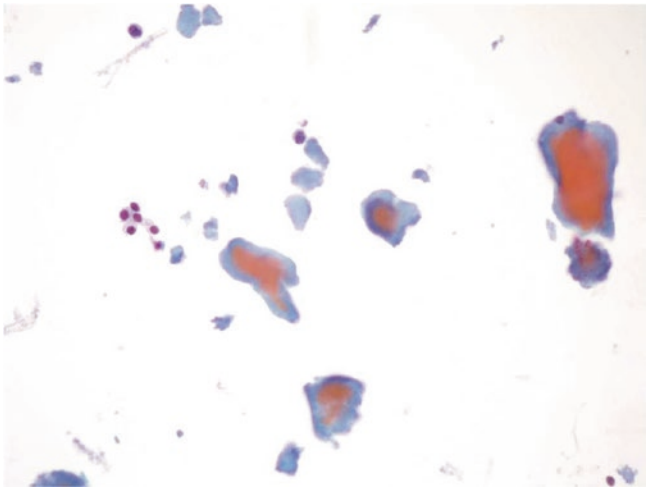

Fig. 16.36 Features of a thyroid goiter on liquid-based cytology. Small-sized thyrocytes and colloid goblets in the background

FNAC of goiter nodule is characterized by a wide range of morphological patterns reflecting different stages of the disease including also the detection of secondary changes such as oxyphilic metaplasia, hemorrhagic and/or cystic component, granulation tissue, fibrosis, and calcifications. The morphological feature is composed of variable amount of colloid, benign follicular cells, some oxyphilic cells, and variable amount of macrophages (Fig. 16.36).

The morphologic picture between LBC and conventional smears (CS) differs in two aspects: (a) the cells in each LBC slide are a monolayered sample of the entire material with a variable amount of cells which remains in the preservative solution and (b) the automated process causes some changes in both cellular and background morphologies (Table 16.1). One of the most important changes occurring in LBC slides is the appearance of the colloid substance which is fragmented during the filtering procedure. On LBC this substance is organized in small droplets in the background of a benign nodule with a quantitative detection, whereas in CS the colloid usually does not require any quantification [194]. Unlike CS, the LBC picture may be safely interpreted, in presence of even a small amount of typical follicular cells, as goiter with hemorrhagic changes.

The cytological findings of a **Graves' nodule** are not specifically similar to a benign goiter with the presence of lymphocytes and oncocytes in the background. The typical pattern is organized in flat sheet and loosely cohesive groups of cells with abundant foamy cytoplasm (Fig. 16.37). The pathognomonic feature is the presence of flame cells characterized by marginal cytoplasm vacuoles with red frayed edges [195]. These flame cells are not only encountered in

Table 16.1 Morphologic criterion for the classification of thyroid lesions on liquid-based and conventional cytology

Diagnosis	Conventional smear	Liquid-based cytology	Hist. corresp.
Benign lesions			
Colloid nodule	Abundant and clumped colloid, large sheets of small thyrocytes with "hyalinized stroma," foamy "colloidophagic" histiocytes	Clusters of small monomorphic thyrocytes with clear, sometimes "granular" cytoplasms; small clumps of dense colloid (colloid globules); foamy "colloidophagic" histiocytes	Diffuse or nodular goiter; macrofollicular adenomatous nodule in a goiter
Thyroiditis	Inflammatory cells (mostly mature lymphocytes) in the background; fragments of reticular tissue with epithelioid histiocytes; small clusters of thyrocytes sometimes with oxyphilic metaplasia; scant colloid; plurinucleated histiocytes (in DQT)	Small clusters of thyrocytes or oxyphilic cells "infiltrated" by small lymphocytes and granulocytes; the same cells are present in the background; small clumps of colloid; large plurinucleated histiocytes (in DQT)	Granulomatous "De Quervain's" thyroiditis (DQT). Lymphocytic "Hashimoto's- type" thyroiditis
Follicular proliferations			
FN (indeterminate, follicular neoplasm/nodule)	Scant colloid; microfollicles or small clusters of medium-sized thyrocytes, sometimes with slight nuclear pleomorphism (hyperfunction) and rounded nuclei; fibrovascular tissue; hemorrhage with hemosiderin-laden histiocytes	Small clusters of medium-sized thyrocytes with pleomorphic nuclei, generally with regular outlines; fibrin flakes; none or scanty colloid globules; hemosiderin-laden histiocytes	Adenomatous nodule; follicular adenoma; minimally invasive follicular carcinoma; foll. variant of papillary carcinoma
SC (suspicious for carcinoma)	Colloid absent; small clusters or microfollicles of medium- to large-sized thyrocytes with moderate nuclear pleomorphism, irregular nuclear membrane, clearing and grooves (no pseudoinclusions or papillae), often oxyphilic cytoplasms; hemorrhage; fibrovascular tissue	Small clusters of medium-sized thyrocytes (see FN) mixed with scattered aggregates of large cells with pleomorphic nuclei, clear chromatin and irregular nuclear outlines; no papillae or nuclear pseudoinclusions; fragments of fibrous tissue. If prominent nucleoli: hyperplastic nodule	Foll. variant of papillary carcinoma; "toxic" or hyperplastic adenoma; follicular carcinoma
OFN (oxyphilic follicular neoplasm)	Scant colloid; sheets or clusters of oxyphilic cells; hemorrhage; scattered inflammatory cells and reticular tissue in the background (if thyroiditis)	Small aggregates of oxyphilic cells with cytoplasmic granules and large hyperchromatic pleomorphic nuclei; fibrous tissue; scant colloid globules (if inflammatory cells admixed: thyroiditis)	Ox. adenomatous nodule; oxyph. neoplasm; oxyph. hyperplastic nodule in thyroiditis
Malignant neoplasms			
Papillary carcinoma	Colloid absent; large irregular sheets or papillae lined by large thyrocytes with severe nuclear pleomorphism and irregularities, with pseudoinclusions and grooves; hemorrhage; fibrovascular tissue	Aggregates of thyrocytes with large and elongated nuclei with grooves and focal pseudoinclusions; plurinucleated giant cells; fibrin filaments; iron-laden histiocytes; small and thin papillae	Papillary carcinoma
Medullary carcinoma	Small clusters of cells with round or ovoid slightly pleomorphic nuclei eccentrically disposed ["plasmacytoid cells"]; fragments of hyaline material; scant colloid; calcitonin positivity	Isolated cells with round or ovoid slightly pleomorphic nuclei eccentrically disposed ["plasmacytoid cells"] or cylinder shaped; calcitonin positivity	Medullary carcinoma
Anaplastic (undifferentiated) carcinoma	Hemorrhage and fragments of necrotic material. Small clusters of large cells with rounded or spindle cytoplasms, pleomorphic nuclei and prominent nucleoli	Necrotic material, isolated large cells with atypical nuclei, prominent nucleoli and round to spindle cytoplasms. Positivity for cytokeratins, often negativity for thyroglobulin and calcitonin	Anaplastic (undifferentiated) carcinoma

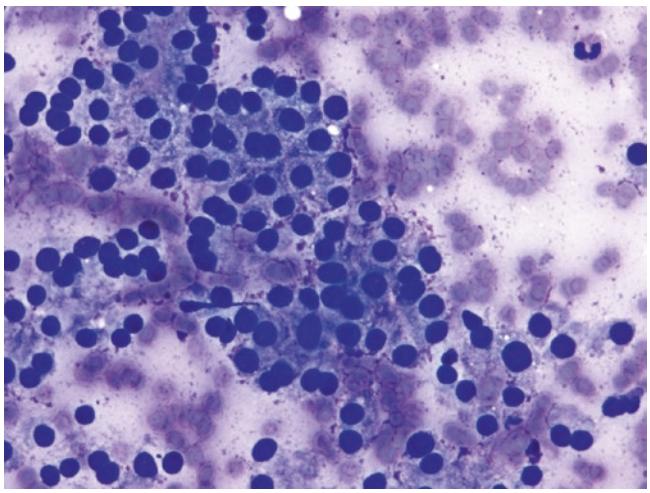

Fig. 16.37 Features of a hyperplastic thyroid nodule on conventional cytology. Note fire flame sign in the periphery of the cells

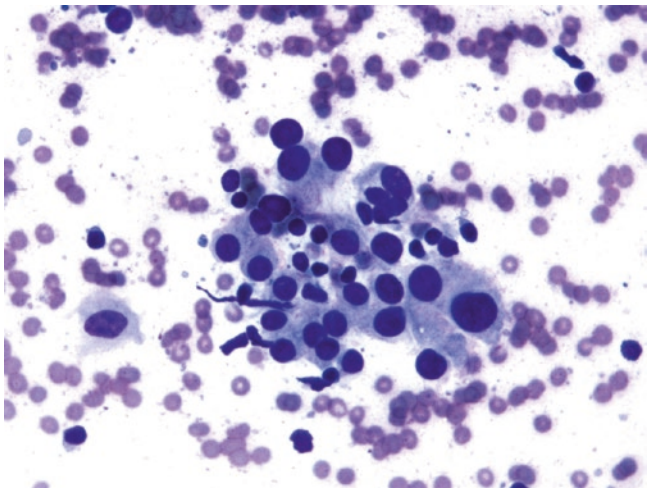

Fig. 16.38 Features of a Hashimoto thyroiditis on conventional cytology. Lymphocytes are intermingled with thyrocytes showing Hurthle metaplastic features

Graves' disease but also in other nonneoplastic conditions, follicular neoplasms, and papillary carcinoma.

The LBC picture of a **Hashimoto thyroiditis** is similar to CS with the exception of the amount of lymphocytes in the background which can be higher than normal because of the spinning of the material before the automated process. When a thyroiditis is suspected, the detection of lymphoepithelial clusters in an inflammatory background is the pivotal clue for the diagnosis [191] (Fig. 16.38). According to the literature, the LBC diagnosis of thyroiditis is reliable [191, 196]. Oxyphilic hyperplastic nodules in a thyroiditis should not be surgically removed because they represent the functional replacement of the parenchyma infiltrated by the inflammatory cells. Alternatively, the hyperplastic follicular cells may undergo changes in nuclear pleomorphism and clearing which can be misdiagnosed as suspicious for papillary carcinoma [196]. However, the occurrence of either oxyphilic carcinoma or non-Hodgkin primary lymphoma within a thyroiditis is exceedingly uncommon compared to the frequency of papillary carcinoma. Thus, the identification of lymphoepithelial clusters, mostly when made up of oxyphilic cells, virtually does rule out an oxyphilic neoplasm and warrants a simple follow-up for the patient.

Granulomatous thyroiditis (subacute, de Quervain's thyroiditis) is a chronic inflammatory condition which might be clinically diagnosed. The cytological pattern is composed of clusters of epithelioid histiocytes and granuloma with multinucleated giant cells [197].

Riedel's thyroiditis is the rarest inflammatory condition, and it is characterized by acellular smears, bland spindle cells, and few rare inflammatory cells [198].

16.11.2 Follicular Lesions

Numerous studies demonstrated that these follicular lesions (FL) represent 20% of all thyroid nodules, and they are defined as the so-called gray zone in which different benign and malignant entities are included [199]. This entity includes the histological diagnoses of nodular hyperplasia, follicular adenoma, and follicular carcinoma but also several cases of follicular variant of papillary thyroid carcinoma (FVPC) in which the absence of the undoubted nuclear features of PTC may fall short of a definitive malignant diagnosis. All these evidences resulted in a high number of unnecessary thyroidectomy with additional morbidity and higher health-care costs. This category is the center of discussion because it reflects the major morphological limit of cytology and its low diagnostic accuracy, which was characterized by the impossibility to evaluate any capsular and vascular invasion.

An articulate debate on this FL/follicular neoplasm (FN) category has emerged in a number of new classification systems for reporting thyroid cytopathology leading to a division in subcategories with different risks of malignancy [200, 201]. The Bethesda Reporting System subclassified it in three categories including (1) follicular neoplasms or suspicious for follicular neoplasm, (2) follicular lesions of undetermined significance and atypia of undetermined significance (AUS/FLUS), and (3) suspicious for malignancy. On the other hand, the majority of European classification maintained the distinction with the category of suspicious for malignancy [201, 202]. In agreement with our European classification, we will discuss this last entity as a distinct part of the chapter.

There are very few differences in the cytologic pictures of follicular neoplasms (FN) in LBC compared to CS. This diagnosis is based upon the identification of microfollicles made up of medium-sized thyrocytes in a background with scant colloid (Figs. 16.39 and 16.40).

The lesion is mostly follicular structured and is made up of medium-sized thyrocytes with rounded nuclei and central inconspicuous nucleolus [199]. The smears show moderate or high cellularity organized in microfollicles with some isolated cell in the background. Colloid is scant or absent, and some atypical cells might be seen. In this category there is an almost complete agreement in the different classification systems in terms of malignancy risk (between 20 and 30 %). A general consensus was evident in the therapeutic action of the surgical removal of the lesion after a collegial discussion. At histology, an FN may correspond to both a follicular adenoma and an adenomatous nodule in a goiter (70–80 % of cases), but a follicular carcinoma or a follicular variant of a papillary carcinoma cannot be ruled out only on morphology.

Another advantage of the LBC technique in a diagnosis of FN is the possibility to apply additional investigations (immunocytochemistry, flow cytometry, molecular biology) to the cells left in the vial, and these procedures are particularly helpful in redefining the malignant risk of the lesion (see below) [183, 184, 203]. Our recent experience involving an immunocytochemical panel made up of HBME-1 (Fig. 16.41) and galectin-3 pointed to an 81 % overall diagnostic accuracy in discriminating between low and high risk of malignancy in follicular proliferations, which increased to 92 % when a concordant positive panel was applied [183, 203].

The same diagnostic criteria and therapeutic action are applied to the FN composed mostly by **oxyphilic (or Hurthle) cells** [204–206]. Cytological smears of an oxyphilic lesion are categorized as "oxyphilic or Hurthle cell neoplasm" when more than 85 % of follicles are oxyphilic cells, and it might be included in the FN category. Oxyphilic cells are characterized by enlarged central or eccentrically round nuclei with abundant granular cytoplasm and different degrees of atypia which is a classical oxyphilic finding (Fig. 16.42). Oxyphilic cells may feature nuclear enlargement and pleomorphism either in benign neoplasms or even in hyperplastic lesions where a striking nuclear pleomorphism of the oxyphilic component may be found [204–206]. Several authors have attempted to correlate the atypia of the oxyphilic cells and some other features (such as transgress-

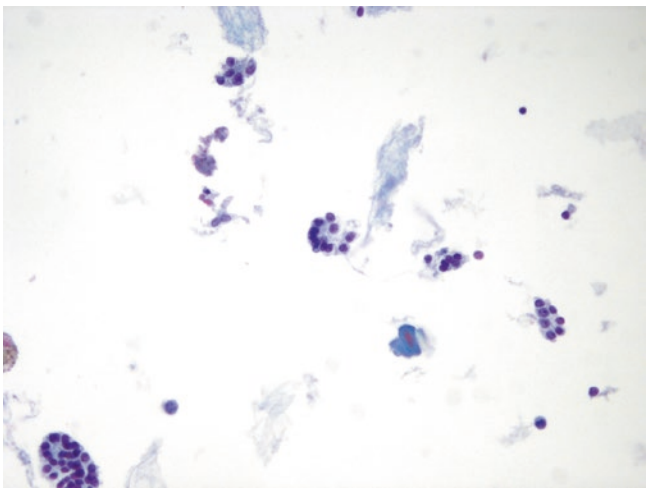

Fig. 16.39 Details of microfollicular structures from a thyroid follicular neoplasm on liquid-based cytology. Medium-sized cellularity characterized by regular thyrocytes organized as small clusters or follicles

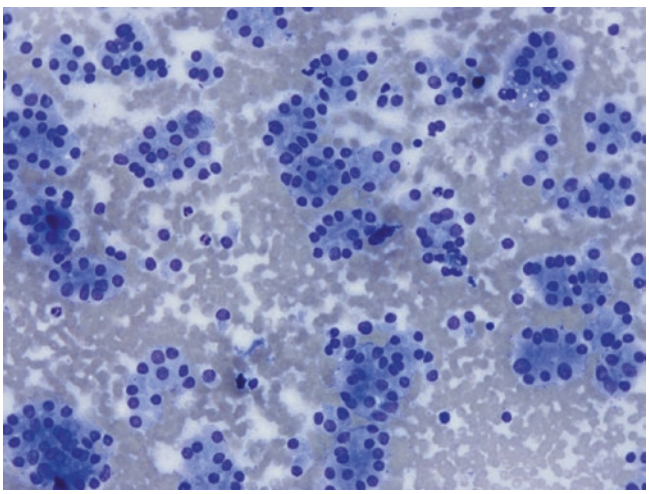

Fig. 16.40 Microfollicular pattern of a thyroid follicular neoplasm on conventional cytology. Medium-sized cellularity characterized by regular thyrocytes organized as small clusters or follicles

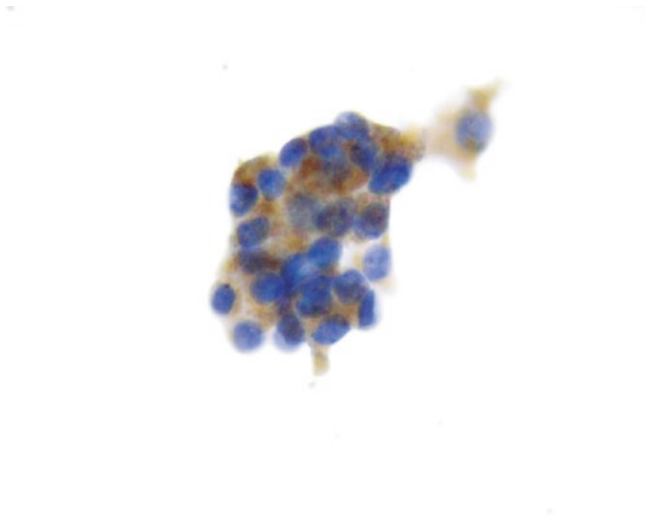

Fig. 16.41 Membranous/cytoplasm positivity for HBME-1 in a thyroid follicular neoplasm on liquid-based cytology

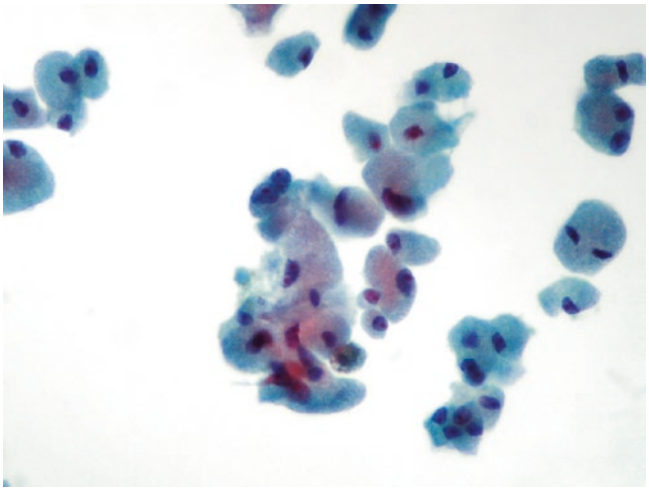

Fig. 16.42 Details of oxyphilic cells from a thyroid oxyphilic lesion on liquid-based cytology. Evidence of enlarged central or eccentrically round nuclei with abundant granular cytoplasm and different degrees of atypia which is a classical oxyphilic finding

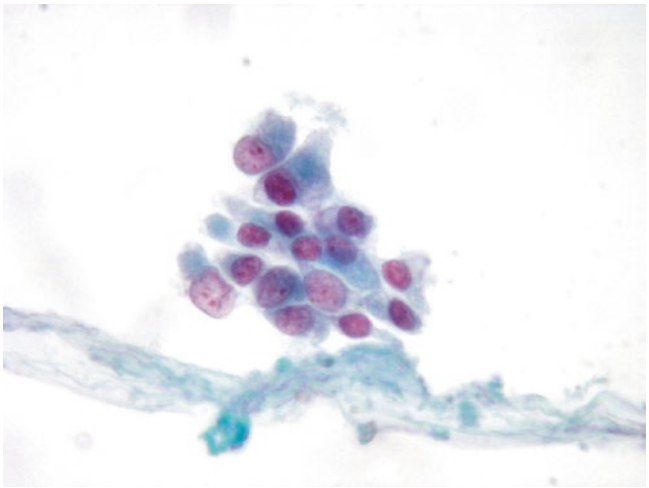

Fig. 16.43 Details from a case diagnosed as suspicious for malignancy on liquid-based cytology. Evidence of pleomorphic nuclear features without nuclear pseudoinclusion in thyrocytes

ing vessels) with the risk of malignancy, but their results are still debatable. Renshaw tried to underline some specific findings of a malignant Hurthle cell lesion. He described five criteria including scant colloid, small cell dysplasia, large cell dysplasia, crowding, and dyshesion even though all these features are not specific for malignancy [205]. Despite the general idea of a worrisome lesion, recent studies support the idea that the majority of these oxyphilic neoplasms are histological benign oxyphilic adenomas [204–206]. The colloid amount is scant, and hemosiderin-laden histiocytes may coexist. The association with both fire-flare cells (detected in hyperfunctioning lesions or in thyroiditis) and small thyrocytes (more than 20 % of the cellular component) suggests a benign lesion with an oxyphilic component.

