Antonio Cardesa
Pieter J. Slootweg
Nina Gale
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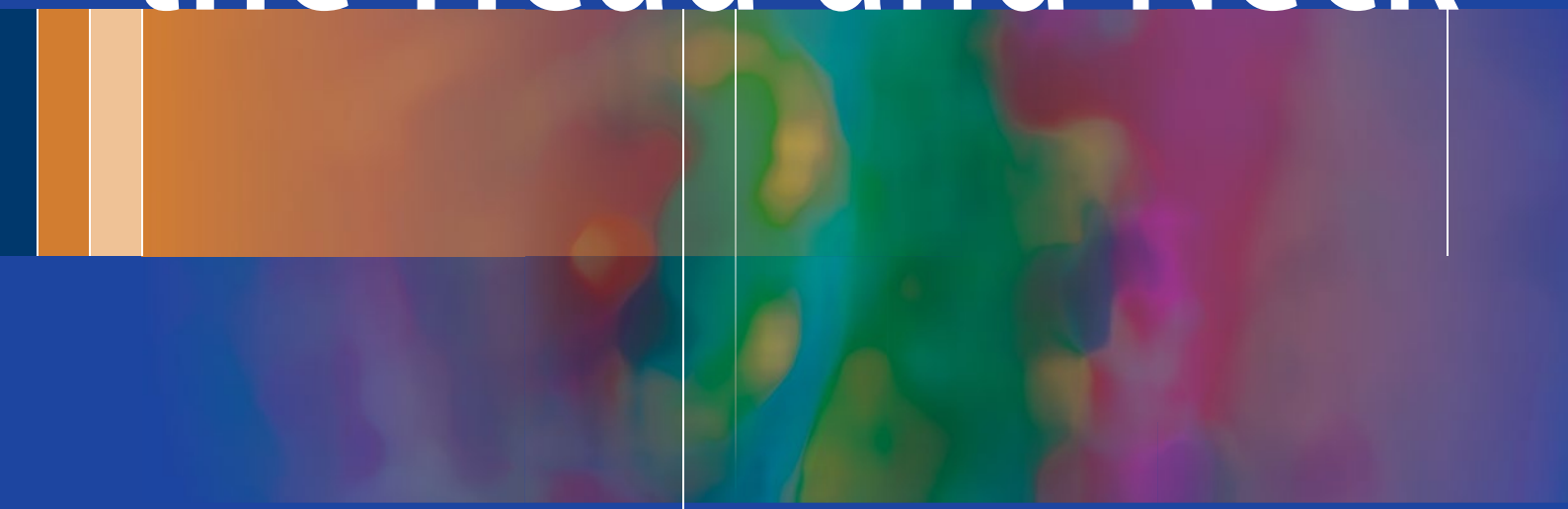
Editors

Pathology of the Head and Neck



Second Edition

Springer

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ISBN 978-3-662-49670-1 ISBN 978-3-662-49672-5 (eBook)
DOI 10.1007/978-3-662-49672-5

Library of Congress Control Number: 2016955862

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer-Verlag GmbH Berlin Heidelberg

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.

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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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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

A. Cardesa et al. (eds.), *Pathology of the Head and Neck*, DOI 10.1007/978-3-662-49672-5_1

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).

