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Chapter

Laryngeal Leukoplakia: A Focus on Histology

Giuseppe Leoncini

Abstract

Leukoplakia is a clinical term referring to a whitish plaque on the mucosal surfaces that cannot be scraped off. Otolaryngologists daily have to face such findings in both the oral cavity and the larynx. In the latter, several pathological conditions ranging from reactive to neoplastic lesions can underlie leukoplakia. Hence, a proper understanding of the histological spectrum of laryngeal diseases sharing leukoplakia as their main clinical presentation plays a critical role in the clinical management of patients. In that setting, the histological assessment of laryngeal dysplasia is known to have represented a matter of disagreement mostly about grading, and several grading systems have been proposed over time. Nonetheless, the histologic assessment of laryngeal leukoplakia is a mandatory requirement in clinical planning, leading to a proper treatment choice.

Keywords: leukoplakia, dysplasia, larynx, grading, differential diagnosis

1. Introduction

Laryngeal leukoplakia (LL) is a clinical term referring to the presence of a whitish plaque on mucosal membranes that cannot be scraped off [1]. Leukoplakia is a descriptive term having not a single histological counterpart but, on the contrary, a broad spectrum of pathological pictures, ranging from benign (reactive-inflammatory) lesions to dysplastic and neoplastic conditions. Establishing a proper diagnosis represents a unique challenge since the optimal clinical management depends on the identification of the reactive or dysplastic nature of that lesion. LL can be found at any laryngeal site, mostly onto the true vocal folds. Regardless of the clinical presentation, LL should be histopathologically examined to rule out both dysplasia and invasive carcinoma, which should be properly treated. Further complexity in managing LL relies on the frequent location along the vibratory side of the vocal folds, where scarring could produce functional consequences and voice quality impairment. Mucosal biopsies performed with flexible laryngoscope represent to date the most widely used diagnostic technique to obtain an earlier diagnosis. Early detection is the strongest prognostic factor affecting the patients’ survival. However, the biopsy technique has some limitations, as mucosal sampling could underestimate the severity of the lesion first because of the poor depth of biopsy, then because of the histological heterogeneity of broader lesions [2, 3].
2. Histopathological assessment

2.1 Vocal folds’ normal anatomy and histology

The true vocal folds are laryngeal structures consisting of both vocalis muscle and vocal ligament, covered by stratified squamous epithelium. The free edge is formed by the vocal ligament and its epithelial lining, representing the mobile top of the vocal folds and contributing to phonation. The vocal ligament is in continuity with the cricovocal (cricothyroid) ligament, extending from the thyroid cartilage to the vocal process of the arytenoid cartilages. The vocalis muscle lies laterally to the vocal ligament, extending from the vocal process of the arytenoids to the vocal ligament [4]. The true vocal folds are lined by nonkeratinized stratified squamous epithelium, as seen in the superficial rim of the ary-epiglottic folds, in the anterior epiglottic surface (Figure 1). In contrast, ventricle, ventricular folds, saccule, and subglottis are covered by ciliated columnar epithelium, with scattered goblet cells. Seromucinous glands can be found in small clusters within the loose stroma of the false vocal fold, whereas they are scattered in the lamina propria of ventricle, saccule, posterior epiglottic surface, subglottis. Seromucinous glands are usually scanty or absent in the true vocal folds [5]. The false vocal fold has no contractile structures, is covered by respiratory-type epithelium, and represents the upper limit of the ventricle, whereas the true vocal fold is the lower limit.

2.2 Histopathology

LL is an umbrella clinical term including several histological conditions. Squamous cell hyperplasia (or keratosis) is qualified by the thickening of a pre-existing squamous epithelium, usually involving basal and prickle cells. It can encompass the presence of conspicuous keratin layer, composed of a nuclear keratin scales (orthokeratosis) or by squamous cells with picnotic nuclei and dense cytoplasm (parakeratosis). A preserved cellular differentiation, based on both normal nuclear-to-cytoplasmic ratio and base-to-top epithelial maturation, qualifies epithelial hyperplasia excluding dysplasia. Squamous cell hyperplasia can show exuberant features, represented by prominent squamous tongues.
simulating an infiltrative growth into the underlying stroma, thus mimicking well-
differentiated squamous carcinoma. Such lesion is referred to as **pseudo-epitheliomatous hyperplasia**, a reactive lesion that can associate with chronic conditions. Squamous cell hyperplasia should be distinguished by **squamous metaplasia**, referring to the replacement of the ciliated respiratory-type epithelium by stratified squamous epithelium. It is a reactive phenomenon involving, by definition, those anatomical sites covered by non-squamous epithelium and can extend to the sero-mucinous glands. The term **diskeratosis** is sometimes used - but not widely accepted - to describe a focal abnormal maturation in the squamous lining, involving one or few cells displaying abnormal keratinization. It belongs to the spectrum of reactive squamous lesions.