16.11.3 Atypia of Undetermined Significance (AUS/FLUS)

The Bethesda classification has established a different category which is defined as "follicular lesion with undetermined significance (FLUS)" or "atypical cells of undetermined significance (ACUS or AUS)" [89, 199, 207]. The definition of this category was based on smears with cells showing architectural and/or nuclear atypia that are not sufficient to be classified as suspicious for follicular neoplasm, suspicious for malignancy, and malignant. This category might not be superior of 7 % of all thyroid diagnosis. The cytological smear is highly cellular, but the cells are monomorphous with occasional enlarged nuclei. The reasons for enclosing a case in this category are briefly three: (1) there are technical problems including poor preservation, drying, and artifact; (2) there are some atypical features, but the amount of material is scant for a diagnosis; and (3) atypia may be architectural or cytologic [199]. This lesion may correspond to the cellular adenomatous nodule of DeMay and is usually included in the nonneoplastic category of the European classifications (Thy 2 of the BTA classification, TIR 2 of the Italian classification). The difference between these two categories resides in the different risk of malignant occurrence which in the American classification is stated in the range between 5 and 15 % (mostly follicular carcinoma) whereas in the European systems is closer to nonneoplastic lesions. Recent papers underline some difficulties in a correct definition of this category and that AUS/FLUS might presents a malignant rate similar to that of the follicular neoplasm category.

16.11.4 Suspicious for Malignancy

This category is one of the three subgroups of FN for the Bethesda system while the Italian and British cytological classifications defines this group as the distinct category of suspicious for malignancy (SM) [199]. These cases had some features of malignancy which raise a strong suspicious for malignancy. In fact these cases included a risk of malignancy ranging between 50 and 70 %. Cytological smear is characterized by a highly cellular samples organized in follicular structures of thyrocytes with elongated and clear nuclei, sometimes with grooves and peripheral nucleoli without papillae, psammomatous bodies, or nuclear pseudoinclusions (Fig. 16.43).

This category warrants the surgical removal of the nodule as a follicular variant of a papillary carcinoma is very likely to be found at the histological examination (more than 90 % of cases). Even though SM morphology alone may be sufficient for the detection of atypia and nuclear pleomorphisms especially in expert cytological hands, several papers highlighted the pitfalls of the morphology alone which can lead to inappropriate treatment (lobectomy vs. total thyroidectomy or frozen section), additional morbidity, and higher health-care costs [208, 209].

A recent review article by Correia-Rodrigues and other papers has highlighted the increasing application of ancillary techniques to thyroid cytology focusing on immunocytochemical panels as a good choice to discriminate between low and high malignant risk lesions which result in an overall diagnostic accuracy spanning from 81 to 92 % with the concordant positive panel [8, 9, 208–210].

16.11.5 Thyroid Malignant Neoplasms

Thyroid malignancy represents 4–8 % of all FNAC, and the majority of them (90 %) are papillary thyroid carcinoma. The diagnostic accuracy of FNAC is at about 96–100 % [199]. This malignancy and its variants (follicular variant of PTC, tall cell PTC, macrofollicular PTC) show evidence of follicular differentiation with the typical distinctive nuclear features.

The cytological diagnosis of thyroid malignancy does not differ substantially in LBC preparations especially because the clear background facilitates the identification and characterization of the cellular details.

16.11.5.1 Papillary Carcinoma and Other Differentiated Variants

The most important and common malignant tumor which should be appropriately identified is **papillary thyroid carcinoma (PTC)**. This entity accounts for 80 % of all cancers. The LBC diagnosis of PTC is straightforward when the nuclear pseudoinclusions (major criterium) are detected even within tridimensional clusters of cells with nuclear elongation and clearing [186, 199]. Cytological smear shows abundant cellularity, enlarged crowded follicular cells with elongated and irregular nuclei, nuclear pseudoinclusions, grooves, and powdery chromatin. Papillary structures, multinucleated giant cells, typical chewing gum colloid, and psammoma bodies are seldom identified (Figs. 16.44 and 16.45).

In this group we can include also the **cystic variant of PTC** in which the cytological smear includes also histiocytes, watery fluid, and macrophages. The diagnosis is based on the evidence of convincing nuclear features of PTC [199].

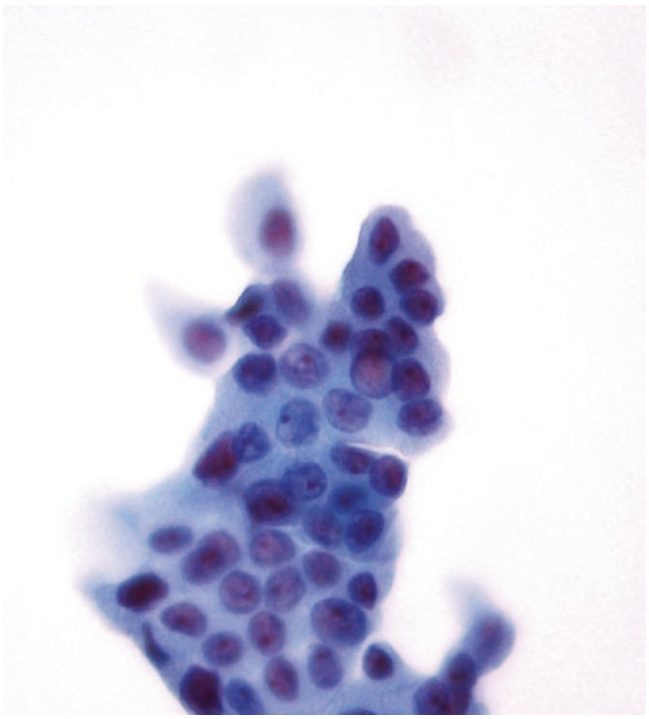

Fig. 16.44 Papillary thyroid carcinoma on liquid-based cytology showing characteristic nuclear pseudoinclusions

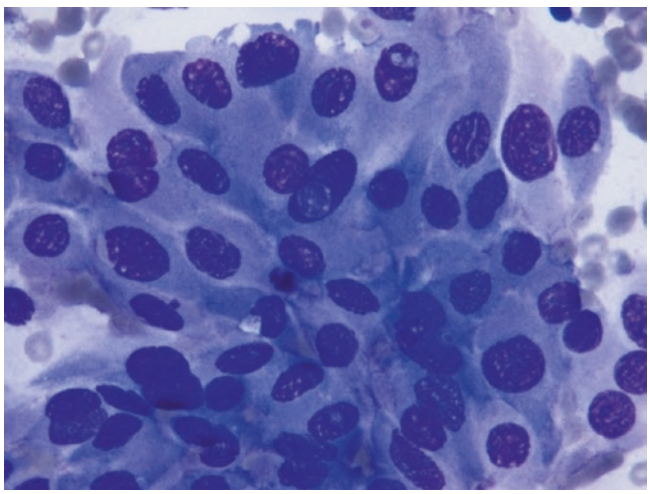

Fig. 16.45 Details of the nuclear features from a papillary thyroid carcinoma on conventional cytology including grooves, crisp, elongation and pseudoinclusions

Some concerns have been moved to LBC at the beginning of its application due to the difficulty in detecting the distinctive nuclear features of PC [193, 194, 211]. However, the most recent investigations have somewhat underlined the feasibility and reliability of LBC in the accurate evaluation of both major and minor diagnostic criteria of PC. On the other hand, on both conventional and LBC, major difficulties

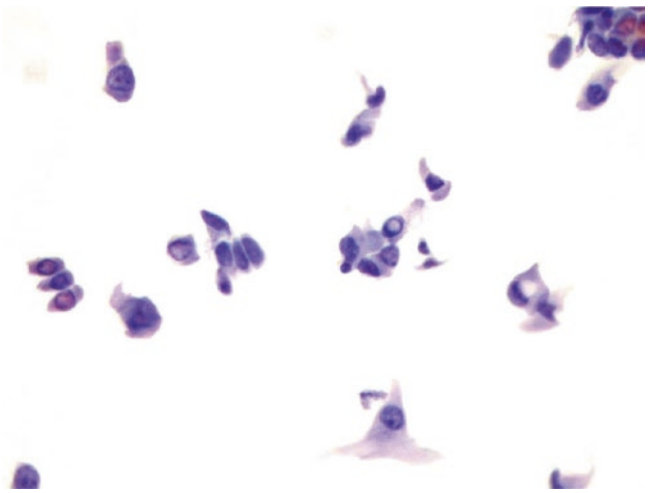

Fig. 16.46 Details of the nuclear features from a tall cell papillary thyroid carcinoma on liquid-based cytology. Evidence of typical and pathognomonic nuclear pseudoinclusion with a high-to-width cell ratio of 3:1

might be encountered in the diagnosis of a **follicular variant of PTC (FVPC)** because of the lack of the typical parameters. The major misleading parameter is the absence of papillary structures and the bland nuclear features which may fall short of a correct diagnosis. A typical smear of FVPC consists on malignant nuclear changes and crowding flat sheet with branching and nuclear elongation and grooves [193, 194, 211, 212]. All these parameters are subtler than on classical PTC smear.

Tall cell variant of PTC represents an aggressive variant of PTC. Cytological feature in adjunct to the classical parameters of PTC adds elongated cells with distinct cell borders, eosinophilic cytoplasm, and a high nuclear-cytoplasm ratio with a high-to-width ratio of 3:1 [199, 213] (Fig. 16.46). In some cases, the typical "soap bubble" appearance of the nucleus is encountered.

Macrofollicular variant of PTC is cytologically composed of monolayered sheet of cells with atypical cells showing the PTC. Also in this variant, as for FVPC, the pattern might show some colloid and subtle nuclear details which are difficult to interpret [214].

Diffuse sclerosing PTC is a common entity in young population. Cytological smear is composed of a lymphoid background with psammoma bodies and malignant cells characterized by metaplastic squamous features [215].

Oncocytic variant of PTC is characterized by the nuclear details of PTC in cells with typical oncocytic features. A typical smear shows oncocytic cells organized in papillae, sheet, or isolated cells with grooves, nuclear pseudoinclusions, nuclear hyperchromasia, and small nucleoli [216, 217]. Usually lymphocytes are absent or scant. On the contrary the evidence of a diffuse lymphoid component might suggest a **Warthin-like variant of PTC** [217].

Columnar cell variant is another aggressive variant of PTC. Cytological smear is composed of papillae, clusters, and flat sheet of oval neoplastic elements with elongated nuclei and subnuclear or supranuclear cytoplasm vacuoles. The absence of nuclear pseudoinclusions and scant nuclear groove represents the major difficulties in diagnosing this variant. The nuclei of this variant are darker than those of the classical PTC and might be mistaken as benign cells. Nevertheless, another interpretation might be of a metastatic colorectal neoplasm. For this reason, an immunocytochemical panel included thyroglobulin, TTF-1, CDX2, and cytokeratin 20 and might help in achieving the correct diagnosis [218].

16.11.5.2 Poorly Differentiated Carcinomas

This poorly differentiated category of malignant neoplasm was proposed by Carcangiu as an intermediate malignancy between differentiated follicular neoplasms and anaplastic carcinomas [219]. This category shows a variety of different degree of pleomorphic features. Cytological smears are composed of abundant cellular component with small epithelial cells organized in microfollicles, clusters, and nests. The cells show a high nucleo/cytoplasm ratio with scant cytoplasm. Nuclei present anysokaryosis, irregular borders, thick chromatin, and small nucleoli. In some cases, mitosis and necrosis are present [219–221].

This diagnosis is easily distinguished from a benign thyroid lesion, but some features might overlap with follicular neoplasms mainly because of the rarity in comparison with well-differentiated carcinomas and medullary carcinoma. The lesion should be differentiated from a medullary carcinoma, primary neuroendocrine carcinoma, and metastatic carcinoma. Immunocytochemistry might be useful because this poorly differentiated carcinomas are negative for calcitonin and CEA and positive for thyroglobulin and TTF-1 and are rarely positive for neuroendocrine markers such as chromogranin and synaptophysin [199].

16.11.5.3 Medullary Carcinomas

Medullary thyroid carcinoma (MTC) represents 7 % of all thyroid carcinomas and can appear as a sporadic (75 %) or familiar disease (25 %) with a dominant autosomal mode of inheritance. The cytological pattern of a medullary carcinoma can vary widely from one case to another due to the large variants of it such as papillary, glandular, giant cells, spindle cells, small cells, neuroblastoma-like, paraganglioma-

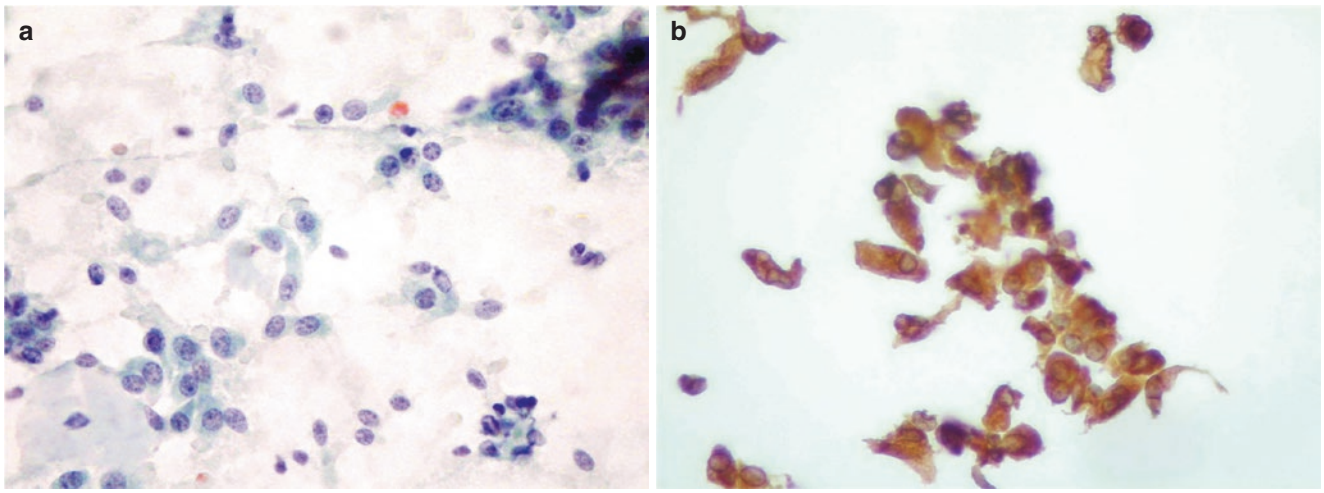

Fig. 16.47 (**a**, **b**) Details from a medullary thyroid carcinoma on conventional cytology. Typical biphasic population of plasmacytoid and spindle features with salt and pepper chromatin (**a**). Calcitonin-positive expression in a medullary thyroid carcinoma, on conventional cytology (**b**)

like, oncocytic, clear cell, squamous cells, and melanin producing [199, 222–224].

For these aforementioned patterns, the aftermath is a difficult cytological diagnosis which relies on the identification of a population of both plasmacytoid and spindle cells with variable nuclear pleomorphism. Cells are organized in syncytial-like structures with some bizarre giant cells. Nuclei are usually round and eccentric located with a typical coarsely (salt and pepper) chromatin (Fig. 16.47a). Very frequently, some binucleate and multinucleate cells are present. In some cases the granular cytoplasm shows small red granules which are easily detected with MGG stain. The typical amyloid showing is dense; amorphous feature is present. Although the diagnosis of medullary carcinoma can be suggested by morphology alone, it is highly advisable to be confirmed by ancillary studies. The immunocytochemical application, which might be easier with LBC method, offers the opportunity to identify the presence of calcitonin (Fig. 16.4b) and CEA in the neoplastic parafollicular cells and the concomitant negativity for thyroglobulin of the same neoplastic parafollicular cells. Positive for TTF1, synaptophysin, and chromogranin should be referred [222–224].

The most relevant differential diagnoses are with Hurthle cell tumor, hyalinizing trabecular adenoma, papillary carcinoma, anaplastic carcinoma, metastatic carcinoma, metastatic melanoma and plasmacytoma. The differential diagnosis with Hurthle cell tumor is based on the evidence in the Hurthle cell of a prominent macronucleoli and fine chromatin with blue cytoplasmic granules with MGG stain. In all the other cases, a combination of morphology and immunocytochemistry might help in leading to the correct diagnosis [224].

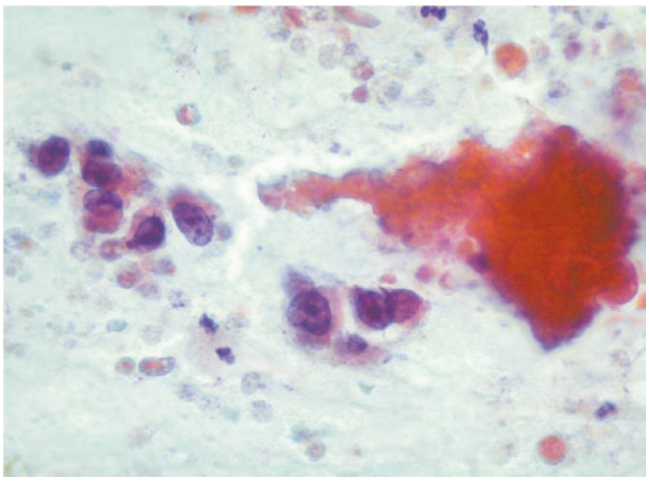

Fig. 16.48 Details from an anaplastic thyroid carcinoma on conventional cytology with poorly differentiated and bizarre nuclei. Presence of necrotic debris in the background

16.11.5.4 Anaplastic/Undifferentiated Carcinoma

Anaplastic thyroid carcinoma (ATC) is a rare and extremely aggressive thyroid carcinoma. It accounts for less than 5 % of all thyroid carcinoma with a very poor outcome, and most of the patients succumb in a ranging period between 6 months and 1 year. This entity is an uncommon finding in thyroid cytology as this tumor presents a rapid growth which frustrates the attempts to plan a surgical strategy [225, 226]. Cytological smears are composed of variable cellularity organized in isolated or groups of cells. The features of the neoplastic cells are epithelioid, with round, polygonal, or spindle shape, and their size can vary between small and giant and with three types of anaplastic cells (squamous,

fusiform, and giant) in a different percentage. The typical nuclei show enlargement, pleomorphisms, and prominent nucleoli. The LBC picture of ATC usually shows similar features with a background of necrotic debris and fibrovascular fragments and pleomorphic cells as described above (Fig. 16.48). Both typical and atypical mitoses and necrosis are present in the smear. Some of these anaplastic carcinomas might have a focus of coexisting well-differentiated carcinoma or poorly differentiated carcinoma which might be identified. The typical immunocytochemical patter expresses positivity for pan-keratin and vimentin while negativity for thyroglobulin and TTF-1.

The most important differential diagnoses are primary lesions such as fibrosarcoma, medullary carcinoma, Hurthle cell carcinoma, epidermoid carcinoma, and hemangioendothelioma but also with metastatic carcinoma from the lung, breast, and kidney and melanoma. The aggregation of the malignant cells and their large granular-vacuolated cytoplasm help in distinguishing this neoplasm from a large cell non-Hodgkin malignant lymphoma, which represents, from a clinical and morphologic viewpoint, the most important differential diagnosis. It is important to underline that the clinical history of a rapid growing mass and the morphological features of the cytological smear are pathognomonic for the correct diagnosis.

16.11.6 Other Neoplasms

This group may include both benign and malignant conditions. One of the most controversial is hyalinizing trabecular tumor (HHT) which is a rare follicular-derived neoplasm which shows trabecular pattern, intratrabecular hyalinization, and some nuclear features of PTC. For this reason, some authors consider it as a variant of PTC [199, 227, 228]. Cytological smear is composed of cohesive neoplastic cells usually organized around a stromal material. This material is an amyloid-like hyaline substance which is easily recognized by a green color on Papanicolaou smear and MGG. Intranuclear pseudoinclusions and grooves are commonly present with also paranuclear cytoplasmic bodies and occasional psammoma bodies in the background [227, 228]. Although the majority of these HHT have benign outcome, few cases of malignant HHT have been reported. A typical cytoplasmic positivity for MIB 1 is characteristic of this lesion and can help in the diagnosis.