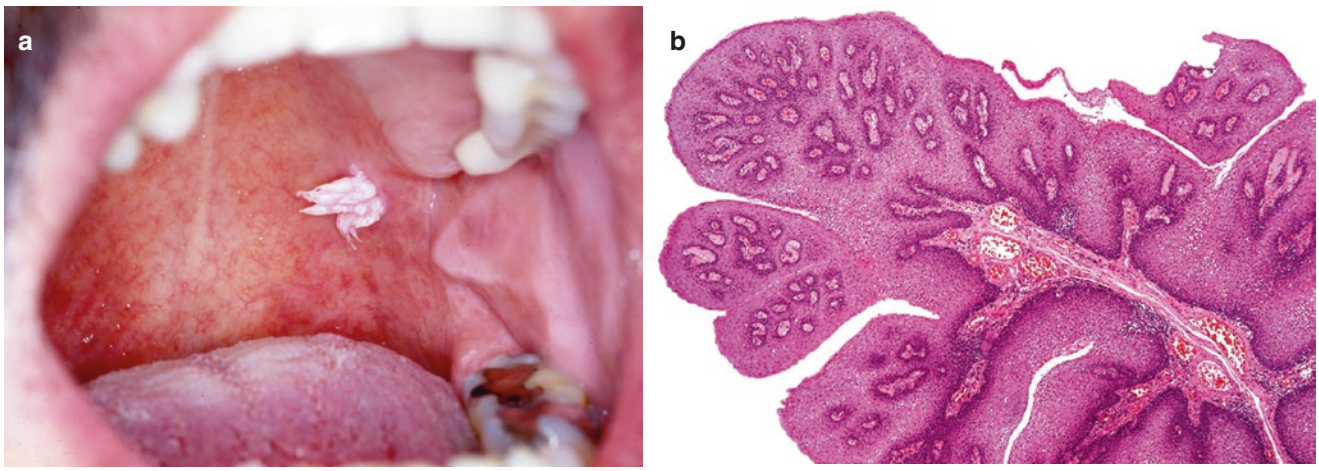

Fig. 1.1 Oral squamous cell papilloma. (**a**) Whitish papillary lesion of the palate (Courtesy of Dr. J. Fischinger, Ljubljana, Slovenia). (**b**) Projections of fibrovascular stroma are covered by parakeratotic squamous epithelium

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).

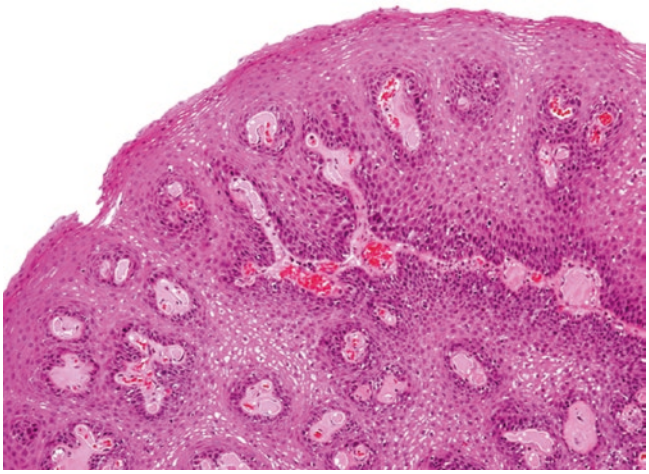

Fig. 1.2 Focal epithelial hyperplasia (Heck's disease). Prominent acanthosis of the squamous epithelium with broad rete ridges, papillary surface is not present

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

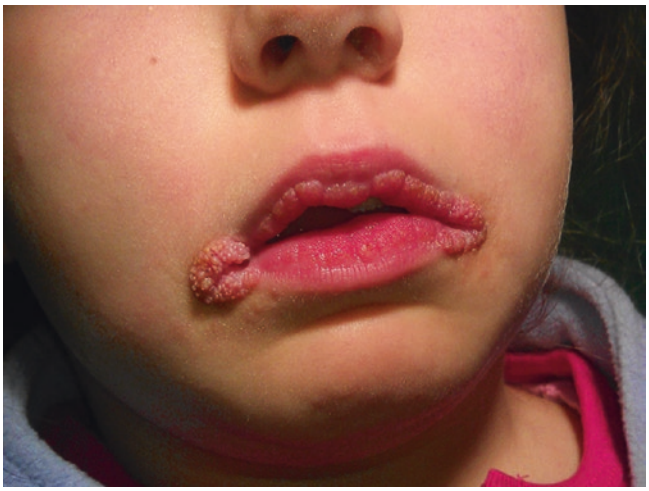

Fig. 1.3 Congenital systemic epidermal nevus syndrome. A confluent papulous and verrucous lesion has to be separated from HPV-induced papillary lesions of the oral cavity (Courtesy of Dr. T. Dovšak, Ljubljana, Slovenia)