### 3. Laryngeal leukoplakia in nonneoplastic diseases

The main histological feature of LL is represented by the epithelial thickening (hyperplasia) with preserved epithelial maturation or cellular atypia. Conversely, the presence of abnormal epithelial maturation and cellular atypia should raise suspicion for dysplasia. Despite the clinical setting of LL, dysplasia should be always suspected and ruled out through a proper histological assessment of mucosal specimens. In this regard, biopsy interpretation represents a critical part of the patient’s management.

#### 3.1 Phonotrauma

LL represents the main clinical presentation of laryngeal dysplasia and carcinoma. Nonetheless, it can also be detected in inflammatory disease of the upper airways. The so-called **phonotrauma** is a common clinical condition deriving from the improper use of phonation and characterized by an altered quality of voice, manifesting with hoarseness, rough or scratchy voice, and vocal fatigue. During phonation, the vocal folds’ vibration represents a major physical threat. Indeed, both the magnitude and directions of the vocal folds’ collision determine mechanical stress and subsequently an injury. The stratified squamous epithelium represents the outermost layer lining the vocal folds, supported by loose stroma and muscle. In some instances, the epithelial damage can extend down to the basal cell layer. Because of impact, stretching, and shearing forces, both the epithelial and the underlying loose connective layers could be affected, the latter being characterized by vascular and fibrotic changes, leading to the vocal folds’ tendency to prolapse toward polyps’ formation. In the setting of phonotrauma, epithelial reactive alterations could be seen, with or without stromal changes. A structural compromission of the vocal folds’ epithelial barrier after acute phonotrauma has been reported by some Authors [6–10]. At histology, the epithelial lining is usually thicker than normal, showing several degrees of dyskeratosis, ranging from apoptotic-like epithelial changes to cytoplasmic clearing and ballooning. Epithelial maturation is preserved, sometimes associated with mild orthokeratosis. Inflammatory cells are usually absent or scanty. At fiberoptic examination, lesions usually appear as a slightly raised white plaque with ill-defined margins. Stromal changes could associate, giving rise to a bulging area or nodular/polypoid lesions.

#### 3.2 Chronic laryngitis

Laryngeal inflammation for at least 3-weeks-long is usually referred to as **chronic laryngitis**. It can result from several etiologies, ranging from infectious (bacterial, viral,
fungal) to chronic inflammatory (tobacco smoke-related and reflux-related) conditions. Both symptoms and laryngoscopy are usually nonspecific. The great majority of bacterial laryngitis has an acute clinical course, characterized by prominent exudates and crusting. Chronic laryngitis should be suspected in those patients with prolonged impairment of the quality of voice. Viral infection is mainly due to Herpes Simplex Virus, which usually causes acute laryngitis, whereas chronic infection is rare. It is characterized by laryngeal edema, mucosal ulceration, and exudates. Fungal infections could run asymptomatic in immune-competent hosts. Such infections are seldom found in the larynx, being more common in the oral cavity. Immune compromise, drugs, previous radiotherapy, intubation, and neoplasms are common predisposing conditions. The most complained symptoms are hoarseness, cough, and local pain. Histology is not routinely obtained in inflammatory laryngeal diseases. When biopsy is performed, the histological picture is characterized by epithelial hyperplasia, ortho- or parakeratosis, and mixed inflammatory cells. The detection of PAS-D positive rod-like structures on the mucosal surface is diagnostic for fungal infection (Figure 2). Particularly, they could be superficially located, with scanty intra-epithelial neutrophil infiltration. Pseudo-epitheliomatous features can be observed. Patients suffering from Gastro-Esophageal Reflux Disease (GERD) frequently complain about laryngeal symptoms over time, such as hoarseness and cough. During the past years, the role of gastric acid reflux in determining laryngeal inflammation has been debated. Though chronic acid gastric reflux can promote laryngo-pharyngeal inflammation, only a minority of patients with clinically diagnosed reflux laryngitis shows pharyngeal reflux, with a similar prevalence in both healthy and reflux-laryngitis patients. Nonetheless, occult laryngeal pathology is known to be common in the adult population and laryngo-pharyngeal reflux has been reported as one of the most prevalent conditions [11, 12]. Mirroring the histologic picture seen at the gastro-esophageal junction, the vocal folds’ mucosa is characterized by a variable degree of epithelial thickening, basal intercellular space dilation (spongiosis), and few intra-epithelial lymphocytes and granulocytes. Mild sub-epithelial edema is not uncommon. Current smokers have a