Primary thyroid lymphomas are 5 % of all thyroid tumors and 2.5–7 % of all extrathyroid lymphoma. Both Hodgkin and non-Hodgkin lymphomas may affect the thyroid gland, whereas non-Hodgkin lymphoma outnumbered the first entity. Several papers report diffuse large cell lymphomas and extranodal marginal zone B-cell lymphoma or mixed

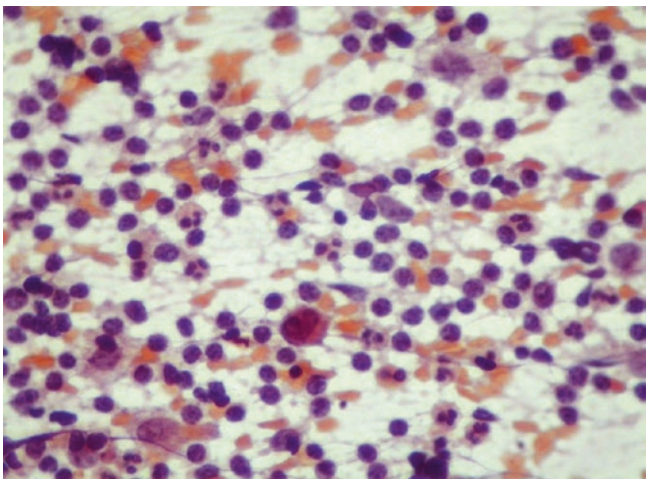

Fig. 16.49 Details from a primary thyroid low-grade non-Hodgkin lymphoma. Observe the heterogeneous lymphocytic population and absence of epithelial cells

patterns. Also other types have been reported including follicular and Burkitt lymphoma but with lower incidence. Cytological smear is cellular with prevalence of round-oval noncohesive lymphocytes (Fig. 16.49). The background may present numerous lymphoglandular bodies. The lymphoid cells and their size vary in agreement with the specific histotype. Nuclei show coarse chromatin with nucleoli and in some types also large basophilic cytoplasm (diffuse large B-cell lymphoma). In cases of low-grade lymphomas, lymphocytes are mixed with immunoblasts and plasma cells, whereas in high-grade lymphoma immunoblasts and centroblasts are the dominant features. The main difficult differential diagnosis is with Hashimoto thyroiditis, diffuse small cell carcinoma, and anaplastic carcinoma. The combining evaluation of morphology and application of ancillary techniques including immunocytochemistry and flow cytometry is useful [229].

Other primary rare entities include mucoepidermoid carcinoma which shows the identical features of the salivary gland histotype, synovial sarcoma, neural tumors (schwannoma and malignant peripheral nerve sheath tumor), and intrathyroid parathyroid adenoma, thymoma, and liposarcoma which are treated in the specific section of the primary lesions [174, 230–233].

Metastatic lesions to the thyroid gland account 5–7 % of all thyroid FNAC, but their incidence is significantly higher in autopsy series. Reporting data show that 71 % of thyroid nodules of patients with extrathyroid carcinomas are benign, and 17 % represent a metastatic location [199, 234, 235]. Lung, breast, kidney, large bowel, and larynx metastatic carcinoma to the thyroid gland may occasionally present as a single nodule mimicking a primary tumor. Cytological

smear is composed of a background with necrotic debris or hemorrhagic material in which clusters of neoplastic cells with features of adenocarcinoma or squamous cell carcinoma are detected. The correlation between the clinical history and the morphological features supported by an adequate immunocytochemical application (firstly for thyroglobulin and TTF-1 to rule out a thyroid origin) might lead to the correct diagnosis [234, 235].

16.11.7 Classifications System of Reporting

The need of a clarity of communication led to some classification systems in order to obtain a clear and clinically useful interpretation of the FNAC [199]. An articulate debate was delivered for the follicular neoplasm category as emerged in a number of new classification systems for reporting thyroid cytopathology which led to a division in subcategories with different risks of malignancy [185–189, 192, 200–202].

In this regard several classifications with different tiered schemes were discussed including the proposals of the Papanicolaou Society, the American Thyroid Association, the American Association of Clinical Endocrinologists, and the British Thyroid Association [199–202]. As discussed in the FN section, a conclusion was reached by the National Cancer Institute (NCI) State of the Science conference in which the Bethesda System of Reporting Thyroid Cytopathology proposed three subcategories of the follicular/indeterminate neoplasms including (1) follicular lesions of indeterminate significance or atypia of undetermined significance (AUS); (2) follicular neoplasms and Hurtle cell neoplasms (FN); and (3) suspicious for malignancy (SM). The stratification of cancer risk for each of these categories ranged from 5–15 %, 15–30 % to 60–75 %. However, as emerged by several papers, one of the limit and confusion of this subclassification of FN reflected the difficulties in reproducibility and practical management even among expert cytopathologists [188, 189, 192]. Recent studies underlined the overall accuracy of the Bethesda System for Reporting Thyroid Cytopathology which can be reckoned as a valid and reliable system [189].

Concerning the European scenario, Great Britain and Italy have a national reporting system. The British Thyroid Association/Royal College of Pathologists subclassified follicular neoplasms (FN) into the two subgroups of Thy3a-AUS of undetermined significance and Thy3f-follicular neoplasm with a good diagnostic agreement among British cytopathologists. In their system, any case categorized as Thy3a, Thy3f, Thy4, or Thy5 is reviewed by a thyroid team in order to make a correct management [202]. Although the current Italian classification included the single category of Follicular neoplasm group with 20 % cancer risk, a new classification with a subdivision of the follicular neoplasm category is going to be published [201]. As discussed in a recent paper by Cochand-Priollet, although the idea to extend the Bethesda unique project in Europe was supported by translations in the native language of the countries, some discrepancies and disagreement regarding the use of the Bethesda system occurred in the categories of AUS/FLUS and FN [236]. Furthermore, one of the problems, which need to be solved, with comparisons of data with different reporting system is how these results have been categorized.

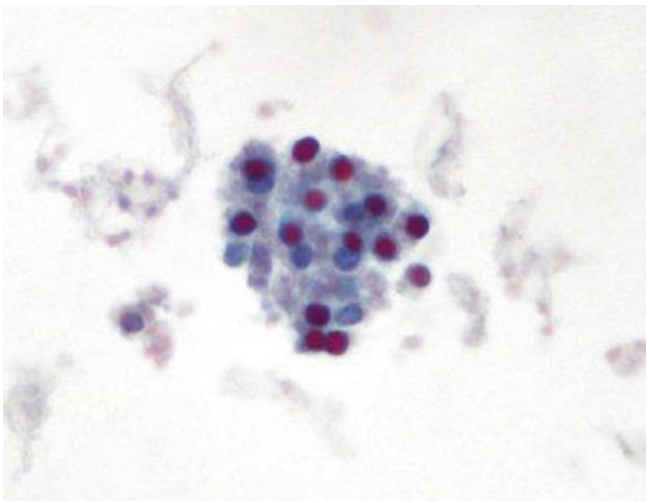

Fig. 16.50 Details from a parathyroid adenoma on liquid-based cytology with small groups of monomorphic cells with round nuclei

16.11.8 Parathyroid Lesions

These parathyroid lesions are rarer than those which occur in the thyroid gland. Some of them might be palpable, and they are usually found as parathyroid lesion on ultrasound guidance. The cystic lesions have been discussed in the section of cysts. In this part we will analyze the cytological features of benign and malignant neoplasms [237–240].

Parathyroid adenomas are usually solid lesion not always associated with functioning symptoms of hyperparathyroidism. Cytological smears are composed of frequent isolated or less commonly small groups of monomorphic cells (Fig. 16.50) with round nuclei and salt and pepper chromatin [232, 237, 238]. The detection of naked nuclei is frequently encountered. These parathyroid cells show a vacuolated oxyphilic cytoplasm. Anisokaryosis is often present. The absence of colloid may be a good sign of a parathyroid origin of the lesion although the support of an immunocytochemical panel with positivity for parathormone, chromogranin, synaptophysin, and negativity for thyroglobulin and TTF1 may lead to a conclusive diagnosis. If this immunopanel is not possible to

apply, the diagnosis might be only suspected and added as a descriptive note in the conclusive cytological report [199].

Parathyroid carcinoma is rare entity which may be clinically suspected if the patient has high level of serum calcium and parathormone and a palpable lesion. Cytological smear is highly cellular with round-oval cells with moderate pleomorphic features and granular chromatin. Colloid and macrophages are absent. Some naked nuclei might be present. In some case, mainly high-grade lesions, mitoses, and necrosis might be present. Cytological features are usually bland, and for this reason, even though a parathyroid malignancy might be suspected, the correct diagnosis is to be referred to the histological detection of the capsular and vascular invasions [239, 240].

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Gross Examination, Dissection, Evaluation, Reporting and Staging of Head and Neck Specimens

17

Tim Helliwell, Julia A. Woolgar, Asterios Triantafyllou, and Pieter J. Slootweg

Contents

T. Helliwell, MD, MB BChir, FRCPath (✉) • J.A. Woolgar, FRCPath, PhD, FDS RCS Eng, BDS • A. Triantafyllou, FRCPath, FDS RCS Eng, BDS
Department of Cellular Pathology, Liverpool Clinical Laboratories, Duncan Building, Daulby Street, Liverpool, Merseyside L69 3GA, UK
e-mail: trh@liv.ac.uk; Julia.anne.woolgar@gmail.com; triantaf@liv.ac.uk

P.J. Slootweg, MD, DMD, PhD
Department of Pathology, Radboud University Nijmegen, Nijmegen, The Netherlands
e-mail: Piet.Slootweg@radboudumc.nl

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17.1 Introduction

This chapter brings together many aspects of pathology practice that complement those required to achieve an accurate diagnosis and which are required to deliver a high quality service that guides the management of patients with diseases of the head and neck region. Although largely based on datasets for the histopathological reporting of head and neck cancers, we also include sections on benign and non-neoplastic diseases and the general context in which head and neck specimens should be handled and reported.

A diagnostic laboratory handling head and neck specimens should have sufficient pathologists and scientific staff with relevant experience in traditional histopathological techniques to provide high quality sections in a timely way for patient management. Increasing, laboratories will provide, or have access to, molecular techniques (immunocytochemistry, in situ hybridisation, PCR-based methods and gene sequencing) to allow more precise disease classification and to guide targeted therapies. In many countries, laboratories will be accredited to international standards (e.g. ISO 15189:2012) that should reassure patients and commissioners that work is performed to a good standard with robust working practices based on continuous quality improvement.

Guidelines are systematically developed statements which assist pathologists when making decisions affecting the delivery of appropriate healthcare for specific clinical circumstances and should be based on the best available evidence at the time the document was prepared. Nevertheless, it may be necessary or even desirable to depart from the guidelines in the interests of specific patients and special circumstances. The clinical risk of departing from the guidelines should be assessed by the relevant multidisciplinary team of professionals involved in a patient's care.

The development of national reporting guidelines and datasets reflects the need for a consistent approach to avoid the proliferation of numerous and varied local guidelines. This is increasingly been developed internationally through the International Collaboration for Cancer Reporting (ICCR). The International Collaboration for Cancer Reporting is a multinational agreement between the College of American Pathologists, the Royal College of Pathologists, the Australasian College of Pathologists and the European Society of Pathology, which is bringing together the various national guidelines into a single set of core data that should be incorporated into histopathology reports on cancers, through a process which can be adapted to respect cultural and geographical differences in the approaches to reporting cancers [http://www.rcpa.edu.au/Library/Practising-Pathology/ICCR]. Preliminary work on the ICCR datasets for head and neck cancers will start in 2016.

The rationale for using datasets for reporting cancer pathology (adapted from [1]) is that certain pathological features of cancers:

- Together with clinical and imaging data are related to clinical outcomes and provide patients with a prognosis
- Assist patients and their clinicians in deciding on the most appropriate treatment, including the extent of surgery and the use and choice of adjuvant radiotherapy or chemotherapy
- Allow the accurate and equitable comparison of surgeons in different surgical units and identify good surgical and pathological practice and the comparison of patients in clinical trials
- Facilitate international comparisons of outcome data
- Facilitate the monitoring changing patterns of disease, particularly by cancer registries

Reporting guidelines and datasets are of interest to pathologists, patients, surgeons and oncologists, politicians and

commissioners of services. They provide a consistent framework for communicating complex concepts relating to the nature of diseases, particularly cancers, so that pathologists can be satisfied that the important information required for patient management has been recorded accurately. Patients and their clinical teams can have confidence in the quality and content of the data and use this as the basis for informed decision making for patient management. Commissioners of services can receive reassurance that pathologists and their scientific colleagues have done a good job and provided the required, agreed output. Politicians, through their public health services, value the provision of robust data that measure the quality of the services in their country and which facilitate comparisons within and between countries. Without a common 'currency' of terminology, comparisons of the quality of care have little value.

For any guidance to be respected, it must be explicitly based on good evidence which is published in the peer-reviewed literature. Internationally accepted guidelines, such as those of the AGREE collaboration [www.agreecollaboration.org], provide a way of monitoring the quality of datasets and guidelines and the extent of involvement of a range of stakeholders. As the literature expands, guidance has to be regularly reviewed to ensure that appropriate consensus is reached on the value of particular data items and to ensure that advanced understanding of diseases is reflected appropriately in the guidelines. This is particularly important with the increasingly rapid introduction of therapeutic agents that are targeted at specific mutations in specific proteins/genes.

A relatively neglected area is the use of datasets to assess the quality of a diagnostic pathology service. Merely 'ticking all the boxes' on a proforma may indicate good practice in maintaining accurate records, but should not be sufficient to demonstrate the accuracy or high quality of clinical reports. Pathologists or a team of pathologists should seek to compare their patterns of diagnosis and evaluation of, for example, tumor size and grade, with colleagues in other centres to provide normative referencing of data quality and to generate research questions where apparently unexpected variations in data between centres occur.

17.2 General Aspects of Datasets for Cancer Reporting

Each dataset for the histopathological reporting of cancers contains *core* (*essential or required*) *data items* that are items that are supported by robust published evidence (large cohort studies or randomised trial data) and are required for cancer staging, optimal patient management and prognosis. The primary reasons for the inclusion of core data are the need for accurate classification and staging and the desire to predict those carcinomas that are likely to recur at local, regional (nodal) or distant sites so that appropriate surveillance, surgery, radiotherapy and/or chemotherapy can be delivered to mitigate the effects of recurrence. TNM staging (Table 17.1), in isolation, does not provide sufficient information for management and prognosis and additional factors need to be considered [2]. In some countries, core data items meet the requirements of professional standards and it is therefore recommended that at least 90 % of reports on cancer resections should record a full set of core data items. Clinical judgement is required in some situations to ensure that guidelines and datasets are optimised for individual patients but, in general, pathologists should aim to work within the guidance.

Other items may be described as *non-core* (*desirable or recommended*). These may be included to provide a comprehensive report or to meet local clinical or research requirements. All data items should be clearly defined to allow the unambiguous recording of data.

The R classifier (in TNM) for residual tumor is not recommended for use in the setting of mucosal head and neck cancers. The method of assessment of mucosal margins described in Sect. 17.8.9 is well established and current surgical practice, particularly the use of laser resection, does not require the assessment of macroscopic or microscopic residual disease. The R classifier is recommended for use in staging thyroid neoplasms (see Sect. 17.14).

The guidance for head and neck cancers arising at mucosal sites is mainly derived from data on squamous cell carcinomas but similar principles may be applied to the reporting of other mucosal malignancies arising in this anatomical area including adenocarcinomas, neoplasms of minor salivary glands and neuroendocrine epithelial neoplasms.

17.2.1 Multidisciplinary Working

The optimal reporting of specimens from the head and neck area requires a partnership between the pathologist and surgeon/oncologist. For some specimens, e.g. bone lesions and lesions related to teeth, imaging information is essential for accurate diagnosis. The regular discussion of cases at clinicopathological meetings (tumor boards, multidisciplinary team meetings) and correlation with preoperative imaging studies are important in maintaining and developing this partnership. The surgeon can help the pathologist to provide the information necessary for patient management by the appropriate handling and labelling of the specimen in the operating theatre.

Table 17.1 General principles of TNM classification of malignant tumors [2]

pT – primary tumor	
pTX	Primary tumor cannot be assessed
pT0	No evidence of primary tumor
pTis	Carcinoma in situ
pT1, pT2, pT3, pT4	Increasing size and/or local extent of the primary tumor (see specific sites)
If there is doubt as to which category a tumor should be allocated, the lower (less extensive) category should be used	
Additional descriptors may be used in special cases:	
'm' suffix indicates the presence of multiple primary tumors in a single site: pT(m)	
'y' prefix indicates those cases in which classification is performed during or following neoadjuvant therapy. The ypTNM categorises the extent of tumor present at the time of examination and is not an estimate of tumor before treatment	
'r' prefix indicates a recurrent tumor when staged after a documented disease-free interval: rTNM	
pN – lymph nodes	
pN0(i+)	Isolated tumor cells (ITC) are small groups of cells <0.2 mm diameter or <200 cells in one section. These are not included in the positive node count but are recorded when they are the sole evidence of potential metastasis
pN1(mi); pN2b(mi); pN2c(mi)	Micrometastasis defined as 2 mm or less in diameter. If only micrometastasis is present, the suffix (mi) is used after the appropriate pN-stage category
pN1, pN2, etc.	Macrometastasis – more than 2 mm in diameter
pM1 – distant metastasis	Only used when metastasis is confirmed microscopically (pM0 and pMx are no longer used)

The request form for laboratory investigation should include patient demographic data, the duration of symptoms, whether surgery is palliative or curative, details of previous histology or pathology reports and the core clinical data items (type of specimen, site and laterality, as appropriate). Clinical TNM stage is useful for correlation with pathological findings. A history of previous radiotherapy or chemotherapy may influence the interpretation of the histological changes and should prompt a comment on the extent of any response to treatment. The request form should provide the opportunity for surgeons to provide annotated diagrams of specimens, either as free-hand drawings or on standard diagrams.

17.2.2 Data Collection and Audit

Core pathological data are conveniently summarised as a proforma, which facilitates comprehensive data collection and provides surgeons and oncologists with data in a consistent format for easier retrieval. Proformas may be used as the main reporting format or may be combined with free text. Individual centres may expand the detail in some sections, e.g. for sites and subsites, or to facilitate the recording of data for particular tumor types.

Many pathology services will have agreements with their local clinicians and commissioners that define the standards of service required. These standards may be subject to regular review and audit. Laboratories might consider including into such agreements the following aspects related to datasets:

- Where biopsies or resections have been taken, the service might record the % of cases where the pathology reports are discussed at clinical meetings.
- Where pathology has been reviewed specifically for the multidisciplinary team (tumor board) meeting, this should be recorded, together with amendments to the report (if any).
- Where it is agreed that reports on resection specimens will be presented as electronic structured reports or locally agreed proformas, there may be regular review of:
  - The % of resection cases where reports are presented in a structured format
  - The % of structured reports on resection specimens that include all data items
- Turnaround times for biopsies and resection specimens:
  - The % of diagnostic biopsies reported within seven calendar days of the biopsy being taken
  - The % of all histopathology specimens (excluding those requiring decalcification) reported within ten calendar days of the specimen being taken
- Normative referencing between local services and nationally or internationally comparable services for the proportions of cases with particular features, for example:
  - Proportion of positive sentinel node biopsies
  - Predictive value of thyroid cytology
  - Size distribution of tumor maximum diameters. It should be expected that the distribution is smooth and continuous, with no rounding of measurements to nearest 0 or 5 mm.
  - Number of lymph nodes found in specific specimen types

17.3 Dissection Guidance for Biopsies and Non-cancer Resections

This section and the following section provide general guidance on the laboratory handling of common specimens received for the assessment of non-neoplastic and neoplastic diseases. While some procedures may be standardised to aid workflow, other procedures are dependent on the clinical context (informed by the request form), the initial morphological assessment or the likely diagnosis.

There is little literature on the management of samples for diagnosis of non-neoplastic disorders, but good overviews are provided [3, 4]. General guidance follows, accompanied by site-specific information in selected areas.

17.3.1 Mucosal Biopsies

Most of these specimens are small and should be measured in three dimensions, identifying any specific lesions, e.g. polyps, ulcers and nodules. Incisional biopsies of sufficient size may be bisected longitudinally, while the margins of excisional biopsies are usually best assessed in transverse sections. Unilateral nasal polyps are usually blocked in their entirety because unilateral lesions have a slightly higher risk of being neoplastic than bilateral lesions.

Resection specimens of tonsil or salivary tissue may be orientated by the surgeon. The orientation should be confirmed, if possible, and the specimen measured in three dimensions. Deep and peripheral excision margins may be inked before cutting into four to five transverse slices. Care must be taken to examine the capsule and record any areas where it is incomplete or ruptured. Describe the location of any abnormality, e.g. nodules or cysts. Dissection should be in planes appropriate to sample the closest excision margins. For tonsils where there is no macroscopic abnormality, two blocks are usually sufficient. In patients with nodal metastatic carcinoma of clinically unknown primary site, a primary tonsillar carcinoma may be very small and the whole of the ipsilateral tonsil should be embedded for histological examination.