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,

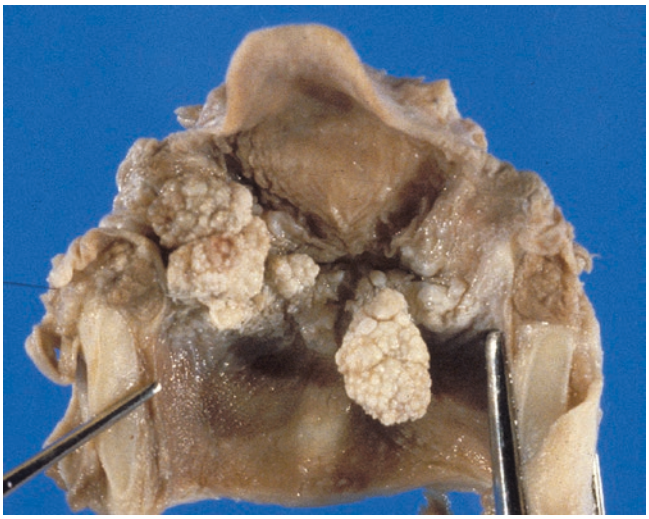

Fig. 1.4 Laryngeal papillomatosis. Numerous clusters of papillomas obliterate the laryngeal lumen

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

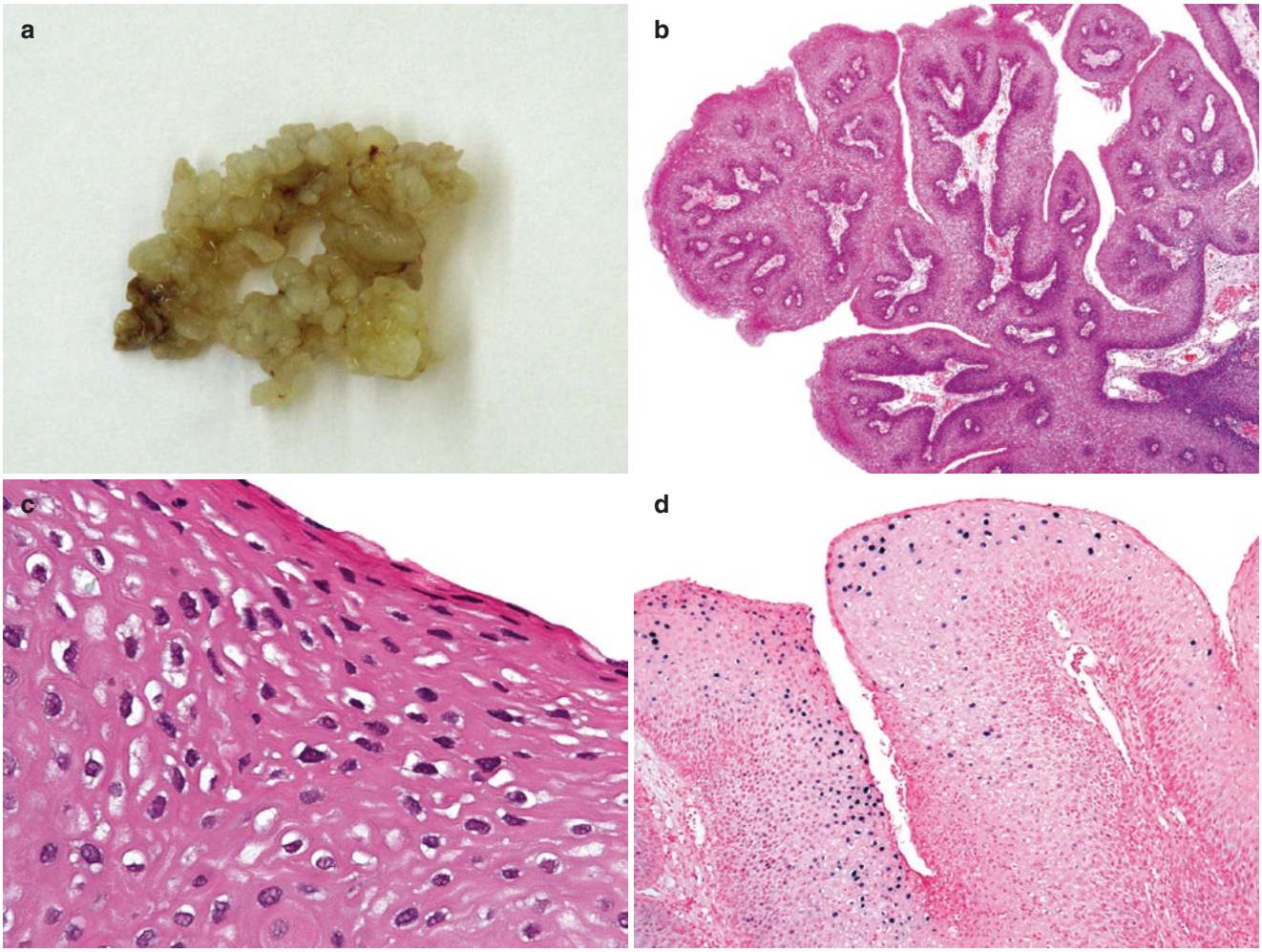

Fig. 1.5 Laryngeal papillomatosis. (**a**) Several pieces of clusters of multilobulated papillomatous lesion. (**b**) Branches of laryngeal papillomatosis is covered with hyperplastic squamous cell epithelium. (**c**) Numerous koilocytes are seen in the upper part of the epithelium. (**d**) Positive 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].

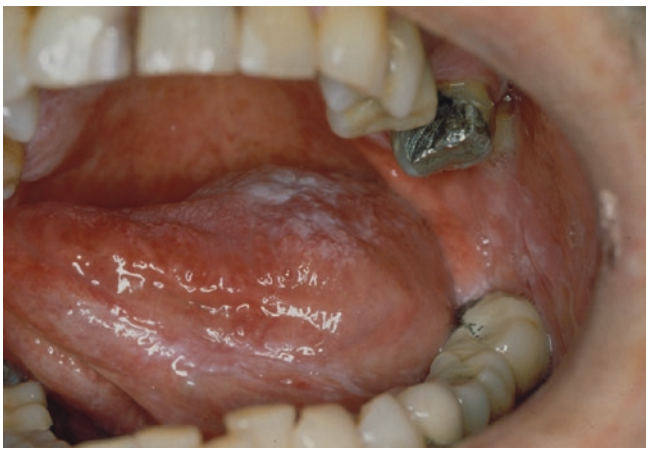