![Figure 2. Mucosal sample of true vocal fold in patient with laryngeal fungal infection. Note the rod-like structures (arrows) over the superficial epithelium (periodic acid-Schiff-diastase [PAS-D] ×10 magnification).](image)
higher risk to develop LL compared to never-smoker patients [13], with or without dysplasia. Chronic laryngitis can frequently underlie LL in smokers. In such circumstances, vocal folds’ inflammation is characterized by mild-to-moderate subepithelial infiltration composed of lymphocytes, plasma cells, and histiocytes. Squamous cell hyperplasia and variable ortho- and para-keratosis are not uncommon findings. Dysplastic foci or invasive squamous cell carcinoma could associate.

3.3 Laryngeal involvement in systemic non neoplastic diseases

Larynx can be rarely involved in systemic diseases, such as autoimmune diseases that could mimic chronic laryngitis. Laryngeal lichen planus (LP) is a rare - and probably under-recognized - autoimmune disease affecting both skin and mucosal membranes. Although mucosal LP is more frequent in the oral cavity, where it should be distinguished from pemphigoid of the mucosal membranes, laryngeal involvement has been reported as well. As in chronic laryngitis, laryngeal LP harbors sub-epithelial inflammation, squamous hyperplasia, and superficial ortho- and para-keratosis without dysplasia but, in contrast, laryngeal LP usually presents at least focally, with a sub-epithelial “band-like” inflammation, cytoplasmic vacuolization in basal keratinocytes and basal apoptotic (cytoid or civatte) bodies, commonly unseen in chronic laryngitis. The hyperkeratotic appearance of laryngeal LP can also mimic squamous cell carcinoma, which should be always ruled out, since LP can be successfully treated with glucocorticoid-based therapy,
avoiding unnecessary surgical procedures [14]. Among systemic diseases potentially involving the larynx, amyloidosis should be also mentioned. It consists of a disease group characterized by extracellular deposition of insoluble protein in tissues, known as amyloid fibers. Larynx can be involved in both the systemic and localized amyloidosis. In the localized variant the laryngeal location is not uncommon, accounting for about 15% of cases, being the glottic region the most involved laryngeal site. At fiber-optic examination, a raised whitish plaque can be detected. Histological examination is useful to recognize the presence of amorphous material in the subepithelial connective tissue. Squamous hyperplasia is usually absent and, in contrast with other nonneoplastic causes of LL, the gross features of the lesions are related to the amyloid protein accumulation rather than to the epithelial thickening. The use of Congo Red dye is useful to highlight a green apple birefringence using polarized light microscopy (Figure 3) [15, 16].