Usually a single, haematoxylin- and eosin-stained section is sufficient for diagnostic purposes. Where dysplasia is suspected (clinically or on microscopy), at least three levels at 100 μm intervals should be examined. White lesions and dysplastic lesions may be stained using PAS with diastase digestion to help identify fungal hyphae and spores. Mycobacterial stains are required in granulomatous conditions. If Wegener's granulomatosis is suspected, an elastic van Gieson stain may be helpful in identifying damaged vessels and further clinical information on the presence of positive c-ANCA tests and ESR is useful.

Fresh samples submitted for suspected vesiculobullous disorders may assessed for IgG, IgA, IgM, C3 and fibrinogen deposition.

17.3.2 Jaw Lesions

These specimens will include a number of benign and non-neoplastic lesions such as ameloblastoma, 'mixed odontogenic tumors' and odontomes, as well as fibro-osseous lesions. The presentation of jaw specimens is variable and includes enucleated specimens composed of fragmented pieces of soft tissue or bone, as well as bone resections. If multiple fragments are included, the number of pieces, total dimensions and dimensions of the largest piece should be recorded. If the sample is small, it should all be processed; otherwise representative sections are usually sufficient. Small hard tissue fragments are common and decalcification overnight is often sufficient. Large fragments of bone and identifiable teeth or tooth fragments should be described, decalcified and blocked separately. The relationship between lesional tissue and a tooth, such as attachment to the cement-enamel junction or root apex, should be recorded. Large cysts require a description of the wall and the presence of mural thickening or nodules, which should be sampled to identify unicystic ameloblastomas.

For larger specimens, identification of the type of operation and orientation are required. Photographs may be labelled to indicate orientation and the origin of blocks. Radiographs are essential to assess the extent of the lesion, tooth resorption and the presence of calcification. Measurements of resection specimens should include the anteroposterior diameter along the alveolar ridge, the maximum bone height, i.e. ramus, the dimensions of a lesion and the distance from the resection margins.

Surgical margins, e.g. mucosal, deep, superior limit of ramus, etc., may be inked. Small specimens can be decalcified in their entirety before sampling or blocking out. For large resections, especially of the mandible, it is often helpful to take slices of 5–8 mm on a band saw. It may also be possible to slice maxillary specimens, although these are sometimes very fragile and decalcification of the entire specimen helps sampling and preserves orientation.

17.3.3 Teeth

Teeth may be received with odontogenic cysts or as part of a resection specimen and may require histological examination to determine the vitality of the pulp; this can inform the pathogenesis of a periapical lesion. Occasionally a clinical

diagnosis of a developmental tooth disorder requires histological confirmation.

The macroscopic description will normally include tooth notation, site, morphology, presence of caries or root resorption and filling material. The structure of the enamel and dentine is assessed for colour, transparency, banding, erosion, abrasion and relative hardness.

Teeth are usually decalcified before dissection and sectioning but a ground section (on one half of a bisected tooth) is required for the diagnosis of enamel defects. Morphology is often better preserved if, after slicing, the tissue is fixed for 1–2 days in formalin.

Unless the tissue is likely to fragment or otherwise be distorted, it is recommended that bone is trimmed to approximate block size before decalcification. This should allow decalcification to be completed in 1–10 days, although very dense bone and teeth will take longer. Detailed protocols for decalcification are described in standard textbooks [5], and pathologists should seek an appropriate balance between slower decalcification for optimal morphology and more rapid decalcification to facilitate patient management. Excessive decalcification affects morphology and strong acids (e.g. nitric acid) interfere with immunocytochemistry and degrade nucleic acids. In general, decalcification in 5 % formic acid is appropriate with the end point confirmed by palpation, ammonium hydroxide or radiography.

Stains for PAS, Alcian blue or mucicarmine may be useful in the diagnosis of glandular odontogenic cysts and a van Gieson stain is useful in identifying dentinoid, e.g. in ghost cell lesions and the mixed odontogenic tumors and odontomes.

17.3.4 Lymph Nodes and Soft Tissue Lesions of the Neck

Most neck specimens of benign disease are small excisional biopsies. Lymph nodes up to 4 mm diameter should be embedded intact, while nodes up to 10 mm diameter can be bisected longitudinally through the hilum and embedded in total. Nodes larger than 10 mm are sliced at 4–5 mm intervals and two or three representative slices embedded.

Thyroglossal cysts usually present as a strip of fibrous tissue surrounded by fat and muscle, often with a segment of central hyoid bone. After measuring in three dimensions, the soft tissue is all embedded as follicular thyroid tissue can be scanty. Histological examination of the hyoid rarely provides additional diagnostic information.

Branchial cysts are usually submitted intact. The description should include the thickness of the cyst wall and the presence of any nodules. Sufficient sampling to rule out the possibility of a cystic metastatic carcinoma is required; small specimens should be bisected or embedded intact, while larger specimens are sliced and two to four representative blocks taken.

17.3.5 Salivary Glands

Biopsies of minor salivary glands are small (less than 15 mm) and, after description and measurement in three dimensions, should be blocked in their entirety to demonstrate lesional tissue in relation to adjacent normal tissue and surgical margins. For surgically orientated excisions, deep and peripheral margins may be marked with ink to aid assessment of the adequacy of resection.

Submandibular and sublingual glands are usually removed for sialolithiasis, with benign lesions of the parotid usually requiring superficial parotidectomy. The description should include the overall dimensions of the specimen, a description of any calculus, nodule or cyst including its dimensions, the presence or absence of a capsule and the distance from the resection margins.

Blocks should include the lesion (one block per 10 mm diameter), sampling of surrounding tissues to determine the adequacy of excision, the ends of any identifiable nerves (if malignancy is suspected) and any intra-glandular or adjacent lymph nodes.

H&E stains may be supplemented by mucin stains, e.g. PAS, Alcian blue or mucicarmine, for the diagnosis of benign salivary gland tumors. Immunohistochemistry is sometimes useful for the diagnosis of salivary gland tumors and for the distinction between benign lymphoepithelial lesions and extranodal marginal zone (MALT) lymphoma. This can be supplemented by molecular analysis for light- and heavy-chain restriction.

17.4 Dissection Guidance for Cancer Resections

17.4.1 General Principles

For cancer cases, the essential/core items that are required for patient management and prognosis are predominantly a mixture of macroscopic and microscopic features. The quality of evidence that supports the recommendations for reporting cancer specimens is generally good (large cohort studies or randomised clinical trials) for the core/essential data items but varies for specific sites and histological types of neoplasm.

Resection specimens should be orientated by the surgeon and, if appropriate, pinned or sutured to cork or polystyrene blocks. The surgeon should indicate surgically critical mar-

gins using metal tags or sutures. Fixation is in a formaldehyde-based solution for 24–48 h (the volume of fixative should be ten times that of the tissue). Photography and radiography of the specimen may be used to record the nature of the disease and the sites from which tissue blocks are selected. Surgical margins should be painted with Indian ink or an appropriate dye to facilitate recording the proximity of the neoplasm to the margins.

Specimens should be cut with a large knife into 3–5 mm parallel slices to demonstrate the relationship of a neoplasm to mucosal resection margins and the maximum size and depth of invasion by the tumor. The selection of blocks of tissue for microscopic assessment should ensure that representative tissue is available for diagnosis, to record essential data for staging and to assess the adequacy of excision:

- Blocks of tumor: at least one block per 10 mm diameter of tumor, including one selected to demonstrate the maximum depth of invasion; the whole tumor should be processed if it is less than 10 mm in maximum diameter.
- Blocks of defined mucosal and soft tissue margins.
- Non-neoplastic mucosa (one block).
- Bone: one of more blocks to confirm involvement by tumor suspected clinically, on imaging or on macroscopic examination.
- Bone surgical margins – only required if bone is involved by tumor.

17.4.2 Site-Specific Considerations for Dissection and Block Selection

17.4.2.1 Oral Cavity, Tongue, Maxilla and Mandible

Specimens from the central and lateral parts of these structures should be cut in the coronal plane, while specimens from the anterior part of the oral cavity and jaw bones should be sliced in the sagittal plane. Illustrative examples are shown in Figs. 17.1 and 17.2, and more detailed guidance is available from detailed texts [3]. If the tumor is close to bone, the specimen should be decalcified with soft tissue in situ so as to avoid distorting the margins.

17.4.2.2 Nasopharynx

The great majority of nasopharyngeal carcinomas are treated non-surgically so that guidance relating to small biopsies is most appropriate for these tumors. Resection specimens of carcinomas from this area should be carefully orientated by the surgeon so that surgically important resection margins can be appropriately sampled.

17.4.2.3 Laryngopharyngectomy

Horizontal slices 3–5 mm thick provide optimal demonstration of the relationship between the tumor and the laryngeal cartilages, although thicker slices may be required if mega-blocks are used (Fig. 17.3). For supraglottic carcinomas, blocks should include the relationship between the carcinoma and the anterior (submucosal) resection margin at the

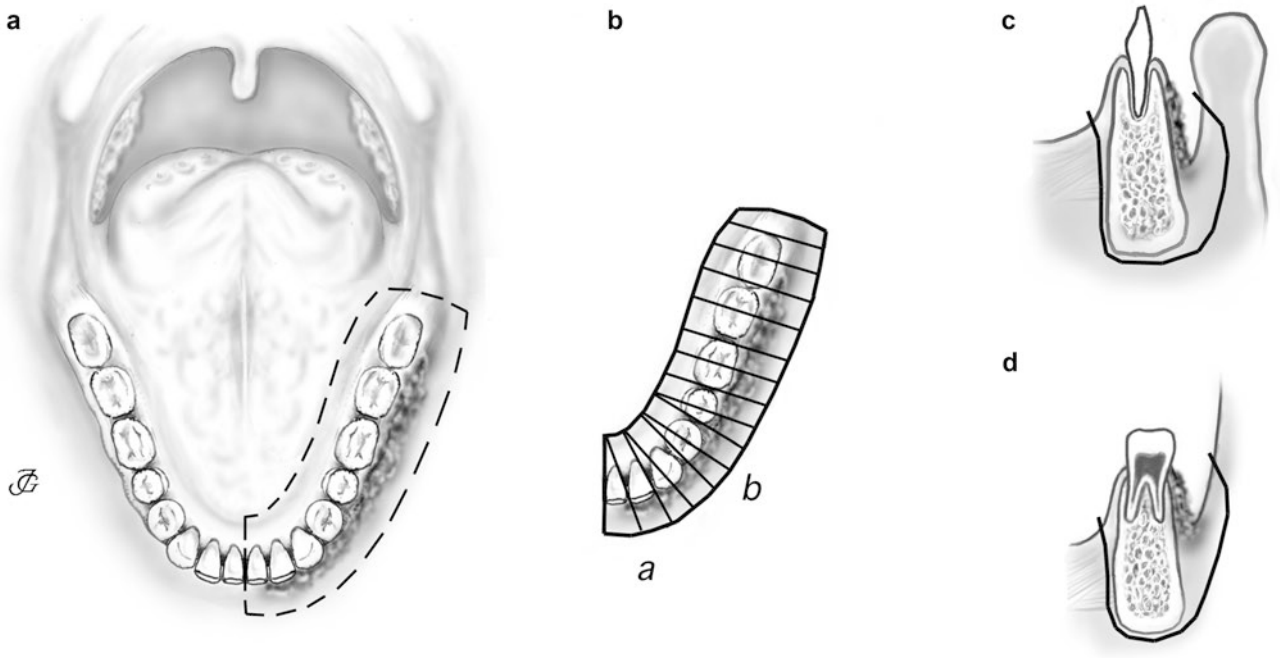

Fig. 17.1 Pathological anatomy and dissection of buccal gingival cancer in the mandible. (**a**) Outline of the resection; (**b**) surgical specimen with plane of sectioning; (**c**) cut surface of resection anteriorly: (**d**) cut surface of resection posteriorly (Figure 2.30 from Slootweg and de Groot [3], with permission)

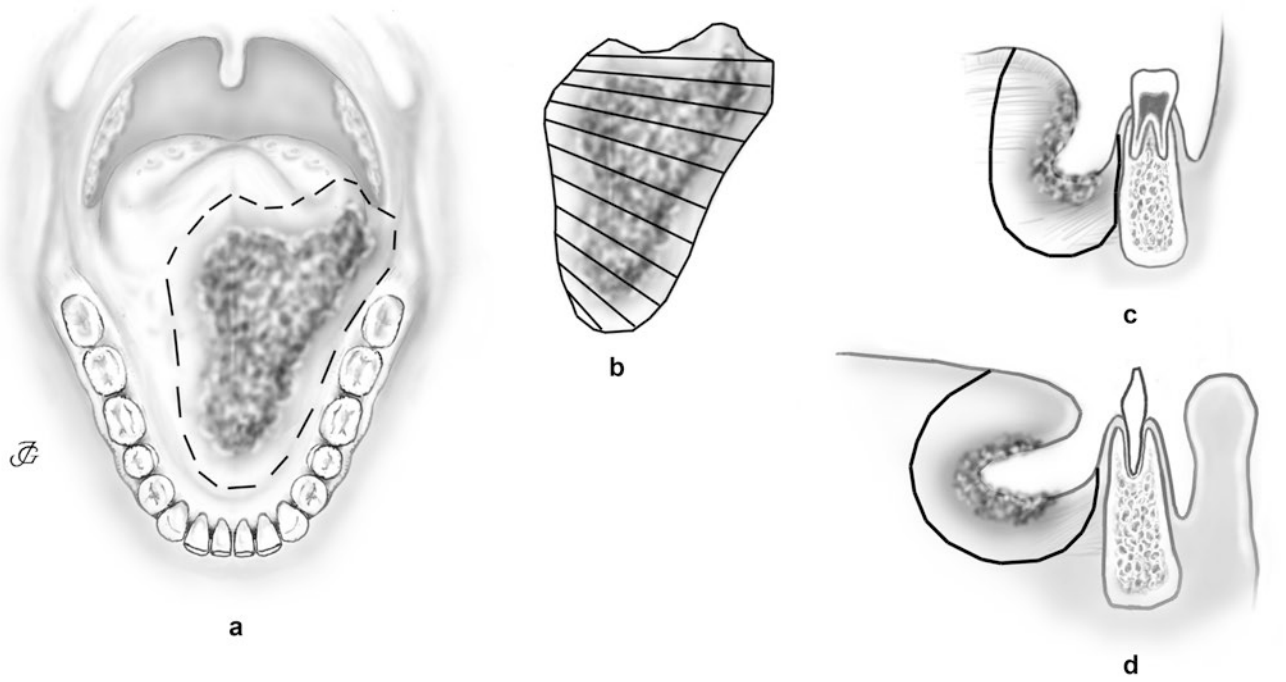

Fig. 17.2 Pathological anatomy of cancer involving the anterior and posterior floor of mouth. (**a**) Outline of the resection; (**b**) surgical specimen and plane of sectioning; (**c**) cut surface of resection posteriorly: (**d**) cut surface of resection anteriorly (Figure 2.46 from Slootweg and de Groot [3], with permission)

base of the tongue; blocks taken in the sagittal plane are appropriate to demonstrate this feature (Fig. 17.4). The description should include the subsite of origin of carcinoma and the extent of involvement of laryngeal cartilages and extra-laryngeal tissues. If thyroid is present in a pharyngolaryngectomy, one block is sufficient if the thyroid appears normal. If the thyroid is abnormal, one or more blocks should be taken to confirm or exclude invasion by carcinoma or other pathology.

17.4.2.4 Salivary Glands

Representative blocks of tumor (at least one per 10 mm of tissue diameter) should include normal tissue and the relationship between tumor and the nearest resection margin. For tumors <30 mm diameter, it is often appropriate to block the entire tumor; for larger tumors, macroscopically different areas should be sampled, particular at the edge of the tumor. Lymph nodes should be sought within the gland and in periglandular soft tissue; all nodal tissue should be blocked for microscopic examination as should the resection margins of specifically identified nerves.

17.4.2.5 Trans-oral Laser Resection Specimens

The handling of trans-oral laser resection specimens requires close collaboration between surgeon and pathologist. The main tumor resection may be in one or more parts and it is usual for separate biopsies of the resection margins to be submitted for examination. The specimens should be pinned onto a board so that the anatomical relationships between the pieces are maintained and an annotated diagram should indicate the nature of each piece of tissue. The radial and deep margins should be linked to facilitate microscopic orientation of the sections. The main tumor should be serially sliced and blocked in its entirety. If possible, biopsies from resection margins should be sliced perpendicular to the margin and blocked in their entirety.

Small biopsies of the vocal cord are often difficult to orientate and may be pinned onto cork board or attached to strips of dehydrated cucumber to facilitate handling in the laboratory.

17.4.3 Thyroid

Thyroid resection specimens should be fixed intact for 24 h before dissection to avoid traumatic artefacts around the capsule. For large specimens, further fixation may be required after initial slicing. The description will include the overall measurements and whether or not the capsule is intact. A lobe is cut (usually transversely) into parallel slices at 3–4 mm. The size of the lesion or largest lesion is recorded as well as any apparent extension through the thyroid capsule.

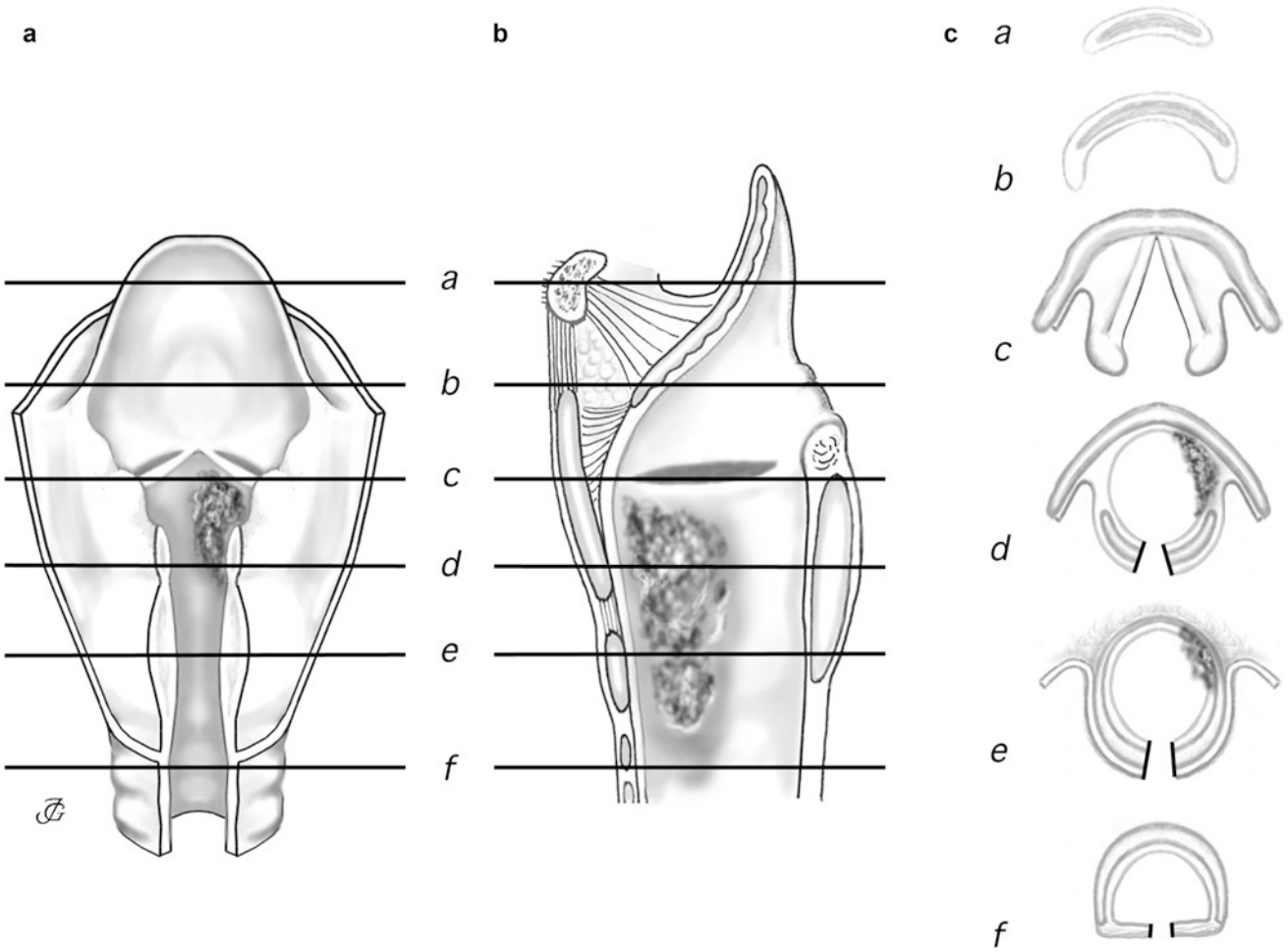

Fig. 17.3 Pathological anatomy of subglottic cancer dissected with transverse slices. (**a**) Clinical appearance viewed from posterior; (**b**) Clinical appearance in sagittal view; (**c**) Transverse slices through larynx corresponding to the lines shown in figures (**a**) and (**b**) (Figure 4.8 from Slootweg and de Groot [3], with permission)

The appropriate number of blocks will vary according to the tumor type [6]; this may be suggested from preoperative cytology. In general, blocks should show the relationship between the tumor and the capsular margin, any possible extrathyroidal extension and the surgical margin. Apparently normal tissue should be examined and any nodules and scars should be sampled to detect possible multifocality.