Fig. 1.6 Leukoplakia of the dorsal tongue. The microscopic diagnosis was low-grade squamous intraepithelial lesion with superficial parakeratotic layer (Courtesy of Dr. J. Fischinger, Ljubljana, Slovenia)

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.

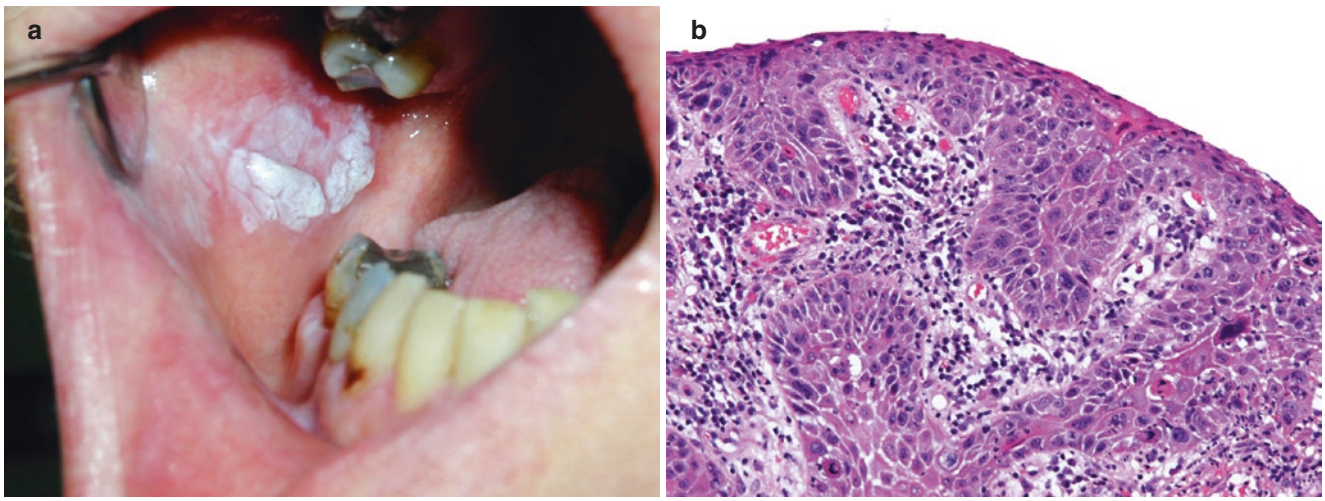

Fig. 1.7 Erythroleukoplakia of the buccal mucosa. (**a**) Thickened whitish plaques with uneven surface and with speckled foci of erythema on the periphery of the lesion (Courtesy of Dr. D. Dovšak, Ljubljana, Slovenia. (**b**) Histologically, architectural and cellular atypias meet the criteria of carcinoma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