4. Laryngeal leukoplakia and dysplasia

Laryngeal dysplasia (LD) is defined by a spectrum of both maturation abnormalities and nuclear atypia involving the laryngeal epithelial lining, that may or may not precede an invasive squamous carcinoma. Even though dysplasia includes atypical cellular features, such a term should not be considered synonymous with atypia, as the latter indicates atypical nuclear features alone, excluding the maturation abnormalities. Thus, the two terms should not be used interchangeably. Dysplastic changes encompass crowded immature epithelial cells, loss of cellular polarity, nuclear pleomorphism and hyperchromas, increased nucleus-to-cytoplasm ratio, and mitoses including atypical forms. Such cellular and architectural abnormalities can be found as either superimposed into pre-existing squamous hyperplasia or raised into non-hyperplastic laryngeal epithelium. Hence squamous hyperplasia should not be considered a prerequisite for developing LD. According to the dysplasia model applied for the uterine cervix, LD is defined as mild, moderate, and severe regarding the level of epithelial involvement. In situ carcinoma (CIS-non-keratinizing type) is defined by the full-thickness mucosal epithelial dysplastic change without infiltration of the basement membrane. Conversely to the uterine cervix, the larynx usually harbors keratinizing dysplasia, which exhibits by definition at least focal squamous maturation, making the concept of a full-thickness dysplastic involvement not suitable for laryngeal CIS. As a consequence, the use of the term severe keratinizing dysplasia (SKD) is likely a more appropriate designation. Abnormal supra-basal maturation with dyskeratotic features, mitoses and surface keratinization are needed to qualify the histologic picture of LD as severe. Nonetheless, histopathologic criteria for evaluating laryngeal keratinizing dysplasia are less defined [17–20].

4.1 Grading systems

LD represents the earliest lesion manifesting, at both microscopic and molecular levels, neoplastic features [5]. Although the progression risk differs according to the grading, LD is widely considered the precursor lesion of squamous cell carcinoma (SCC). The LD grading has been a matter of disagreement among clinicians and pathologists, because of terminology was not uniform, grade designation seemed burdened by subjectivity and the risk stratification was often imprecise. The role of grading is mainly focused on the definition of the progression risk toward SCC. Squamous hyperplasia carries a very low risk of developing invasive SCC, whereas the presence of dysplasia increased such a risk [21]. In order to improve uniformity in diagnostic terminology,
several grading systems and classification schemes have been proposed. It was previously suggested that squamous intra-epithelial neoplasia (SIN) was the most suitable term to refer to these epithelial changes since they are regarded as a morphologic manifestation of the noninvasive neoplasia [22, 23]. The Ljubljana Classification improved the concordance degree of histopathologic assessment of LD [24], even though the SIN classification system and the Ljubljana Classification were conceptually different, beyond their terminology. Subsequently, the concept of laryngeal intraepithelial neoplasia (LIN) was introduced, including both dysplasia and CIS. In 2017, the World Health Organization (WHO) recommended a two-tier classification, consisting of low and high-grade dysplasia/intraepithelial neoplasia, based on the severity of both architectural changes and cytological atypia, in order to improve the diagnostic reproducibility (Figure 4) [25, 26].

The concept of laryngeal CIS was introduced in the previous WHO classification (2005) and subsequently removed from the SIN classification, which considered both severe dysplasia and CIS in the SIN3 category. A transient reappraisal of CIS was seen in the amended version of the Ljubljana Classification, distinguishing the high-grade squamous intra-epithelial lesions (SIL) from CIS. Actually, laryngeal CIS has been included in the high-grade dysplasia, according to the latest WHO classification (Table 1).

<table>
<thead>
<tr>
<th>Abnormal maturation</th>
<th>WHO 2005 SIN classification</th>
<th>Ljubljana classification (amended version)</th>
<th>LIN classification</th>
<th>WHO 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal third</td>
<td>Mild dysplasia</td>
<td>SIN 1</td>
<td>Low Grade SIL</td>
<td>LIN 1</td>
</tr>
<tr>
<td>Lower half</td>
<td>Moderate dysplasia</td>
<td>SIN 1 or SIN 2</td>
<td>LIN 2</td>
<td></td>
</tr>
<tr>
<td>More than half</td>
<td>Severe dysplasia</td>
<td>SIN 2</td>
<td>High Grade SIL</td>
<td>LIN 3</td>
</tr>
<tr>
<td>Upper third</td>
<td>CIS</td>
<td></td>
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<td></td>
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<tr>
<td>Full thickness</td>
<td>CIS</td>
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Table 1. Comparison of following grading systems for laryngeal dysplasia.
4.2 Carcinogenic mechanisms