Completion thyroidectomy specimens should be sliced and blocks taken from any nodules or cysts and from background thyroid. Pragmatically, two blocks of macroscopically normal thyroid are sufficient.

17.4.3.1 Papillary Carcinoma

For papillary carcinoma, there is little evidence on which to base sampling. In practice, for tumors <30 mm, the whole tumor can be blocked while at least two blocks per cm diameter of a larger tumor should permit diagnosis and assessment of whether the tumor is of uniform type. One block per 10–20 mm of normal-looking tissue should be taken from each lobe to assess whether the lesion is single or multifocal. Multifocal lesions have a poorer prognosis.

17.4.3.2 Follicular Carcinoma

Follicular lesions that are grossly invasive should be sampled widely enough to allow identification of any poorly differentiated carcinoma, documentation of vascular invasion, distance to resection margins and spread beyond the thyroid capsule. Follicular lesions and encapsulated follicular variant papillary carcinomas that are not grossly invasive should be extensively sampled at the interface between the tumor capsule and normal gland. Lesions <30 mm in diameter can be blocked in their entirety, and for larger lesions, at least ten blocks should be examined. Where there are multiple nodules, the largest should be processed as above, and other encapsulated or solid, pale nodules sampled.

17.4.3.3 Medullary Carcinoma

Blocks should be taken to confirm the diagnosis and recognise the relationship to the thyroid capsule and any extrathyroidal

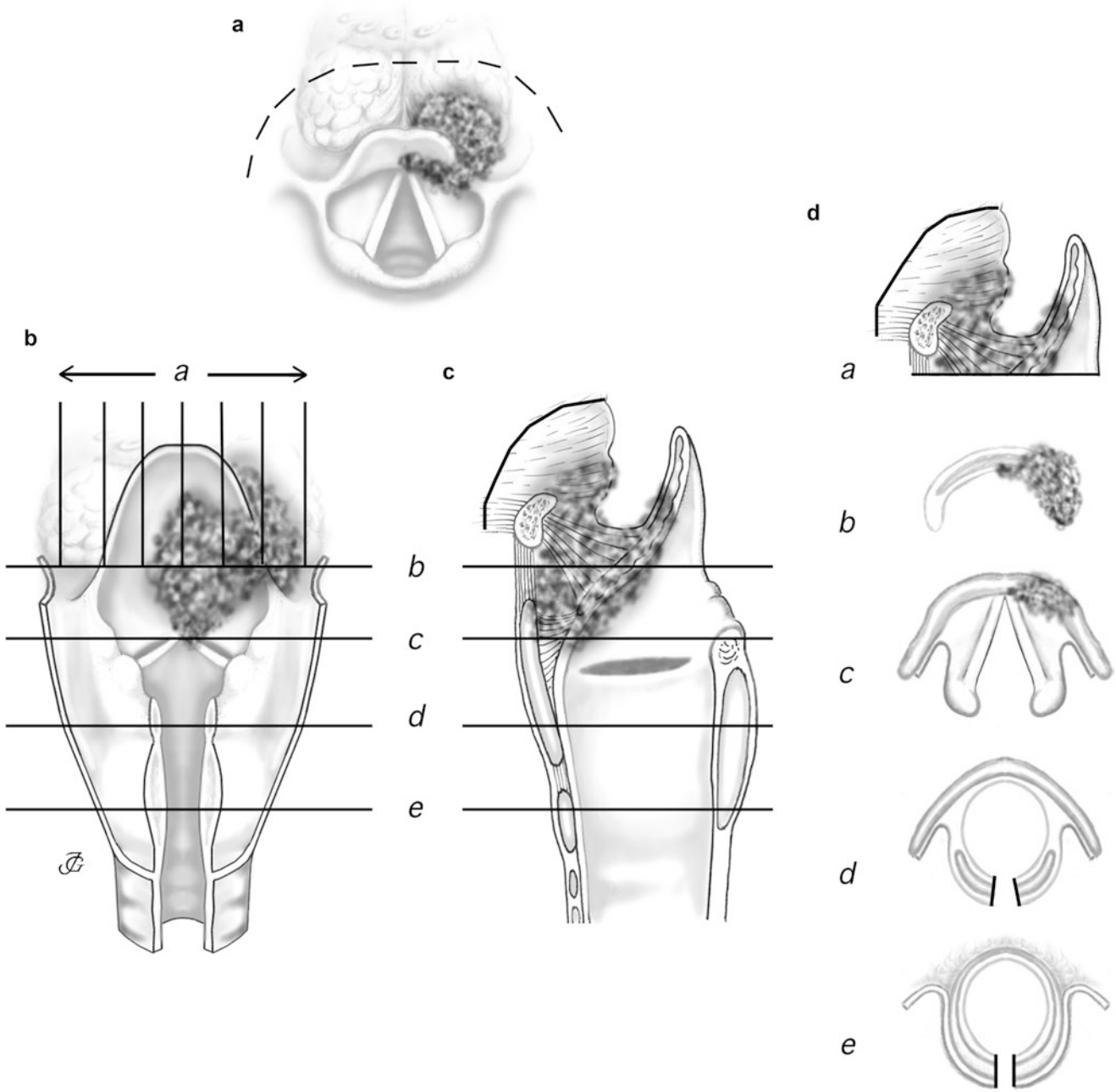

Fig. 17.4 Pathological anatomy of supraglottic endolaryngeal cancer illustrating how a combination of transverse and sagittal slices provides optimal exposure of the extent of spread. (**a**) Clinical appearances on laryngoscopy with outline of anterior resection plane; (**b**) Clinical appearance viewed from posterior; (**c**) Clinical appearance in sagittal view showing extension of tumor into base of tongue; (**d**) Transverse slices through larynx corresponding to the lines shown in figures (**a**) and (**b**); the superior supraglottis has been sliced sagitally (Figure 4.16 from Slootweg and de Groot [3], with permission)

extension. The non-involved gland may be examined for evidence of C-cell hyperplasia in an attempt to identify familial cases, although this has been largely superseded by genetic testing for *RET* mutations. Prophylactic thyroidectomies for MEN2 or familial medullary thyroid carcinoma should be blocked in total to recognise multifocal lesions and C-cell hyperplasia. Calcitonin immunohistochemistry is usually necessary to identify lesions suspected of being medullary carcinoma and to confirm the presence of C-cell hyperplasia.

17.4.4 Skin Excisions

The description and dissection of skin excision specimens are highly context dependent [7]. In general, it is usual to measure the size of the specimen in three dimensions and the maximum diameter and thickness of any lesion and its distance from the nearest margin. Primary excision specimens are sliced transversely at 3–4 mm intervals and blocks taken to demonstrate the nature of the lesion and its relationship to

the resection margins. For lesions less than 10 mm diameter, the whole lesion may be blocked, while for larger lesions, sampling to show important aspects is acceptable. When there is no easily identifiable lesion, the whole of the specimen should be blocked. Re-excision specimens should be sampled to include the whole length of a scar and the associated margins.

For macroscopically atypical melanocytic lesions or biopsy-proven melanoma, the whole lesion should be embedded, to include all margins less than 2 mm from the lesion.

17.4.4.1 Sentinel Lymph Nodes for Cutaneous Malignant Melanoma

Sentinel lymph node biopsy (SLNB) is an important prognostic investigation [8]. The dissection protocol should follow that used in EORTC trials (or equivalent) and should detect metastasis in 25–33 % patients [9]. The EORTC protocol slices the node along its long axis (2 mm transverse slicing is equally sensitive) and all tissue is sectioned at 50 μm intervals with H&E and immunocytochemistry being performed on sections from each level. In larger nodes, the sectioning interval may be increased to 100 μm. EORTC recommends S100 immunocytochemistry to provide the greatest sensitivity, but other markers such as melan-A may be used (and audited for performance in identifying metastases). Molecular methodology (PCR) is probably insufficiently specific to be applied to melanoma. If a potential tumor deposit is seen macroscopically, it is acceptable in the first instance to examine the abnormality using H&E section.

17.4.5 Resections of Soft Tissue Neoplasms

Specimens of soft tissue tumors are not usually orientated. The description should include the overall dimensions, the dimensions of a localised lesion and the distance from the lesion to identifiable margins. The description of a soft tissue tumor will usually include the colour, whether encapsulated or infiltrative, and the presence of haemorrhage and necrosis. The tumors can be cut into 3–5 mm slices and representative blocks to include, as a minimum, one block per cm of tumor.

Blocks are taken to include the nearest resection edge and the deep margin where appropriate. It is not necessary to take a resection margin which is more than 30 mm from the main tumor, with the exception of some superficial low-grade myxofibrosarcomas and epithelioid sarcomas which can infiltrate microscopically along fascial planes. Lesions that are smaller than 50 mm in diameter are normally processed in their entirety. For larger tumors, 1 block per 10 mm of the longest dimension of the tumor, up to a maximum of 12 blocks, is usually sufficient. Areas that appear visibly different may require extra sampling.

The diagnosis of some types of soft tissue lesion relies on genetic and molecular investigations. While this is often possible on paraffin-embedded material, it may be useful to have fresh tissue samples for cytogenetics and to store tissue at −80 °C for molecular studies.

17.4.6 Bone Specimens

Biopsy specimens for histological examination are usually obtained by either open (surgical) biopsy or closed (percutaneous) needle biopsy. Most specimens require at least 3 h of fixation. Core needle biopsy specimens, if properly fixed, may be decalcified overnight in acid or a chelating agent. If the core is 5 mm or more in thickness, it should be divided.

It is often useful to receive specimens in a fresh (unfixed) state in the laboratory. This allows the appropriate use of frozen material, specific fixatives for histochemistry and electron microscopy and snap freezing of tissue for molecular genetic work and flow cytometry. Most ancillary techniques can be used on tissues that are decalcified even in strong acids. However, strong acids lead to fragmentation of nucleic acids and are likely to interfere with molecular genetic analysis.

Large resection specimens for primary bone tumors should be carefully described to include the type of bone resection and its dimensions and the presence or absence of exposed tumor in bone or soft tissues. With regard to the tumor, the description should include (as appropriate) the anatomical location of the tumor within bone and its gross appearance including size, shape, colour, border and the presence of cystic change, haemorrhage or necrosis. Invasion from the bone into adjacent soft tissues or joints and the distance to resection margins are important features.

Before dissection, correlation with clinical or ex vivo radiographs can be helpful in orientating the specimen and selecting sites for sampling. Bone tumor specimens will often be photographed before dissection and the location of the blocks taken for histological examination recorded on a photograph of the slab specimen of tumor.

Blocks of a complete slice through the tumor will normally be taken as well as blocks of the site of previous biopsy and biopsy tract and blocks to show the relationship between the tumor and margins.

17.4.7 Neck Dissections

Neck dissections may be elective (clinically N0) or therapeutic (clinically N+) procedures and are most simply described according to the tissues included in the dissection (Table 17.2).

Table 17.2 Classification of neck dissections (ND) based on structures removed

Categories	Comments
Extended radical ND	Standard radical ND plus additional structures such as the skin, parotid gland
Standard radical (classical) ND	Levels I–V with sacrifice of internal jugular vein, sternocleidomastoid muscle and spinal accessory nerve
Function-preserving ND	
Comprehensive (modified radical, Bocca)	Levels I–V with preservation of internal jugular vein and/or sternocleidomastoid muscle and/or spinal accessory nerve
Selective ND	Removes nodal levels at greatest risk of metastasis
Supraomohyoid	Selective ND, levels I–III
Extended supraomohyoid	Selective ND, levels I–IV +Vb
Posterolateral ND	Selective ND, levels II–V
Lateral ND	Selective ND, levels II–IV
Central compartment ND	Selective ND, bilateral level VI

Table 17.3 Cervical lymph nodes: anatomical levels and nodal characteristics

Anatomical level/sublevel	Boundaries	Usual number of nodes
Sublevel IA (submental)	Within the triangular boundary of the anterior belly of the digastric muscles and the hyoid bone	3–4
Sublevel IB (submandibular)	Within the boundaries of the anterior and posterior bellies of the digastric and the mandibular body. Close to submandibular salivary gland and facial artery	3–7
Level II (upper jugular)	Around the upper third of the internal jugular vein and adjacent spinal accessory nerve extending from skull base to inferior border of hyoid bone and from posterior border of sternocleidomastoid muscle to the lateral border of the sternohyoid muscle and the stylohyoid muscle anteriorly	
Sublevel IIA	Sublevel IIA nodes are located anterior to the spinal accessory nerve	10–20
Sublevel IIB	Sublevel IIB nodes are located posterior to the spinal accessory nerve	10–20
Level III (middle jugular)	Around the middle third of the internal jugular chain, extending from carotid bifurcation superiorly to the omohyoid muscle inferiorly and from the posterior border of the sternocleidomastoid muscle to the lateral border of the sternohyoid muscle anteriorly	5–10
Level IV (lower jugular)	Around the lower third of the internal jugular vein, extending from omohyoid muscle superiorly to clavicle inferiorly and from posterior border of sternocleidomastoid muscle to lateral border of sternohyoid muscle anteriorly	5–10
Level V (posterior triangle)	Extends from union of sternocleidomastoid and trapezius muscles at superior nuchal line of occipital bone to clavicle inferiorly and from anterior border of trapezius posteriorly to posterior border of sternocleidomastoid muscle anteriorly	20–30
Sublevel VA	The spinal accessory nodes within the upper occipital triangle above inferior belly of omohyoid muscle	
Sublevel VB	The transverse cervical and supraclavicular nodes within the lower supraclavicular triangle below inferior belly of omohyoid	
Level VI (anterior compartment)	From the hyoid bone superiorly to the suprasternal notch inferiorly and from the common carotid arteries posteriorly. Includes the pre- and paratracheal nodes, pre-cricoid (Delphian) node and the perithyroidal nodes	10–20

Six major anatomical levels (groups) of lymph nodes are described, based on their topographical anatomy, surgical zones and functional drainage routes (Table 17.3). Surgeons and pathologists should agree how a resection specimen should be sent to the laboratory. If the specimens are submitted intact, orientation is easier if the tissue is pinned onto a polystyrene or cork tile with marker sutures or tags indicating nodal levels and points of clinical interest. If the anatomical groups of nodes are placed in separate containers, care must be taken to avoid disruption of the relationship between nodal disease and the deep margin of a large primary tumor, the extent of extracapsular spread and the relationship of the tumor to nerves, vessels and other critical structures. Nodes additional to the main anatomical groups, i.e. outside the

Table 17.4 Additional nodal groups

Nodal group	Nodal characteristics	Metastatic involvement
Parotid	20 (range 10–35), <10 mm. Grouped as extraglandular and intraglandular	Removed during total parotidectomy for salivary gland malignancies or metastases from facial skin and scalp
Facial	6 (range 3–10), <10 mm. Named according to location (mandibular, buccal, infraorbital, malar)	Mainly metastases from skin but also in about 15 % of retromolar and 5 % of mandibular alveolar and buccal mucosal carcinomas
Sublingual and lingual	Inconsistent, 2–4, <10 mm. Close to sublingual glands and along midline lingual raphe	Involved in about 5 % of oral tongue and floor of mouth SCCs
Retropharyngeal	1–3, <15 mm. Lie within the retropharyngeal space behind the upper part of the *pharynx* and in front of the *arch of the atlas*	Involved in about 20 % of nasopharyngeal and oropharyngeal carcinomas

internal jugular chain, should be submitted in separate containers (Table 17.4).

The component parts of the dissection (salivary glands, muscles; internal jugular vein and spinal accessory nerve according to the extent of the neck dissection) should be identified. The internal jugular vein, when included, is usually obvious on the deep aspect. Inspection and palpation of adipose tissue allow identification of nodes down to 3 mm diameter; this is easier if fixation is prolonged to 48 h as the nodes are firmer. Smaller nodes may be transparent and difficult to distinguish from adipose tissue and sometimes it is easier to process slices of adipose tissue rather than attempt to dissect nodes from the gross specimen.

Nodal masses and discrete nodes should be dissected out with a small amount of pericapsular adipose tissue. Large nodes may be cystic. Nodal masses should be measured intact and the number of nodes contributing to the mass estimated after slicing.

Tissue blocks should be selected to show the maximum extent of tumor and its relationship to perinodal structures [10, 11]. If the node is adherent to the internal jugular vein, blocks should be taken to assess whether the adherence is due to fibrosis or actual tumor infiltration. Small or flat nodes can be processed whole. Larger nodes may be bisected or, if more than 15–20 mm in diameter, can be sliced at 3–4 mm intervals. If a node appears negative on slicing, all the tissue slices should be processed.

Other methods include serially slicing the neck dissection specimen every 3–4 mm rather than dissecting it, which can produce a large nodal yield, but care must be taken to ensure each node is only counted once. Lipid-clearing techniques are generally considered too time consuming for routine use.

Generally, radical neck dissections (without prior radio/chemotherapy) yield around 45 nodes (typically 30–60) and level I–IV and I–III dissections, 20–40 and 15–35 nodes, respectively, but the number depends on operator expertise, the patient's age and gender, the extent of metastatic disease and the adiposity of the neck.

Salvage neck dissections following surgery or radiotherapy can be problematic due to distortion by fibrosis. Anatomical landmarks may be missing and some anatomical levels may yield few nodes. Macroscopically, recurrent tumor may be indistinguishable from scar tissue and it is important to sample extensively as viable tumor cells may be widely dispersed amongst scar tissue.

In routine diagnostic practice, the thoroughness of the assessment must be balanced against available resources. The most widely used compromise is to assess one H&E-stained section from each tissue block; additional sections and immunohistochemistry are required infrequently.

17.4.7.1 Sentinel Lymph Nodes for Mucosal Squamous Cell Carcinoma

Sentinel nodes should be subjected to rigorous pathological evaluation [12]. This protocol involves bisecting or cutting the fixed node(s) into 2 mm thick slices, processing all the tissue and assessing an H&E-stained section from each tissue block. If metastasis is not seen, then the full thickness of the block is cut through at 150 μm levels with six sections taken at each level. One H&E-stained section from each level is examined, and if these are negative, immunocytochemistry is performed on a second section using a pancytokeratin antibody. Any cytokeratin-positive cells should be compared with the adjacent H&E-stained section to avoid false-positive readings due to cross-reactivity

17.5 Reporting Guidance for Small Biopsies and Non-malignant Cases

17.5.1 Mucosal Biopsies

The report should refer to the overlying epithelium, lamina propria and other identified tissues including an indication of the depth of the biopsy (e.g. by reference to muscle on the deep aspect). Any infective agents or dysplastic features (graded according to the agreed guidelines) must be highlighted. For excision specimens of leukoplakia, the presence and grade of atypia at surgical margins must be noted (this is not relevant for small, incisional biopsies).

17.5.2 Nasal Cavity and Paranasal Sinuses

Specific diagnoses should be provided for any polypoid lesion, i.e. allergic/inflammatory-type, inverted papilloma, in view of the potential risk of malignant transformation in the latter.

17.5.3 Small Diagnostic Biopsies for Malignancy

The information that can be obtained from small biopsy specimens which show malignancy will be determined, in part, by their size. The type of carcinoma and its provisional grade are the minimum data, as these may influence treatment. It is acknowledged that the grade in superficial biopsy material may not be representative of the most aggressive part of the invasive tumor front. If severe dysplasia/in situ carcinoma is present, this should be recorded as it may influence the plane of excision. It is not realistic to assess reliably the depth of invasion or presence of vascular invasion in small biopsies. For larger diagnostic biopsies, the pattern and depth of invasion can be determined.

17.5.4 Intraepithelial Neoplasia, Dysplasia and In Situ Carcinoma

Epithelial atypia provides a continuous spectrum of appearances, from mild to severe dysplasia/carcinoma in situ. Detailed discussion of the criteria and reproducibility of grading systems is provided in Chap. 1. The options include the WHO system, the system based on grades of squamous intraepithelial neoplasia, a two-grade system for the oral cavity and the Ljubljana classification for laryngeal lesions. Whichever system is used, it is important that the grading process and its clinical implications are understood by the clinical team.

The presence of moderate dysplasia or severe dysplasia/high-grade intraepithelial neoplasia/carcinoma in situ adjacent to the primary carcinoma and within 5 mm of the resection margins may predict local recurrence and therefore should be reported.

17.5.5 Teeth

Clinical information including family history, extent of teeth affected and the presence of metabolic bone disorders, as well as the examination of radiographs, is required for accurate diagnosis of developmental disorders of the teeth. This assessment requires polarising light microscopy.