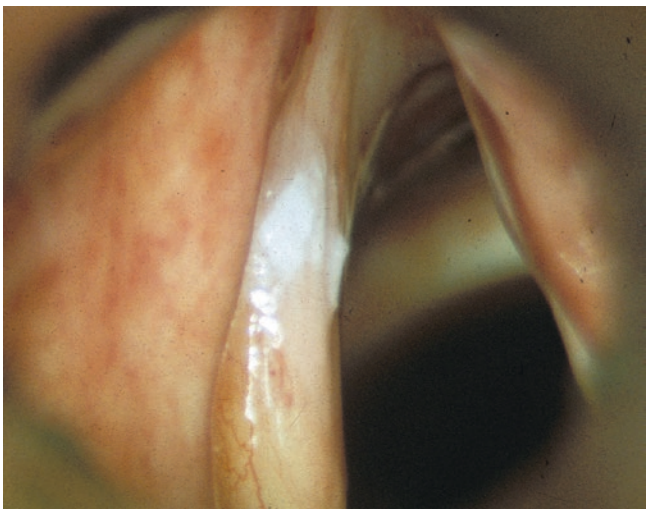

Fig. 1.8 Leukoplakia of the left vocal cord. The middle third of the left vocal cord is covered with a well-circumscribed whitish plaque. The microscopic diagnosis was low-grade squamous intraepithelial lesion with a prominent superficial keratotic layer

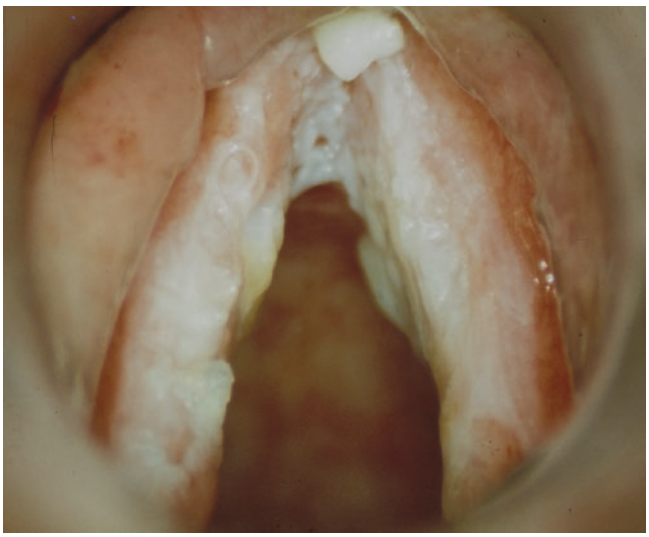

Fig. 1.9 Chronic laryngitis. Both vocal cords are irregularly thickened and covered by whitish plaques. The microscopic diagnosis was high-grade squamous intraepithelial lesion