Several causative agents and carcinogenic mechanisms have been suggested in SCC. The concept of field cancerization (FC) was introduced to describe pathologic atypia in epithelial cells surrounding oro-pharyngeal carcinomas. The role of multiple independent carcinogenic events involving different cells has been postulated, suggesting the role played by carcinogen activation on the whole exposed mucosa. Hence, mutant cellular clones can develop within that field giving rise to metachronous secondary tumors, that should be interpreted as secondary primary rather than recurrent tumors [27, 28]. The concept of FC was confirmed by further studies on head and neck SCC recurrences despite therapy [29, 30]. Alternatively, it has been proposed that neoplastic clones could spread laterally, running within the epithelial lining, from the site of the neoplastic primary toward adjacent normal mucosa [31–34]. Besides the oral cavity, FC has been described in the larynx, which can be exposed to tobacco smoke and other environmental carcinogens. Tobacco and alcohol consumption are the most important risk factors, implicated in 75% of all head and neck SCC [35, 36]. Recent molecular findings suggested that such a phenomenon could be promoted by the acquirement of genetic alteration in a cellular subset with stemness properties, giving rise to a clonal cellular progeny characterized by p53 mutation [37]. Moreover, some nutritional, environmental, and occupational factors were claimed to be implied in the development of head and neck malignancies [38].

The carcinogenic role of the Human Papilloma Virus (HPV) infection has been advocated as a causative agent in a subset of LD and SCC. More than 200 different HPV genotypes have been characterized and subclassified into low-, intermediate-, and high-risk types according to their carcinogenic potential. HPV infection was found to play a role in the earliest stage of carcinogenesis, but a direct causative effect lacked to be fully established in laryngeal SCC [39] Moreover, high-risk HPV (hr-HPV) DNA was also detected in healthy laryngeal tissue where it was considered a bystander [40]. The significance of HPV laryngeal infection lacks to be fully elucidated. The carcinogenetic role of HPV infection relies on the viral integration in the host genome

Figure 5.
Laryngeal HPV-related invasive SCC. Note the immunohistochemical p16(red) and Ki67(DAB) co-staining (x 40 magnification).
and disruption of intracellular control pathways. Indeed, the interaction of the viral subunits E6 and E7 with cell-cycle regulatory proteins p53 and retinoblastoma respectively has been established for integrated and transcriptionally active hr-HPV and contributed to promoting carcinogenesis [41, 42]. Low-risk genotypes such as HPV-6 and HPV-11 are related to recurrent laryngeal papillomatosis [43]. Despite the carcinogenetic role of HPV infection being a controversial topic in the larynx, the causative involvement of hr-HPV (e.g., HPV-16) in the pathogenesis of peculiar SCC subtypes, such as the verrucous [44] and papillary [45] variants have been reported [46, 47]. HPV-related SCC of the larynx and hypo-pharynx are mostly non-keratinizing cancers. In papillary SCC the prevalence of HPV infection is variable, and its oncogenic role remains a matter of concern [48]. Non-keratinizing SCC is an emergent variant of HPV-related laryngeal SCC. It is the most frequent histologic pattern in HPV-related SCC of the head and neck (Figure 5) [49]. In contrast with laryngeal SCC, the potential role of HPV infection in lung cancer is actually not supported [50].

5. Laryngeal leukoplakia and squamous cell cancer

Any neoplastic infiltration beyond the basement membrane into the underlying connective tissue should be referred to as invasive SCC. The micro-invasive SCC is considered the earliest invasive lesion. It is defined by the presence of scattered malignant cells or discrete foci or tongues of neoplastic cells within the submucosa, just below the basement membrane. They are excluded from microinvasive laryngeal carcinomas both CIS, because non-invasive by definition, and those lesions show vascular invasion and muscle or cartilage involvement. Some authors established 1–2 mm as a cut-off to identify an SCC as microinvasive, others proposed the extension into the stroma by <0,5 mm, as measured from the basement membrane of the closest non-neoplastic epithelium [51–53]. The assessment of microinvasion on biopsy can be challenging because mucosal specimens could be superficial and not comprehensive of the invasive component. Thus, caution should be paid in excluding an invasive component when full-thickness malignant cells’ replacement is seen on small biopsy specimens since it could lead to an underestimation of micro-invasive SCC. Furthermore, integrity gaps in the basement membrane alone do not stand for a micro-invasive carcinoma, as the evidence of neoplastic foci in the sub-epithelial connective tissue is a necessary diagnostic requirement. Once evaluated on biopsy, such findings should be accepted as noninvasive carcinoma with reservations, until the assessment of the full surgical excision [54]. Multiple sections of the whole surgical specimen should be examined to confidently rule out invasion. The colonization of seromucinous glands by dysplastic epithelial cells should not be misinterpreted as micro-invasion. Microinvasive SCC can be connected to the overlying dysplastic epithelium or not. The lack of such a morphologic continuum does not exclude the diagnosis of microinvasive SCC, as severe dysplasia is not a prerequisite for developing an invasive SCC, in the larynx as well as in the whole upper aerodigestive tract. The invasive nests must have unequivocal malignant cytological features and mitoses, including atypical forms.