The report should provide details about the enamel including thickness, structure, presence of enamel matrix and appearance of amelodentinal junction and about the dentine including the appearance and presence of mantle zone, predentine, primary, secondary, tertiary and inter-globular dentine, specifically the appearance and presence of dentine tubules including relative width and orientation as well as the location of dysplastic dentine. Pulp examination includes assessment of the root apex, vitality, inflammation, relative size and location [4].

17.5.6 Cysts: Odontogenic and Non-odontogenic

The report should describe the cyst lining and the type and nature of the epithelium, e.g. the presence of keratinization or basal palisading, mucous metaplasia, hyaline bodies or atypical features. The capsule must be described, particularly the presence or absence of inflammation and features such as daughter cysts, calcification, odontogenic rests or foreign material.

17.5.7 Odontogenic Tumors

An accurate description of any epithelium, including the formation of duct-like structures, as well as the presence of atypical features such as mitotic figures, is required. Atypical features such as pleomorphism are common in some odontogenic tumors, including the calcifying epithelial odontogenic tumor. If no odontogenic epithelium is identified, this must be stated. The appearance of the stroma must be described including the presence of enamel, dentine, bone or other calcified materials. The report should comment on the relationship to normal structures, e.g. teeth, bone and the presence of a capsule and nature of the surgical margins.

17.5.8 Major and Minor Salivary Glands

Reports on salivary gland cysts will usually include the nature of a cyst and its lining along with a comment on any associated inflammation and changes in the adjacent salivary tissue, for example, the presence of atrophy, mucus extravasation and duct ectasia. Benign tumors should be diagnosed according to WHO guidelines [13] and the report should indicate the distance to nearest peripheral and deep margins as well as any breach in the capsule that may have resulted in tumor spillage.

17.5.9 Skin, Thyroid, Soft Tissue and Bone

Reports on non-malignant cases will focus on an accurate description of the pathological changes which justify a

specific diagnosis. The clinical implications of the diagnosis may be included and, where appropriate, additional abnormalities in the surrounding tissues.

17.6 Cytological Diagnosis of Mucosal Malignancies and Neck Lumps

17.6.1 Specimen Preparation

A fine needle aspirate is smeared onto three to four slides and stained with Papanicolaou, Giemsa and H&E methods. Sections may also be stained with PAS and a water-mounted section can be prepared for polarised light microscopy. Cell blocks are useful for detailed immunocytochemical and molecular studies, if sufficient material is available.

17.6.2 Mucosal Malignancies

Exfoliative or fine needle aspiration of mucosal lesions is rarely used, as most lesions are susceptible to conventional biopsy techniques. Fine needle aspiration may be used for the rapid assessment of bulky intra-oral lesions.

17.6.3 Cysts of the Jaws

Cytology is occasionally used on aspirates from cystic lesions of the jaws. The primary purpose is to differentiate inflammatory cysts from keratocystic odontogenic tumor (odontogenic keratocyst) by searching for keratin. This has a high rate of insufficient specimens or false negatives and is therefore rarely used. Many inflammatory cells or cholesterol crystals suggest an inflammatory cyst but are not diagnostic.

17.6.4 Neck Lumps

Fine needle aspiration cytology is widely used in the diagnosis of salivary gland tumors and for neck lumps, particularly for the diagnosis and management of neoplasms and for staging head and neck cancer [14]. Occasionally FNA cytology will reveal non-neoplastic lesions, but this is not its primary purpose.

Fine needle aspiration cytology is useful for the diagnostic triage of salivary masses but has well-recognised limitations. The ability to distinguish inflammatory masses, lymphoid and epithelial proliferations is helpful in a rapid diagnostic clinic, but cytopathologists need to be aware of the complexity of histological patterns in salivary neoplasms that makes the precise diagnosis of many carcinomas difficult on limited cytological samples. The cytological opinion on a fine needle aspirate from a salivary mass should always be interpreted in the context of clinical and imaging findings.

Cytology has a limited role in the diagnosis of cutaneous squamous or basal cell carcinomas. Imprints or aspirates for cytological diagnosis may be used for provisional diagnosis in clinics on a 'one-stop' basis.

Most thyroid lesions should have had FNA before surgery, so a differential diagnosis may be available and will influence decisions on management. The use of numerical categories is suggested, for example, Thy1-5 as proposed in the guidelines of the British Thyroid Association [15].

The initial cytological diagnosis and grading of soft tissue sarcomas require considerable experience, and FNAC therefore has a limited role outside specialist centres. Fine needle aspiration cytology can be of use in confirming recurrence or metastasis in a patient with a previously diagnosed sarcoma.

17.7 Frozen Section Diagnosis

The initial diagnosis of carcinoma will usually be made before definitive surgery. On occasions, intraoperative frozen section diagnosis of the nature of a neoplasm will be required. While it will usually be possible to identify the presence of neoplastic tissue, the nature of a poorly differentiated neoplasm may be impossible to determine on frozen sections.

The assessment of the presence or absence of carcinoma at surgical resection margins is the most common indication for intraoperative frozen section diagnosis. When selecting tissue for frozen section analysis, the surgeon should help the laboratory to concentrate on the most relevant areas by submitting tissue less than 10 mm diameter from clinically close margins. The report on the frozen section specimen(s) should normally form part of, or accompany, the final diagnostic report on the case.

In the context of skin malignancies, the use of frozen sections should be limited to Mohs' micrographic surgery where horizontal sections are used to accurately assess margin status. Vertical frozen sections should not be used to assess margins as they are insufficiently representative of the entire margin.

For thyroid disease, intraoperative frozen section is occasionally used to confirm the diagnosis of papillary carcinoma or medullary or anaplastic carcinoma or to identify lymph node involvement. It should not be used to differentiate follicular carcinoma from adenoma.

Frozen section diagnosis of soft tissue sarcomas is rarely required if there have been preoperative needle biopsies or cytology. It is occasionally requested for confirmation of the presence of tumor, but is not indicated for assessment of margins.

Frozen section examination of bone tumors requires experience of osteoarticular pathology and knowledge of the clinical background and radiological appearances of the lesion. Potentially, frozen section histology may guide the adequacy of a tissue specimen, the nature of the lesion, the use of ancillary investigations for diagnosis and assessment of resection margins.

17.8 Reporting Guidance for Resections of Mucosal Carcinomas

17.8.1 Core Data

The clinical and pathological data that should normally be included in reports on primary mucosal malignancies of the head and neck are summarised in Table 17.5. Some data are required for complete TNM staging, the strength of the evidence supporting the inclusion of the data varies from site to site and some features will not be relevant in all cases. For convenience, one list of data items is presented here as an *aide memoire*; more detailed discussion of the evidence and site-specific variations is available elsewhere [1, 16–18]. Subsequent paragraphs provide more detailed information on how these features might be consistently recorded.

17.8.2 Site and Laterality of the Carcinoma

For carcinomas that involve more than one site, the principal site of involvement should be recorded; this may not be the site of origin. If required, the involvement of associated sites can be noted to assist in data analysis. Sites and subsites should be recorded according to the UICC nomenclature to facilitate TNM staging.

17.8.3 Type of Specimen

The type of specimen should be described as incisional biopsy, excisional biopsy or resection. The designation of resection specimens may be refined according to site-specific criteria, e.g. partial or total.

17.8.4 Maximum Diameter of Tumor

The macroscopic diameter (in millimetres) should be used (Fig. 17.5), unless the histological extent is greater than macroscopically apparent, in which case the microscopic dimension is used. Measurements are made pragmatically, acknowledging distortion of tissues by fixation and processing. Maximum diameter informs staging.

17.8.5 Maximum Depth of Invasion

The maximum depth of invasion (in millimetres) below the luminal aspect of surface should be recorded; if the tumor has ulcerated, then the reconstructed surface should be used (Fig. 17.5).

The aim should be to provide a best estimate of tumor depth; for large carcinomas this may be an approximation. Additional information on the tissues involved (mucosa, muscle, etc.) may be included as free text. Depth of invasion is significantly related to nodal metastasis for oral T1/T2 carcinomas, although the optimal cutoff point for prognostic purposes has been debated. Recent reviews and meta-analysis suggest that 4 mm is the optimal threshold for prediction of cervical node metastasis [19–21].

Table 17.5 Core data items recommended for inclusion in the histopathology reports for primary carcinomas

Data item	Source of information
Site and laterality of carcinoma	Surgeon (request form)
Type of operation (excision, resection, laser procedure)	Surgeon (request form)
Maximum diameter of carcinoma	Macroscopic size, confirmed histologically
Maximum depth of invasion	Macroscopic depth, confirmed histologically
Histological type of carcinoma	Microscopy
Grade of differentiation	Microscopy
Pattern of invasion	Microscopy
Distance(s) to resection margins	Macroscopic distance, confirmed histologically
Vascular invasion	Microscopy
Invasion of perineural plane	Microscopy
Invasion of bone	Macroscopic observation, confirmed histologically
p16/HPV status (oropharynx only)	Microscopy
pT stage	Macroscopic and microscopic features; site-specific considerations apply

17.8.6 Histological Type of Carcinoma

The best evidence for the use of datasets is for conventional squamous cell carcinomas. Subtypes of squamous carcinoma, such as papillary, verrucous, basaloid, adenosquamous, acantholytic and spindle cell carcinomas, should be recognised and potential prognostic implications noted. Basaloid squamous cell carcinomas tend to present with more extensive disease but are also more radiosensitive than conventional squamous cell carcinomas and should be diagnosed using standard criteria [13, 22].

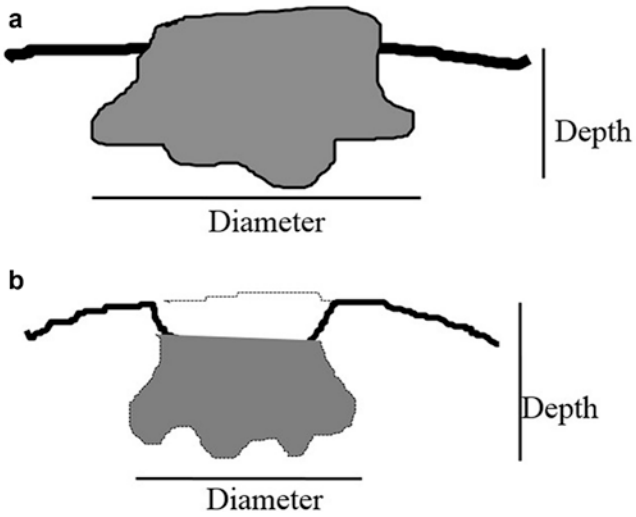

Fig. 17.5 Diagrammatic representations of nodular (**a**) and ulcerated (**b**) carcinomas illustrating the principles of measuring the tumor diameter and thickness (Fig. 1 from Helliwell and Woolgar [1], with permission). The depth of invasion refers to the depth of greatest spread in presumed continuity below the *top* of the adjacent mucosa. For both nodular and ulcerated tumors, the line of the original mucosal surface is reconstructed to determine the true thickness

17.8.7 Degree of Differentiation (Grade)

Grading is based on the degree of resemblance of the carcinoma to the normal epithelium and follows the descriptions in the WHO classification [13]. The most aggressive area (approximating to a microscopic field at ×100 magnification) is graded as well, moderately or poorly differentiated. This system is widely used and prognostically useful, even though it suffers from inter-observer variability and sampling problems. While most squamous carcinomas will be moderately differentiated, it is important for prognostication to separate well-differentiated and poorly differentiated tumors. Where a tumor has a varied appearance, then the highest grade (poorest differentiation) is recorded as core data; other features can be included as free text (Fig. 17.6).

17.8.8 Pattern of Invasion

The pattern of invasion by the carcinoma at its deep margin is of prognostic value for oral carcinomas, with weaker

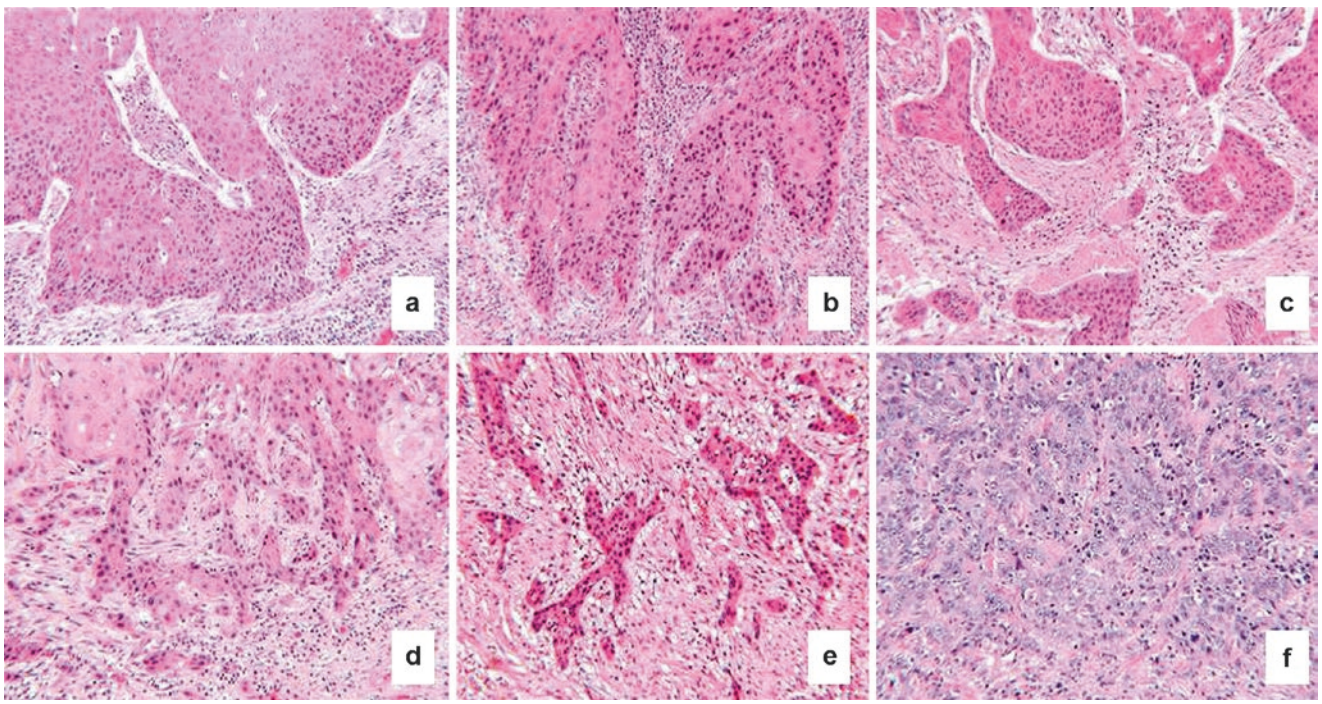

Fig. 17.6 Illustrative patterns of invasive growth by squamous cell carcinoma; (**a–c**) examples of cohesive patterns; (**d–f**) examples of non-cohesive patterns (Fig. 2 from Helliwell and Woolgar [1], with permission)

evidence at other sites [23, 24]. Scoring systems for histopathological features of squamous carcinomas include features related to differentiation and to the tumor/stromal interaction. These may improve the consistency of reporting but are not in widespread use and it is suggested that differentiation and invasive pattern are recorded separately. The patterns of tissue invasion by carcinoma form a continuous spectrum. For prognostic purposes, two groups are recognised: carcinomas composed of broad cohesive sheets of cells or strands of cells >15 cells across (Figs. 17.6a–c) and carcinomas composed of narrow strands, non-cohesive small groups or single cells (Figs 17.6d–f).

17.8.9 Distance from Invasive Carcinoma to Surgical Margins

The distance (in millimetres) is measured for both mucosal and deep margins. From a surgical point of view, >5 mm is clear, 1–5 mm is close and <1 mm is involved. Incomplete resection or the presence of dysplasia at the margin is associated with an increased risk of local recurrence. The practical implications of a close margin are influenced by the pattern of invasion; an infiltrating pattern of invasive front (or vascular or perineural spread ahead of the invasive front) and a close margin may be associated with a high risk of local recurrence [25, 26]. Conversely, a close margin for a well-circumscribed tumor with a cohesive growth pattern may be clinically acceptable. These aspects may be recorded as free text in the report.

17.8.10 Vascular Invasion

The presence or absence of vascular invasion should be mentioned if it is an obvious feature on medium magnification examination of the tumor. The presence of carcinoma cells within an endothelial-lined space is the essential criterion and should be distinguished from retraction artefact. It is not necessary to distinguish between small lymphatic and venous channels. Vascular invasion is a relatively weak predictor of nodal metastasis [24].

17.8.11 Nerve Invasion

The presence or absence of invasion of the perineural plane ahead of the invasive front of the carcinoma should be recorded, regardless of the size of the nerve. Perineural invasion predicts local recurrence, nodal metastasis and survival and may indicate a need for adjuvant therapy [27, 28].

17.8.12 Bone Invasion

Maxillary or mandibular bone may be eroded (non-invasive) or may be diffusely infiltrated by carcinoma. If bone invasion is present, the presence or absence of carcinoma at the bone margins should be recorded.

17.8.13 Laryngeal Carcinoma

The maximum depth of invasion of laryngeal carcinomas is less important than the nature of the tissue planes involved. If desired, tumor thickness may be recorded as a non-core data item. The important tissues for TNM staging are the paraglottic space, the pre-epiglottic space and the thyroid and cricoid cartilages. Invasion of the thyroid and cricoid cartilages is an important criterion for the staging of laryngeal carcinomas. If present, the extent of invasion (inner table only or full thickness) should be recorded.

17.8.14 Sinonasal Adenocarcinoma

Sinonasal adenocarcinomas are classified into salivary type carcinomas, intestinal and non-intestinal adenocarcinomas. Histological type and grade are of prognostic importance.

Intestinal-type carcinomas are morphologically and immunophenotypically similar to colonic neoplasms (usually expressing CK20 and CDX2, but not CK7) and are aggressive neoplasms with papillary, exophytic tumors having a better prognosis than colonic pattern, solid and mucinous carcinomas [29, 30]. Non-intestinal adenocarcinomas are not immunoreactive for colonic markers and are grouped as either low-grade, indolent tumors or more aggressive, high-grade adenocarcinomas on the basis of marked cytological atypia, a high mitotic rate and/or necrosis.

17.8.15 Sinonasal Undifferentiated Carcinoma

Sinonasal undifferentiated carcinomas (SNUC) are highly aggressive epithelial malignancies composed of nests, lobules or sheets of atypical cells with a high mitotic rate, necrosis and apoptosis. They show minimal, if any, squamous or glandular differentiation and immunocytochemical expression of neuroendocrine markers is uncommon. Differentiation from other poorly differentiated, non-epithelial malignancies is important.

17.8.16 Human Papillomaviruses (HPV) and Head and Neck Carcinomas

There is substantial evidence to link high-risk human papillomaviruses (particularly HPV16) to a subset of oropharyngeal carcinomas. HPV-associated carcinomas are usually non-keratinizing, arise in the tonsils or base of tongue and tend to have better overall and disease-free survivals than non-HPV-associated carcinomas [31, 32]. Although there is currently insufficient evidence to modify treatment intensity in these patients, this is a subject of active research. The association between HPV and oral and laryngeal carcinomas is less strong and does not currently have clear prognostic value and HPV status is not core data at sites other than oropharynx.

To allow the stratification of treatment outcomes, the HPV status of oropharyngeal carcinomas should be assessed using validated methods with appropriate controls. The immunocytochemical identification of overexpression of p16 protein is a useful screening method as HPV-associated carcinomas show strong nuclear and cytoplasmic expression of p16 in >70% malignant cells (block pattern of expression), and p16-negative cases are almost certainly not HPV associated. Carcinomas showing p16 overexpression should have the presence of HPV confirmed by in situ hybridisation, if possible, as pathways other than HPV may be associated with p16 overexpression. The report should indicate the methods used to evaluate HPV status (p16 immunocytochemistry and/or in situ hybridisation).

17.8.17 Transoral Laser Resection Specimens

In transoral laser resections of mucosal neoplasms, an estimate of the tumor diameter, thickness and pattern of invasion should be made, incorporating all parts of the specimen, as well as the presence or absence of vascular and perineural invasion. The resection margins of the main resection specimen are usually distorted by thermal damage to the tissues and may not be assessable histologically [33]. Assessment of the overall adequacy of excision should explicitly include the main resection specimen and separate marginal biopsies.

17.8.18 Site-Specific T Codes

Site-specific T codes derived from UICC TNM v7 [2] are shown in Tables 17.6, 17.7, 17.8, 17.9, 17.10, 17.11, 17.12, 17.13, and 17.14.