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

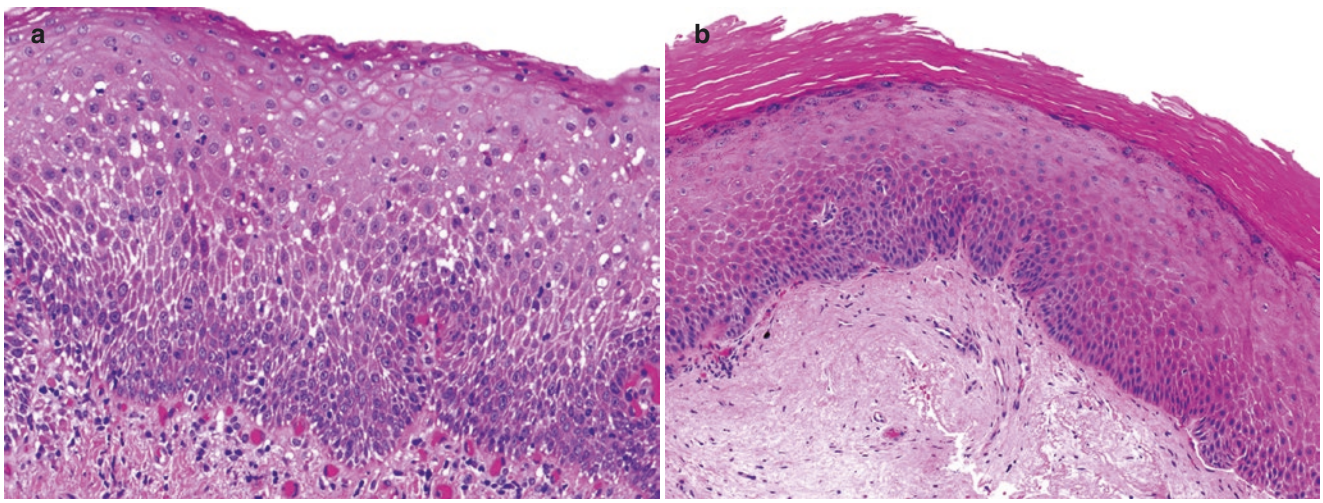

Fig. 1.10 Low-grade squamous intraepithelial lesion. (**a**) Thickened squamous epithelium with increased number of reactive basal–parabasal cells in the lower part of the epithelium. The upper part of the epithelium is unchanged. (**b**) Thickened squamous epithelium with prominent keratotic layer shows 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.

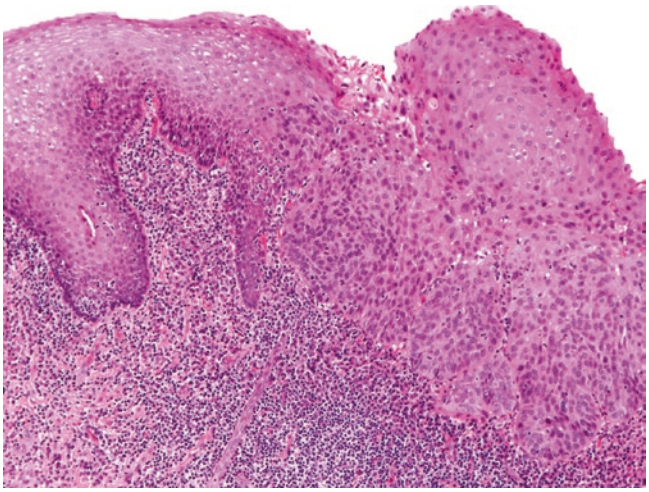

Fig. 1.11 Transition from low-grade to high-grade intraepithelial lesion. Hyperplastic squamous epithelium with a slightly increased number of basal–parabasal cells transfers sharply to the high-grade lesion with atypical epithelial cells occupying partially the whole epithelial thickness