The superficially extending SCC identifies a more advanced laryngeal lesion, invading beyond those histological limits introduced for defining microinvasion, without muscle or cartilage involvement [23]. The neoplastic invasion beyond the basement membrane makes the tumor capable of gaining access to the lymph-vascular channels, resulting in metastatic disease. A peculiar behavior is seen in glottic SCC, which
is usually not associated with metastatic spread in the early stage of disease compared to supra- and sub-glottis since this laryngeal compartment is characterized by a paucity of lymphatic drainage [55].

Invasive SCC is characterized by a spectrum of gross and histopathological features. Grossly, the larynx can harbor exophytic, flat, and ulcerated masses. From a histological perspective, both keratinizing and nonkeratinizing tumors can be found. Several growth patterns can be detected, ranging from conventional to papillary, verrucous, spindle cells, basaloid, and undifferentiated SCC. Neoplastic invasion can be characterized by both neoplastic cords or tongues attached to the superficial epithelium and scattered cells or dyscohesive neoplastic clusters in the lamina propria. Squamous differentiation can vary from well-differentiated to poorly differentiated forms. Association to CIS is not an uncommon finding.

5.1 Histological variants of laryngeal SCC

5.1.1 Papillary SCC

It affects men more than women. The larynx is the most common location, even though it can be seen in the oral cavity and hypopharynx. Papillary SCC usually occurs de novo since the occurrence of cancer at the site of previous papilloma has been rarely reported. The role of HPV infection has been established by in situ hybridization study in such cancer variants [56]. The tumor presented as an exophytic mass, histologically characterized by finger-like projections supported by fibrovascular cores or by a broad-based cauliflower-like growth pattern. Surface keratinization is usually scanty or absent, differentiating the papillary from the verrucous subtype of SCC. Cytologic malignant features are evident in the neoplastic epithelium of the papillary SCC, again differentiating it from verrucous SCC.

5.1.2 Verrucous SCC

It is a highly differentiated variant of SCC affecting men more than women, mostly in the laryngeal glottis. Tumor growth is locally destructive, without metastatic potential. Tobacco smoking and viral induction have been suggested as etiologic factors. The tumor presented as an exophytic mass, histologically characterized by a benign-appearing proliferation composed of uniform squamous cells without significant atypia nor mitoses, prominent surface warty-like keratinization, and a broad base with pushing-type margins. A mixed chronic inflammatory cells infiltrate can be seen in the stroma. Hybrid tumors composed of conventional and verrucous SCC have been described in the head and neck [57].

5.1.3 Spindle cells SCC

It is a highly aggressive biphasic tumor composed of both SCC (CIS or invasive) and spindle cells malignant neoplasm, affecting men more than women. In the larynx, both the glottis and the supra-glottic region can be involved. Previous irradiation has been involved as a risk factor, whilst there was no significant correlation with tobacco smoking, alcohol consumption, and occupational/environmental factors [58, 59]. Spindle cells SCC are not related to HPV infection [60]. Tumor mass usually presents as exophytic neoplasm consisting in a malignant undifferentiated spindle cells proliferation with SCC nests. The spindle cells’ component
usually predominates, and it is characterized by prominent cytological atypia and frequent mitoses, often associated with necrotic foci. The growth pattern can vary from fascicular to storiform and palisading. Areas of stromal collagenization and myxomatous degeneration can be seen. Heterologous elements could be detected, mostly consisting of chondro- and osteo-sarcomatous foci. Epithelial derivation is supported by the intimate relationship with conventional SCC and by epithelial markers’ expression. Spindle cells were found to express cytokeratins in the majority of cases, even though vimentin expression and myogenic differentiation have been reported [18, 60, 61]. Overall, spindle cells SCC usually behave malignantly, even though flat and ulcerating lesions have a worse prognosis if compared to exophytic variants [62]. Reactive myo-fibroblastic proliferations, mucosal malignant melanomas, and sarcomas should be considered in the differential [63–65].