Table 17.6 Site-specific T codes for the lip and oral cavity

T stage	Descriptor
T1	Tumor 20 mm or less in greatest dimension
T2	Tumor 21–40 mm in greatest dimension
T3	Tumor >40 mm in greatest dimension
T4	Tumor invades adjacent structures
T4a	Moderately advanced local disease
	Lip: tumor invades through cortical bone, inferior alveolar nerve, floor of mouth or skin of face
	Oral cavity: tumor invades adjacent structures, e.g. through cortical bone of the mandible or maxilla into deep extrinsic muscles of tongue, maxillary sinus, skin of face
T4b	Very advanced local disease
	Tumor invades masticator space, pterygoid plates or skull base and/or encases internal carotid artery

Table 17.7 Site-specific T codes for the oropharynx

T stage	Descriptor
T1	Tumor 20 mm or less in greatest dimension.
T2	Tumor 21–40 mm in greatest dimension
T3	Tumor >40 mm in greatest dimension
T4	Tumor invades adjacent structures
T4a	Moderately advanced local disease
	Tumor invades larynx, deep/extrinsic muscle of tongue, medial pterygoid muscles, hard palate or mandible
T4b	Very advanced local disease
	Tumor invades tumor invades lateral pterygoid muscle, pterygoid plates, lateral nasopharynx or skull base or encases carotid artery

Table 17.8 Site-specific T codes for the nasopharynx

T stage	Descriptor
T1	Tumor confined to nasopharynx or extends to oropharynx and/or nasal cavity
T2	Tumor with posterolateral parapharyngeal extension
T3	Tumor invades bone of skull base and/or paranasal sinuses
T4	Tumor with intracranial extension and/or involvement of cranial nerves, infratemporal fossa, hypopharynx or orbit

Table 17.9 Site-specific T codes for the hypopharynx

T stage	Descriptor
T1	Tumor limited to one subsite and 20 mm or less in greatest dimension
T2	Tumor involves more than one subsite or measures 21–40 mm in greatest dimension
T3	Tumor >40 mm in size or with fixation of hemilarynx or extension to oesophagus
T4	Tumor invades adjacent structures
T4a	Moderately advanced local disease
	Tumor invades adjacent structures (thyroid cartilage, cricoid cartilage, hyoid bone, thyroid gland, oesophagus, central soft tissue)
T4b	Very advanced local disease
	Tumor invades prevertebral fascia, encases carotid artery or invades mediastinum

Table 17.10 Site-specific T codes for the supraglottic larynx

T stage	Descriptor
T1	Tumor limited to one subsite with normal vocal cord mobility
T2	Tumor invades more than one adjacent subsite without fixation of larynx
T3	Tumor limited to larynx with vocal cord fixation and/or invades postcricoid area, pre-epiglottic tissues, deep base of tongue and/or inner cortex of thyroid cartilage
T4	Tumor invades adjacent structures
T4a	Moderately advanced local disease
	Tumor invades through thyroid or cricoid cartilage and/or invades tissues beyond the larynx, e.g. soft tissues of neck, thyroid or oesophagus
T4b	Very advanced local disease
	Tumor invades prevertebral space, encases carotid artery or mediastinal structures

Table 17.11 Site-specific T codes for the glottic larynx

T stage	Descriptor
T1	Tumor limited to vocal cords with normal mobility
	T1a – tumor limited to one vocal cord
	T1b – tumor involves both vocal cords
T2	Tumor extends to supraglottis and/or subglottis and/or with impaired vocal cord mobility
T3	Tumor limited to larynx with vocal cord fixation and/or invades the paraglottic space and/or with invasion of inner cortex of thyroid cartilage
T4	Tumor invades adjacent structures
T4a	Moderately advanced local disease
	Tumor invades through thyroid or cricoid cartilage and/or invades tissues beyond the larynx, e.g. soft tissues of neck, thyroid or oesophagus
T4b	Very advanced local disease
	Tumor invades prevertebral space, encases carotid artery or mediastinal structures

Table 17.12 Site-specific T codes for the subglottic larynx

T stage	Descriptor
T1	Tumor limited to subglottis
T2	Tumor extends to vocal cords with normal or impaired mobility
T3	Tumor limited to larynx with vocal cord fixation
T4	Tumor invades adjacent structures
T4a	Moderately advanced local disease
	Tumor invades through thyroid or cricoid cartilage and/or invades tissues beyond the larynx
T4b	Very advanced local disease
	Tumor invades prevertebral space, encases carotid artery or mediastinal structures

Table 17.13 Site-specific T codes for the maxillary sinus

T stage	Descriptor
T1	Tumor limited to antral mucosa with no bone involvement
T2	Tumor causing bone erosion or destruction, except for posterior wall
T3	Tumor invades posterior wall of sinus, subcutaneous tissues, floor or medial wall of orbit, infratemporal fossa, pterygoid plate, ethmoid sinuses
T4	Tumor invades adjacent structures
T4a	Moderately advanced local disease
	Tumor invades anterior orbital contents, skin of cheek, pterygoid plates, infratemporal fossa, cribriform plate, sphenoid or frontal sinuses
T4b	Very advanced local disease
	Tumor invades any of the following: orbital apex, dura, brain, middle cranial fossa, cranial nerves other than maxillary division of trigeminal nerve (V2), nasopharynx or clivus

Table 17.14 Site-specific T codes for the nose and ethmoid sinus

T stage	Descriptor
T1	Tumor restricted to one subsite in the nasal cavity or ethmoid sinus, with or without bone erosion
T2	Tumor involves two subsites[a] within one site or extends to involve an adjacent site within the nasoethmoidal complex, with or without bone erosion
T3	Tumor extends to involve the medial wall or floor of the orbit, maxillary sinus, palate or cribriform plate
T4	Tumor invades adjacent structures
T4a	Moderately advanced local disease
	Tumor invades any of the following: anterior orbital contents, skin of nose or cheek, minimal extension to anterior cranial fossa, pterygoid plates, sphenoid or frontal sinuses
T4b	Very advanced local disease
	Tumor invades any of the following: orbital apex, dura, brain, middle cranial fossa, cranial nerves other than maxillary division of trigeminal nerve (V2), nasopharynx or clivus

[a]Sites for classification are the individual maxillary and ethmoidal sinuses and the nasal cavity. The nasal cavity is divided in the following subsites: septum, floor, lateral floor and vestibule

17.9 Reporting Guidance for Neck Dissections and Sentinel Nodes

17.9.1 Core Data

The histological findings are most usefully recorded using a core dataset supplemented as appropriate by free text or additional data to suit local interests. The laterality of any metastases and core data items determine the pN-stage category and stage group [2]. Each side of the neck is recorded independently. Published data are based on the evaluation of one H&E-stained section for each block (except for sentinel nodes; see below); more rigorous examination of the nodes is therefore not necessary in routine practice. Core data which influence post-operative management and correlate with regional recurrence, distant metastasis and survival [28, 34–36] include:

- Total number of nodes
- Total number of positive nodes and their anatomical location
- Size (in mm) of the largest metastasis
- Extracapsular spread – presence or absence and anatomical location of affected nodes

Isolated tumor cells (ITC) are not included in the positive node count; these can usually be recognised on H&E staining, but immunocytochemistry using a pancytokeratin antibody may be helpful in confirming their presence. Micrometastases are positive nodes in which the deposit is 2 mm or less in diameter, while macrometastases are larger than 2 mm diameter. The detection of metastatic squamous cell carcinoma is straightforward when keratinization and/or desmoplasia is present. When these features are absent, and particularly in discohesive metastases, tumor deposits may be difficult to distinguish from nodal reactions, such as sinus histiocytosis. Subtle changes in colour and nodal architecture, nucleolar and increased cytoplasmic eosinophilia, some preservation of intercellular cohesion, mitotic activity and admixture with eosinophils can be helpful.

Note that the size of the largest metastatic deposit is recorded and not the size of the largest node. For a nodal tumor deposit consisting of multiple islands, the total profile diameter is recorded. When there is extensive metastasis with fusion and matting of involved nodes, the maximum dimension of the mass should be recorded together with an estimate of the number of contributing, positive nodes and the anatomical level(s).

Extracapsular spread (ECS) is present when there is spread through the full thickness of the node capsule, with malignant cells impinging on adipocytes or other soft tissues. Clusters of tumor cells in capsular or perinodal lymphatics do not constitute ECS. ECS is more likely in large nodal deposits but can occur in small nodes, in cN0 necks and in association with tumor deposits of <2 mm; such tiny deposits should not be recorded as micrometastases because of the different prognosis conferred by the ECS.

The extent of ECS may be described as macroscopic if seen with the naked eye (provided that this is confirmed histologically) or microscopic, and the extent documented as the distance of the advancing tumor front from the nodal capsule.

Extranodal nodules of tumor in the connective tissue may represent nodal metastases that have completely destroyed the node, soft tissue metastases or discontinuous extension of the primary tumor. Absolute distinction is not always possible. A practical solution is to regard tumor nodules in the lymphatic drainage area as a nodal metastasis and nodules within 10 mm of the primary tumor as discontinuous extensions. In practice, soft tissue deposits are usually found in conjunction with matted nodes and an estimate of the number of positive nodes is sufficient.

In oral squamous cell carcinoma, ECS is a clear indication for post-operative radiotherapy or chemoradiotherapy [36, 37]. In oropharyngeal SCC, the prognostic value of ECS is less clear cut, and only soft tissue deposits without residual nodal structure together with high T-stage and positive resection margins seem strong prognosticators irrespective of HPV status.

Other information that may be added, as appropriate, in a comprehensive report includes the presence of necrosis, cystic degeneration (as often seen in metastatic tonsillar carcinomas) and dystrophic mineralisation. Nodes may show an immunological response to the presence of the tumor with florid follicular hyperplasia, sinus histiocytosis and sarcoid-type granulomas. Vascular transformation of lymphoid sinuses is occasionally seen and may be related to mechanical obstruction by tumor deposits. A histological response to previous adjuvant treatment is reflected by fibrosis and keratin granuloma. If no viable malignant cells are seen post treatment, the prefix "y" is used before N0 in TNM staging.

The extent of nodal involvement may be summarised in a grid (Table 17.15) supplemented by free text as appropriate and a statement of the pN stage.

17.9.2 Sentinel Nodes

Sentinel nodes are reported using the same principles as for node dissections. It should be noted that in current trial protocols, the presence of ITC in a sentinel node (as the only evidence of disseminated disease) is regarded as sufficient to

Table 17.15 Grid for summarising nodal data

Anatomical level	Total no. of nodes	No. of positive nodes	Largest metastasis (mm)	ECS present	Extent of ECS
Ia					
Ib					
IIa					
IIb					
III					
IV					
Va					
Vb					
VI					
All levels					

recommend formal neck dissection. More evidence is required as to the real prognostic significance of minimal nodal involvement (ITC and micrometastasis) [38].

17.9.3 Nodal Metastasis with No Known Primary

The histological pattern of the metastasis (squamous carcinoma versus adenocarcinoma) and immunocytochemistry may be helpful in establishing the primary site. For adenocarcinomas, an immunocytochemical panel should cover the common primary sites for the age and sex of the patient. For squamous cell and poorly or undifferentiated carcinomas, p16 immunocytochemistry and, when positive, in situ hybridisation for high-risk human papillomaviruses suggest an oropharyngeal primary, and expression of Epstein-Barr virus protein or RNA suggests a nasopharyngeal primary. Cytokeratin expression profiles do not reliably assist in the identification of likely primary sites. Modern imaging techniques, e.g. PET-CT scans, often provide clinically useful information on the likely primary site, minimising the need for extensive immunocytochemistry.

17.10 Reporting Guidance for Mucosal Malignant Melanoma

The majority of mucosal malignant melanomas arise in the sinonasal tract, with approximately 25 % in the oral cavity and a few at other sites. Even small melanomas tend to behave aggressively, with high rates of recurrence and death. Melanoma should be considered in the differential diagnosis of any poorly differentiated mucosal malignancy and immunocytochemical analysis performed when appropriate. The 7th edition of the TNM staging system [2] reflects this aggressive behaviour by designating primary melanomas limited to the mucosa as T3 lesions (Table 17.16). Advanced and very advanced mucosal melanomas are classified as T4a and T4b, respectively. In situ mucosal melanomas are excluded from staging as they are extremely rare.

17.11 Reporting Guidance for Mucosal Neuroendocrine Neoplasms

17.11.1 Neuroendocrine Neoplasms of the Larynx

Laryngeal neuroendocrine neoplasms are rare and include paraganglioma (usually benign), typical carcinoid tumor, atypical carcinoid tumor and small cell carcinoma. The diagnostic criteria are described in the WHO classification and elsewhere [13]. Large cell neuroendocrine carcinoma has been defined [39], but it is uncertain whether or not this differs significantly from atypical carcinoid tumor. The biological behaviour of these tumors differs, with typical carcinoids being locally aggressive, atypical carcinoids spreading to regional nodes and small cell carcinoma showing distant metastases. For practical purposes, the core dataset for reporting mucosal neuroendocrine neoplasms should follow the principles used for mucosal squamous cell carcinoma.

17.11.2 Olfactory Neuroblastoma (Esthesioneuroblastoma)

Olfactory neuroblastoma is an uncommon neuroectodermal malignancy that usually arises from the olfactory membrane of the upper nasal cavity, although origin at other sites has been described. The tumors typically have a lobular architecture and a highly vascular fibrous stroma and express neuroendocrine markers (synaptophysin, neurofilament protein and chromogranin). Histological grading (Hyams' grade) is of prognostic significance (Table 17.17).

17.12 Reporting Guidance for Salivary Carcinomas

17.12.1 Core Data

As salivary carcinomas are uncommon and of diverse histology, the published evidence for the prognostic importance of

Table 17.16 T and N staging of mucosal malignant melanoma [2]

Stage	Descriptor
T3	Mucosal disease
T4a	Moderately advanced disease
	Tumor involving deep soft tissue, cartilage, bone or overlying skin
T4b	Very advanced disease
	Tumor involving brain, dura, skull base, lower cranial nerves (IX, X, XI, XII), masticator space, carotid artery, prevertebral space or mediastinal structures
No	No regional lymph node metastases
N1	Regional lymph node metastases present

Table 17.17 Main histological criteria for grading olfactory neuroblastoma

Histological feature	Grade 1	Grade 2	Grade 3	Grade 4
Lobular architecture	Present	Present	Partial	Partial
Pleomorphism	Minimal	Present	Prominent	Marked
Neurofibrillary matrix	Prominent	Present	May be present	Absent
Rosettes	Present	Present	May be present	May be present
Mitoses	Absent	Present	Prominent	Marked
Necrosis	Absent	Absent	Present	Prominent

Adapted from Barnes et al. [13]

Table 17.18 T staging of malignancies of the major salivary glands

Stage	Descriptor
T1	Tumor 20 mm or less in greatest dimension without extraparenchymal extension
T2	Tumor more than 20 mm but not more than 40 mm in greatest dimension without extraparenchymal extension
T3	Tumor more than 40 mm and/or tumor with extraparenchymal extension
T4a	Tumor invades skin, mandible, ear canal or facial nerve
T4b	Tumor invades base of skull or pterygoid plates or encases carotid artery

specific histological features is less strong than for mucosal carcinomas. The core data items represent a summary of published evidence and consensus expert opinion and include:

- Histological type of carcinoma
- Histological grade of malignancy
- Maximum diameter of the tumor
- Distance from carcinoma to nearest resection margin
- Macroscopic extraparenchymal extension of carcinoma
- Perineural invasion
- Lymph node involvement

The histological type of carcinoma according to the WHO classification [13] is required for classification and cancer registration and is a predictor of biological behaviour on univariate analysis in most cohort studies; histological type is less important than stage on multivariate analysis [40]. The size of a salivary malignancy (T stage) is consistently reported to be a major prognostic factor for treatment outcome and survival [40–42]. The maximum diameter of the tumor should be recorded in millimetres based on macroscopic assessment, confirmed or amended after microscopy.

The adequacy of surgical clearance is an independent factor in determining local control of disease and survival [42]. The distance from the tumor to the nearest resection margin should be measured in millimetres based on macroscopic assessment, confirmed or amended after microscopy. In the report summary, the same criteria as for squamous cell carcinomas at mucosal sites may be used: >5 mm is clear, 1–5 mm is close and <1 mm is involved.

Macroscopic extraglandular extension to involve adjacent structures is a predictor of local recurrence and nodal metastasis for parotid carcinomas [40, 43, 44]; the evidence at other sites is by extrapolation. Note that microscopic extraglandular extension is less significant. Perineural invasion is a common finding and may be diagnostically useful in adenoid cystic carcinomas and in polymorphous low-grade adenocarcinomas. Neurological symptoms suggesting invasion of the VII or VIII cranial nerves are predictors of nodal disease and poor outcome, but the independent prognostic relevance of histological invasion of the perineural plane varies between published studies [44]. The involvement of cervical lymph nodes by a salivary malignancy is a major prognostic factor for treatment outcome and survival [41]. The T staging of malignancies of the major salivary glands is shown in Table 17.18.

17.12.2 Grading of Salivary Malignancies

The grade of salivary carcinomas is related to the risk of local recurrence, regional and distant metastasis but is of less importance than stage [44, 45]. The histological type of carcinoma is broadly related to grade and to the risk of local or regional recurrence, although there are exceptions to the general schema (e.g. low-grade variants of salivary duct carcinoma) and some carcinomas may show progression to higher grade or may dedifferentiate. Details of the criteria for grading acinic cell carcinomas, mucoepidermoid carcinomas and adenoid cystic carcinomas are provided in Chap. 5.

17.12.2.1 Carcinoma in Pleomorphic Adenoma

Carcinomas arising in pleomorphic adenomas may be of any histological type, but are thought to be particularly

aggressive, and the prognosis of the carcinomatous component is poorer than that of comparable carcinomas developing de novo. Evidence for a pre-existing adenoma (remnants of myxochondroid stroma, focal scarring, hyalinised nodular 'ghost') should be sought in all carcinomas, particularly those showing multiple histological types and a varied histological appearance. The extent of invasion should be measured in these tumors as it is prognostically useful, although precise criteria are not defined. Invasion more than 5–6 mm from the capsule of the residual adenoma is associated with a high risk of local recurrence and distant metastasis [46, 47].

17.12.3 Minor Salivary Gland Tumors

The core data items and grading criteria for malignancies of minor salivary glands are the same as for major glands, although the evidence is less extensive. Multivariate analysis in a large series of minor salivary carcinomas suggests that the main predictors of nodal disease are T3–T4 carcinomas, high-grade carcinomas and those arising in the pharynx [48]. When reporting minor salivary gland tumors, we suggest that the dataset for squamous cell carcinomas of the appropriate primary site is used and adapted to include the histological type and, where appropriate, grade of salivary carcinoma.

17.12.4 Molecular Markers for Diagnosis, Prediction and Prognosis

Gene expression profiling correlates fairly well with the morphological classification of salivary carcinomas, although the MECT1-MAML2 (also known as CRTC1-MAML2) fusion oncogene separates a prognostically favourable subset of mucoepidermoid carcinomas from the more aggressive mucoepidermoid carcinomas [49, 50].

17.13 Reporting Guidelines for Skin Malignancies

17.13.1 Core Data Items for Basal and Squamous Cell Carcinoma

Core/essential data items for skin malignancies vary according to the type of malignancy but generally include diagnosis, stage and margin status. In addition, clinical management guidelines for basal cell carcinoma and squamous cell carcinoma recommend categorisation into low- and high-risk carcinomas. Risk status is a combination of clinical and pathological features [51, 52]; as all clinical factors may not be available to the pathologist, it is recommended that pathological features of high risk are recorded (Table 17.19).

The measurement of tumor size and margins is based pragmatically on the macroscopic measurement provided that this is confirmed microscopically. In practice, for small lesions and most margins of skin excisions, the microscopic measurement is the easiest to verify, acknowledging that there is likely to be some tissue shrinkage during specimen handling in the laboratory.

Tumor thickness should be measured as a Breslow thickness from the granular layer or, if the surface is ulcerated, from the ulcer base to the deepest extent of invasion by contiguous tumor cells. Tumor thickness should be recorded in mm, and whole integers suffice. The core requirement is measuring peripheral and deep margins and categorising as <1 mm, 1–5 mm and >5 mm.

Vascular invasion requires the presence of tumor within an endothelial-lined space; it is not necessary to distinguish lymphatic and venous invasion.

Table 17.19 Pathological features of basal and squamous cell carcinomas associated with a high risk of progressive disease [7, 53]

Basal cell carcinoma	Squamous cell carcinoma
Morphological subtype:	Morphological subtype:
Infiltrating/morphoeic carcinoma	Invasive squamous cell carcinoma associated with in situ squamous cell carcinoma (Bowen's disease)
Micronodular carcinoma	Acantholytic, desmoplastic and spindle cell variants
Basosquamous carcinoma	Poorly differentiated carcinomas (necrosis and a high mitotic rate)
	Tumor thickness:
	>2 mm has metastatic risk
	>10 mm very high metastatic risk and increased mortality
Level of invasion into subcutis (Clark level 5) or deeper tissues	Level of invasion into or beyond the reticular dermis (Clark level 4)
Lymphatic invasion	Lymphatic invasion
Invasion of the perineural plane	Invasion of the perineural plane
pT stage greater than pT1 (>20 mm diameter)	pT stage greater than pT1 (>20 mm diameter)

17.13.2 Nodal Involvement by Cutaneous Squamous Cell Carcinoma

As for primary mucosal carcinomas of the head and neck, the number of nodes involved and maximum size of metastatic deposit and the presence or absence of extracapsular spread are stage determinants and markers of biological aggression [7].