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

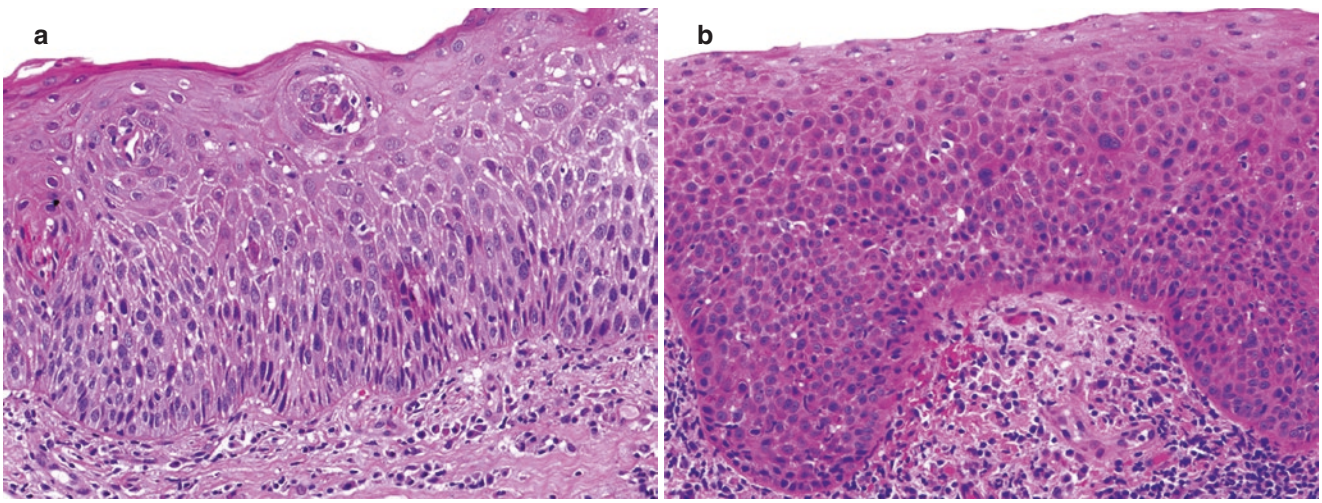

Fig. 1.12 High-grade squamous intraepithelial lesion. (**a**) Augmented epithelial cells showing mild to moderate grades of atypias, the cells are aligned perpendicularly to the basement membrane. (**b**) Atypical epithelial cells occupy almost the whole epithelial thickness with increased nuclear/cytoplasmic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

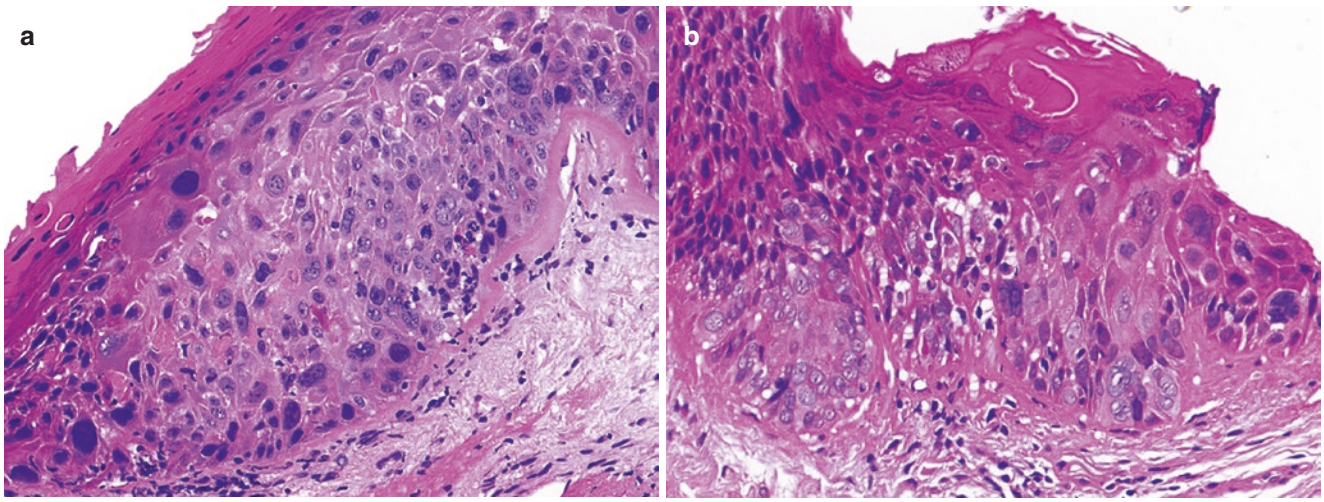

Fig. 1.13 Carcinoma in situ. (**a**, **b**) The epithelium shows loss of stratification and polarity, polymorphic malignant cells replace almost the entire epithelial thickness, mitotic activity is increased; the surface of the epithelium is covered by a few layers of compressed, horizontally oriented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

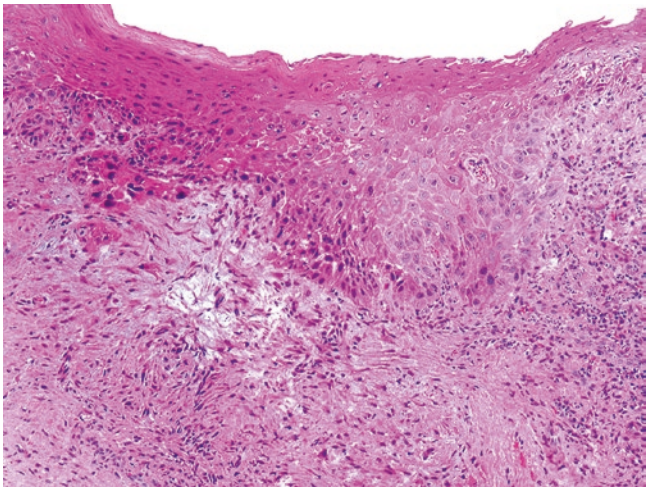

Fig. 1.14 Microinvasive squamous cell carcinoma: invasion of carcinoma into superficial stroma

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].