5.1.4 Basaloid SCC

It is a high-grade variant of SCC involving palatal tonsils, tongue, hypopharynx, and larynx, the latter being mostly affected in the supraglottic region. Known etiologic factors are tobacco smoking and alcohol consumption. Tumor grossly presents as a firm whitish mass, associated with central necrosis. Basaloid cells, characterized by atypical hyperchromatic nuclei and scanty cytoplasm, increased mitotic activity, and peripheral nuclear palisading, are intermingled with conventional SCC. Mucoid hyaline stromal deposition can be seen. Basaloid cells usually expressed epithelial markers, even though Vimentin could be expressed in a subset. Adenoid cystic and neuroendocrine carcinoma should be considered in the differential [66, 67].

5.1.5 Undifferentiated (lymphoepithelioma-like/nasopharyngeal type) SCC

Undifferentiated (lymphoepithelioma-like/nasopharyngeal type) SCC can rarely affect larynx and hypopharynx. Such tumors resulted more prevalent in the Chinese population and related to EBV infection. At histology, keratinizing and nonkeratinizing forms have been described, being the latter further subdivided into differentiated and undifferentiated types [68].

5.2 Molecular prognostic markers

The risk of progression is known to vary in dysplastic LL according to the grading of dysplasia [69, 70]. The use of biomarkers to highlight the cumulative effect of genetic mutations can aid in a more accurate establishment of progression in LL. Prognostic biomarkers can be subdivided into four categories: (1) proliferation; (2) cell cycle control; (3) cell adhesion and invasion; (4) immune checkpoints. Malignant cells are known to acquire a high proliferative rate, that can be monitored by using several markers. Ki67 is a nuclear protein widely studied as proliferative marker, even though it does not represent a reliable marker of malignant transformation in laryngeal dysplasia [71–74]. TP53 is a well-established tumor suppressor gene involved in head and neck SCC. The loss of wild-type p53 activity, as well as p16 and cyclin D1, were frequently detected in many cancer types and were found to be involved in tumor progression [75, 76]. A specific isoform of CD44 (CD44v6) was established to interact with Osteopontin, which is known to be elevated in many cancer types and correlates with laryngeal SCC progression in the larynx [73]. Beta-catenin protein is coded by CTNNB1 gene and is involved together with E-cadherin in intercellular adherence.
and epithelial structure maintenance. Alteration in beta-catenin protein expression plays a role in cancer progression and invasiveness [77]. In the past few years, tumor inflammatory microenvironment has gained more attention. In that setting, both tumors devoid of immune infiltrates and others marked by abundant T cell infiltrates have been detected. Programmed cell death protein 1 (PD1/CD279) is a member of the CD28 family of T cell co-stimulatory proteins that includes CTLA-4, ICOS, and BTLA. It has two specific ligands PD-L1 (B1-H1/CD274) and PD-L2 (B7-H2/CD273) which down-regulate T cell activation on binding to PD1. The PD-1/PD-L1 interaction represents a critical immune checkpoint in the adaptive immune resistance of SCC. Immunohistochemical assays have been employed to evaluate the expression of immune checkpoints in the tumor microenvironment, but limitations have been outlined by many authors, including the use of different antibody clones (including 5H1, E1L3N, SP142, 28–8, 22C3, SP142, and SP263) and the lack of a standardized scoring system. [78–80].

6. Conclusions

The clinical management of LL is a daily critical challenge for Otolaryngologists, who must be aware of the broad spectrum of pathological conditions that could underlie leukoplakia. An effective clinical-pathological correlation represents the basis for proper treatment planning in such patients.

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Conflict of interest

The author declares no conflict of interest.

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Updates on Laryngology