17.13.3 Melanoma

Staging of cutaneous malignant melanoma should be based on AJCC 7th edition for Skin Cancer [54]. An important change in this edition is the use of mitotic index rather than Clark level for early melanoma staging. AJCC regards primary cutaneous staging as also applying to primary melanomas of the skin of the lips and skin of the external ear. The core data that should be recorded for cutaneous melanomas is summarised in Table 17.20.

The prognostic value of the TNM classification is largely based on the grouping of nodular and superficial spreading malignant melanomas. Pure desmoplastic malignant melanoma (>90 % desmoplastic phenotype) has a better prognosis, reduced tendency for lymph node metastasis but greater propensity for local recurrence. Other subtypes are of uncertain prognostic relevance.

Increasing thickness of melanoma correlates with increasing metastatic risk and decreased survival and is a principal T-stage parameter. Thickness should be measured to the deepest extent of invasion by tumor cells from the granular layer or, when present, the ulcer base. Deep extension along peri-appendigeal sheaths, microsatellites and areas of regression are not measured. Melanoma thickness is a continuous variable and all staging criteria are therefore arbitrary. In general, Breslow thickness should be measured to a minimum of one decimal place to allow accurate staging, although measurement to two decimal places is required at the staging boundaries – pT1/2 (1.01 mm), pT2/3 (2.01 mm) and pT3/4 (4.0 mm).

Table 17.20 Core pathological data for cutaneous melanoma [8]

Primary site	Histopathological (sub)type
	Breslow thickness
	Ulceration
	Mitotic index
	Vascular invasion
	Microsatellites and in-transit metastases
	Perineural invasion
	Growth phase
	Tumor-infiltrating lymphocytes
	Regression
	Clark's level 4/5
	Margin status
Lymph nodes	Number of positive nodes
	Extracapsular spread

Ulceration is a dominant independent prognostic factor for clinically localised primary cutaneous malignant melanoma. The extent of ulceration adds prognostic information, with a worse prognosis if the ulceration is greater than 5 mm diameter or more than 70 % of the lesion.

A mitotic index of one or more per mm^2 (in the most proliferative area) is an adverse prognostic feature for invasive melanoma.

The presence of lymphovascular invasion correlates with a worse survival. Angiotropism, with tumor cells surrounding blood vessels, appears to have a particularly bad prognosis.

The presence of melanoma in the perineural plane or within nerve fibres (intraneural invasion) correlates with a higher recurrence rate and is particularly common in desmoplastic malignant melanoma.

Tumor-infiltrating lymphocytes (TIL) are a specific host immune response, an AJCC7 site-specific prognostic factor and an essential parameter for use of the AFIP 8-year survival prognostic model. The AFIP survival model defines three levels of immune response: absent, non-brisk (patchy/discontinuous lymphocytes in the tumor) and brisk (continuous lymphocytic infiltration around the periphery or within the tumor).

Regression is identified through a mixture of the destruction of melanoma cells in the dermis, a variable lymphohistiocytic infiltrate, fibrosis and melanophages and is a site-specific prognostic factor and an essential parameter in the AFIP 8-year survival model [8].

17.13.3.1 Microsatellite/In-Transit Metastasis

Microsatellite/in-transit metastasis is a principal pN-stage parameter in AJCC7 (stage pN2c) and associated with increased locoregional recurrence and reduced disease-free and overall survival.

The definitions for local metastasis vary between tumor types. For cutaneous melanoma, the AJCC recommends [54]:

- Satellite metastasis is visible grossly within 20 mm of the primary cutaneous melanoma.
- Microsatellite metastasis is more than 0.05 mm diameter and is visible microscopically at least 3 mm and less than 20 mm of the primary cutaneous melanoma.
- In-transit metastasis is more than 20 mm from the primary melanoma.

17.13.3.2 Growth Phase and Clark's Levels

Growth phase is a site-specific prognostic for melanoma and an essential data item for the use of the AFIP 8-year survival prognostic tables [55].

Radial growth-phase melanoma theoretically has no metastatic potential and a 100 % survival rate and can have either an in situ or invasive component (the so-called microinvasive melanoma). The dermal cells in an invasive radial growth phase are either solitary or in small clusters and lack mitotic activity. The lesions are usually less than 1 mm thick and restricted to Clark level 2.

Vertical growth-phase melanoma is always invasive and is defined as one or more nests of more than ten melanoma cells within the dermis. The presence of one mitotic figure within malignant dermal melanocytes indicates vertical growth phase. Melanomas that invade into Clark level 3 and below are usually vertical growth phase and the tumors are often thicker than 1 mm.

Clark levels are defined as follows:

Level 1. Confined to the epidermis.
Level 2. Tumor cells within the papillary dermis and/or periadnexal connective tissue sheath. The cells do not fill or expand the papillary dermis.
Level 3. Tumor cells fill and expand the papillary dermis. They form an almost curvilinear line at the interface between the papillary and reticular dermis. This is usually identified by the position of the superficial vascular plexus.
Level 4. Invasion of the reticular dermis.
Level 5. Invasion of the subcutaneous fat.

In the AJCC guidance, 'mitotic rate' replaces Clark level as the staging parameter defining pT1a versus pT1b, in the absence of ulceration [54].

17.13.3.3 Margin Status

Local recurrence of primary cutaneous malignant melanoma is influenced by the completeness and adequacy of primary excision, each of which in turn is influence by the clinical context. In general, reports should include measurements of peripheral (radial) and deep margins rather than judgements on completeness and adequacy. A practical grouping of histological margin status is: involved (0 mm), less than 1 mm, and 1 mm or greater, measured to the nearest millimetre. Tissue shrinkage after excision and during processing is estimated at 10–20 % for skin specimens [56], so 'clinical margins' will be greater than histological margins.

17.13.3.4 Lymph Nodes

The number of nodes positive for micrometastasis or macrometastasis is a primary determinant of pN stage [2]. In sentinel node biopsies, the microanatomic location of micrometastases is important, with micrometastases confined to the subcapsular sinus being associated with a lower incidence of nodal involvement elsewhere.

Extracapsular invasion is a manifestation of potential biological aggression, is associated with a worse prognosis and prompts consideration of adjuvant chemotherapy.

17.13.4 Merkel Cell Carcinoma

A staging system for cutaneous Merkel cell carcinoma and regional lymph nodes was introduced in 2010 [54]. This applies to Merkel cell carcinomas on the vermilion lip but not to those on the eyelid (staged as AJCC7 eyelid carcinoma). The AJCC7 staging thresholds between pT1, pT2 and pT3 are at 20 mm and 50 mm [54]. Invasion of bone, muscle, fascia or cartilage is a determinant for stage pT4. A positive node with microscopic disease is stage pN1a and with macroscopic disease pN1b.

Merkel cell carcinoma is characterised by small blue cells with a high mitotic count and apoptosis. The diagnosis must be supported by immunohistochemical expression of cytokeratin 20 and no expression of TTF-1, CD45, S100 or melan-A. Negative immunocytochemistry is required to designate a node as free from carcinoma [57].

In-transit metastasis defines stage N2. The criteria for in-transit metastasis are different from those for melanoma, and it is defined as any discontinuous nest of cells greater than 0.05 mm in diameter that is clearly separated from the main invasive carcinoma by at least 1 mm of normal dermis [57]. Over 30 % of Merkel cell carcinomas are associated with another malignancy.

Core data for Merkel cell carcinoma [57] are:

- In-transit metastasis.
- Lymphovascular invasion.
- Presence of second malignancy.
- Margins (recorded as for cutaneous malignant melanoma).
- Maximum diameter.
- Lymph node involvement.
- Lymph node extracapsular invasion and margin status are adverse prognostic features.

17.14 Reporting Guidance for Thyroid Malignancies

17.14.1 Core Data Items

The core data items applicable to all thyroid malignancies [6] are:

- Type of malignancy
- Presence of minor component of poorly differentiated tumor

- Presence of any anaplastic carcinoma
- One lesion or multifocal disease
- Maximum diameter (macroscopic diameter, confirmed histologically)
- Vascular invasion
- Extrathyroidal invasion (macroscopic or microscopic)
- Distance to nearest surgical margin (R stage)
- Lymph nodes – site(s), number of nodes and number of involved nodes

Differentiated tumors which contain a minor (<50 %) component of more poorly differentiated tumor (recognised by necrosis and/or mitoses) may have a worse prognosis. If there is any focus of anaplastic carcinoma, the tumor is classified as stage pT4.

Any thyroid neoplasm may have oncocytic features and the diagnosis of malignancy is based on the same criteria as for non-oncocytic neoplasms. Oncocytic variants of thyroid carcinomas, by definition, comprise >75 % oncocytic cells. The stage-stratified prognosis for oncocytic carcinomas is worse than for their non-oncocytic equivalents, largely due to oncocytic carcinomas having poor radio-iodine uptake.

The total number of lymph nodes sampled and the number involved are prognostic in papillary carcinoma, while the site of involved nodes affects staging and prognosis. There is insufficient evidence on the impact of extracapsular spread to include this as a core data item.

Unlike other head and neck malignancies, the pathological resection (R) stage of the tumor is considered to be a useful assessment [6]. This is applied as follows:

- RX. Cannot assess presence of residual primary tumor, e.g. a disrupted specimen
- R0. No residual primary tumor – i.e. definite non-tumor tissue between the tumor and the surgical margin microscopically. The distance should be stated in the report.
- R1. Microscopic residual primary tumor: any microscopic tumor at the surgical margin.
- R2. Macroscopic residual primary tumor: this may be recognised by the surgeon or on macroscopic examination of the specimen and should be confirmed microscopically.

Needle core biopsies have limited value and cannot be used to distinguish between follicular carcinoma and adenoma. Core biopsies may be used to distinguish between inflammatory or fibrotic conditions and diffusely infiltrating tumors or for the diagnosis of tumors other than those of follicular type.

17.14.2 Papillary Carcinoma

The prognosis for classical papillary carcinomas is similar for encapsulated and infiltrating disease, and there is no prognostic impact for the solid variant. Tall cell and columnar variants of papillary carcinoma are associated with poorer prognosis [58]. The outcome of the diffuse sclerosing variant is uncertain. The literature does not provide consistent guidance on the prognostic value of BRAF mutations.

Cystic PTC is assigned a diameter which equates to that of the cyst containing it, but insufficient data exist to determine whether its prognosis is that which would be expected from the diameter of the cyst or is that of the volume of tumor within the cyst.

Follicular variant papillary thyroid carcinoma (FVPTC) shows an exclusively or almost exclusively follicular architecture with papillary carcinoma nuclei. Non-encapsulated FVPTC is clinically and genetically similar to classical papillary carcinoma. Encapsulated variant of FVPTC has a clinical behaviour and molecular genetic profile similar to follicular adenomas and carcinomas and should be assessed on the presence or absence of capsular and vascular invasion. Encapsulated, non-invasive FVPC has a very low risk of recurrence or metastasis. Encapsulated, invasive FVPC requires the presence of capsular and/or vascular invasion but only vascular invasion relates to a low risk of distant metastasis [59].

Diffuse, multinodular variants are rare, occur typically in younger patients and have more frequent vascular invasion, lymph node and distant metastases.

17.14.2.1 Papillary Microcarcinoma

'Microcarcinoma' is defined as <10 mm in diameter and is staged pT1a. A solitary, classical papillary microcarcinoma discovered incidentally probably does not have a significant risk of recurrence or metastasis. Histological markers of potentially more aggressive disease [60] are:

- Multifocal and/or bilateral disease
- Six millimetre or more in diameter
- Extrathyroid extension, especially if macroscopic
- Desmoplastic fibrosis and/or infiltrative growth pattern
- Poorly differentiated component
- Lymphovascular invasion

17.14.3 Follicular Neoplasms

A follicular neoplasm has no nuclear features of papillary carcinoma and is defined as follicular carcinoma on the basis of capsular and/or vascular invasion. Capsular invasion is the complete penetration of the tumor capsule; vascular invasion is the invasion of blood vessels within the tumor capsule or adjacent thyroid tissue; the tumor cells should project substantially into the vessel lumen and may have attached thrombus.

Minimally invasive follicular carcinomas are encapsulated and show focal microscopic vascular and/or capsular invasion. Tumors showing only capsular invasion have a minimal risk of metastasis and no treatment beyond lobectomy may be appropriate. Tumors with any vascular invasion (angioinvasive) have a risk of metastasis. It is likely that the risk of metastasis is greater if four or more foci of vascular invasion are present [61].

Widely invasive follicular carcinoma shows gross invasion or extensive microscopic infiltration of thyroid parenchyma, capsular or extratumoral vessels or extrathyroidal tissues. Widely invasive tumors have a worse prognosis than minimally invasive lesions, and the prognosis worsens as the number of foci of vascular invasion increases and if there is macroscopic extension beyond the thyroid capsule.

17.14.4 Medullary Carcinoma

In best practice, the diagnosis should be confirmed by calcitonin immunoreactivity. In poorly differentiated tumors, where calcitonin expression is lost, expression of carcinoembryonic antigen may be useful. Loss of calcitonin immunoreactivity, the presence or absence of amyloid and tumor desmoplasia do not affect prognosis or management [62].

17.14.5 Poorly Differentiated Carcinoma

This group of tumors, which includes insular carcinomas, has a prognosis intermediate between differentiated follicular cell-derived tumors and anaplastic carcinomas [61]. Poorly differentiated carcinomas are follicular neoplasms in which >50 % of the tumor shows necrosis and/or a mitotic count of five or more per 2 mm^2.

17.14.6 Undifferentiated/Anaplastic Carcinoma

Where a follicular or papillary carcinoma shows even a minor undifferentiated (anaplastic) component, the diagnosis is undifferentiated/anaplastic carcinoma. Most undifferentiated tumors will be diagnosed by FNA or open biopsy and are not usually treated by surgery. Immunohistochemistry for markers of differentiated thyroid cells (thyroglobulin, TTF1 and calcitonin) is almost always negative. Immunocytochemistry for cytokeratins may confirm the epithelial nature. PAX 8 expression is useful in distinguishing anaplastic thyroid carcinoma (generally positive) from metastatic lung carcinoma (generally negative). Often, however, the diagnosis is one of exclusion achieved through correlation with an aggressive imaging appearance.

17.15 Reporting Guidance for Soft Tissue Malignancies

17.15.1 Core Data Items

Core data that may be derived from resection specimens [63] are:

- Site.
- Depth from surface.
- Size of tumor in millimetres – macroscopic measurements should be given unless different on histology.
- Histological type and subtype according to the WHO consensus classification of 2013 [64].
- Grade.
- Tissue planes involved – skin/subcutis or deep fascia/subfascial tissue.
- Relationship to margins.
- Stage.
- Cytogenetic and molecular genetic findings (for small round cell tumors).

17.15.1.1 Grade

The grading system commonly used in Europe is that of the French Federation of Cancer Centres Sarcoma Group [65]. This has three categories based on the summation of scores for differentiation, mitotic index per 2 mm^2 (in the most proliferative area) and amount of necrosis (macroscopic assessment). For low-grade smooth muscle tumors where the mitotic index is critical for assessing malignancy or metastatic potential, mitoses should be counted in 10 mm^2.

17.15.1.2 Relationship to Margins

The clearance (in millimetres) and the type of tissue (e.g. fascia, fat, muscle or skin) at the nearest surgical margin should be given along with comments on the nature of the invasive margin (infiltrative versus pushing) and on vascular invasion.

17.15.1.3 Stage

Soft tissue sarcomas are staged using the staging system of the UICC (TNM) [2] or the American Joint Committee on Cancer (AJCC) [54]. Isolated pathologic staging is of limited value and formal tumor staging should be completed after correlation with imaging.

17.15.2 Specific Aspects of Individual Tumors

The terms ‘atypical lipomatous tumor’ and ‘well-differentiated liposarcoma’ are used synonymously, with the latter preferred for deeply located tumors because of their increased risk of dedifferentiation.

Extremity pleomorphic sarcomas with myogenic differentiation have a worse prognosis when matched for other variables. Myogenic differentiation is assessed on the immunohistochemical expression of myogenic markers such as smooth muscle actin, desmin, smooth muscle myosin, h-caldesmon, MyoD1 or myogenin.

In most sarcomas it is not necessary to assess the proliferation index as well as the mitotic count. However, for grade 2 or grade 3 sarcomas, the proliferation index (Ki67 index) might have predictive value [66].

17.16 Reporting Guidance for Bone Tumors

17.16.1 General Aspects

The 2013 World Health Organization (WHO) classification of bone tumors [64] combines morphological and genetic data to provide a uniform system of classification and standardised nomenclature for the diagnosis of benign and malignant bone tumors. The diagnosis and management of bone tumors depend on close cooperation between the surgeon, oncologist, radiologist and pathologist as the diagnosis may be influenced by the age and sex of the patient as well as the location of the lesion and the bone involved. Where relevant, information should be available on the presence or absence of a pre-existing skeletal disease, a history of a familial syndrome or any occupational history or previous chemotherapy or irradiation that may predispose to bone malignancy.

17.16.2 Core Data Items

The core data items for reporting resection specimens of bone malignancies [67] are:

- Tumor location (which bone and where in that bone)
- Tumor size
- Histological diagnosis (WHO histological classification [64])
- Tumor grade, where relevant
- Tumor necrosis (approximate percentage) in response to any preoperative therapy
- Extent of local tumor spread
- Excision margin status (distance in millimetres to margin and nature of tissues present)
- Cytogenetic and molecular genetic findings (where relevant)

17.16.2.1 Grading and Staging

Histological grading provides a guide as to the biological behaviour of the tumor based on morphological and cytological features, mitotic activity and the extent of tumor necrosis [63]. For some high-grade monomorphic tumors (such as Ewing's sarcoma), the grade is defined by histological type.

Bone sarcomas are staged using either the AJCC staging system [54] or the Musculoskeletal Tumor Society (MSTS) Staging System [68]. The AJCC and MSTS systems have many similarities and separate stage 1 and 2 sarcomas on whether they are intraosseous or extraosseous. The AJCC system subdivides stage I and stage II tumors on the basis of tumor size (threshold of 8 cm diameter) and recommends that tumors with skip metastases are classified as stage III. Formal staging of a bone tumor should be carried out in a multidisciplinary setting where clinical, radiological and histological information are correlated.

17.16.2.2 Extent of Local Tumor Spread

The extent of local bone and soft tissue spread should include comment on tumor involvement of specific anatomical components or compartments, e.g. medulla, cortex, joints and extraosseous soft tissues. This assessment is made macroscopically and confirmed or amended microscopically. The extent of local spread determines whether the tumor is intracompartmental or extracompartmental.

17.16.3 Assessment of Preoperative Chemotherapy

The effectiveness of preoperative chemotherapy for the treatment of high-grade sarcomas, particularly osteosarcoma and Ewing's sarcoma, is determined by the extent of tumor necrosis as a percentage of the total tumor area. For both types of sarcoma, greater than 90 % necrosis is associated with a more favourable prognosis [69]. The extent of necrosis is assessed on the blocks of the slab specimen of resected bone. Large atypical cells with hyperchromatic nuclei, smudged or clumped chromatin and vacuolated cytoplasm in areas of necrosis, calcification or fibrosis are often seen after neoadjuvant chemotherapy of osteosarcomas and currently considered to represent viable tumor cells.

17.16.4 Specific Aspects of Individual Tumor Types

17.16.4.1 Ewing's Sarcoma

The morphological diagnosis of Ewing's sarcoma should be confirmed by immunohistochemical expression of CD99, which is usually strong, diffuse and membranous. CD99 expression is not specific for Ewing's sarcoma and can be seen in other round cell tumors such as in lymphoblastic lymphoma, small cell osteosarcoma and mesenchymal chondrosarcoma. Cytogenetic or molecular techniques should

ideally be used to identify the presence of a characteristic Ewing's sarcoma-associated translocation.

17.16.4.2 Osteosarcoma

The morphological diagnosis of osteosarcoma requires the demonstration of osteoid or bone formation by malignant tumor cells. Specific morphological subtypes of intramedullary osteosarcoma, such as telangiectatic osteosarcoma, small cell osteosarcoma, giant cell-rich osteosarcoma and low-grade well-differentiated osteosarcoma, have prognostic and therapeutic significance. Surface osteosarcomas should be subclassified as either parosteal (juxtacortical), periosteal or high-grade surface osteosarcoma.

17.16.4.3 Chondrosarcoma

The clinical and radiological features of a suspected cartilage tumor should be carefully correlated with the pathological findings. Histological grading based on the degrees of cellularity, nuclear atypia and myxoid change is prognostically relevant [70]. The recognition of specific subtypes, including periosteal, mesenchymal, clear cell and dedifferentiated chondrosarcomas, is important as it influences prognosis and may alter treatment.

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