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PRACTICAL DERMATOLOGY

Oral Leukoplakia: Clinical, Histopathologic, and Molecular Features and Therapeutic Approach

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Abstract. Dermatology departments are currently seeing an increase in the number of cases of oral diseases. Of note among the range of lesions covered by this broad area of knowledge is oral leukoplakia—the most common precancerous lesion and the most problematic in terms of both diagnosis and therapeutic management.

In addition to defining leukoplakia, this review also establishes a differential diagnosis with the other most important oral diseases and analyzes the different clinical, histologic, and molecular features that can provide an indication of the risk of malignant transformation. Finally, a therapeutic algorithm is presented to help us optimize clinical management of the disease.

Key words: leukoplakia, tobacco, therapy, risk of transformation.

LA LEUCOPLASIA ORAL: DEFINICIÓN DE PARÁMETROS CLÍNICOS, HISTOPATOLÓGICOS Y MOLECULARES Y ACTITUD TERAPÉUTICA

Resumen. Actualmente existe un incremento del número de casos de patología oral en los servicios de Dermatología. Dentro de las diversas lesiones que forman parte de esta amplia área de conocimiento destacamos la leucoplasia oral, por ser la lesión mucosa precancerosa más frecuente y la que mayores problemas origina tanto en su diagnóstico como en su manejo terapéutico. En esta revisión, además de definir la leucoplasia, establecemos un diagnóstico diferencial con el resto de las patologías orales más importantes y analizamos los diversos parámetros clínicos, histológicos y moleculares que definen el riesgo de transformación maligna de esta patología. Finalmente se establece un algoritmo terapéutico que nos ayuda a optimizar su manejo clínico.

Palabras clave: leucoplasia, tabaco, terapia, riesgo de transformación.

Introduction

Oral cancer is the most common type of cancer of the head and neck, with an annual worldwide incidence in excess of 300 000 cases. The disease is an important cause of death and morbidity, with a 5-year survival of less than 50%.[1,2] One of the new approaches for control of this cancer is early detection of leukoplakia—considered as the most common premalignant oral lesion of the oral cavity and present in 60% of patients diagnosed with oral squamous cell carcinoma.[3] The presence of such lesions is also a risk marker for oropharyngeal cancer.[4]

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Definition of Leukoplakia

The term leukoplakia refers to a clinical entity defined by the World Health Organization (WHO) as "a white patch or plaque that cannot be characterized clinically or histologically as any other disease."[5] In daily practice, the definition is completed by including the observation that it cannot be removed by simple scraping, thereby distinguishing it from pseudomembranous candidiasis.[6] The prevalence of oral leukoplakia ranges from 0.4% to 0.7% of the population. The distribution by sex is variable and its incidence is higher in patients who are habitual smokers or drinkers.[7]

Table 1. Clinicopathological Features That Define Dysplastic Oral Epithelium

Increased cell and nuclear volume and pleomorphism
Hyperplasia of basal cells
Presence of hyperchromatic nuclei
Prominent enlarged nucleoli
Increased nuclear:cytoplasmic ratio
Premature keratinization of independent cells
Increase in mitotic index
Mitosis above the basal layer
Loss of cell polarity
Decreased cell adhesion
Enlarged/fused epidermal ridges

The importance of these lesions lies in the risk of developing squamous cell carcinoma as between 3% and 17.5% are thought to undergo malignant transformation. Oral leukoplakia has been found in between 16% and 62% of squamous cell carcinomas at the time of diagnosis, and so leukoplakia is closely related to head and neck cancer.[8-10] These differences in percentages of malignant transformation can be explained by the different criteria used to define leukoplakia, and by the geographic diversity associated with different social habits and genetic variations.

Leukoplakia, along with erythroplasia, lichen planus, and submucosal fibrosis, forms part of a set of precancerous oral lesions. Prevention of malignant transformation is critical as the 5-year survival rate after diagnosis of squamous cell carcinoma is 50%.[1,2]

A comparative study between leukoplakias that did and did not undergo malignant transformation in the long term identified a series of clinical, histological, and molecular features that define a group of leukoplakias known as "at high risk of malignant transformation." The possibility of detecting this variant would allow closer monitoring to be established and a more aggressive therapeutic approach to be followed.

Etiology of Leukoplakia

Diagnosis of leukoplakia can be ruled out if an etiologic factor for the whitish plaque can be established, except in the case of smoking. Infections by *Candida*, human papillomavirus (HPV), and, more recently, Epstein Barr virus (EBV) have been identified as cofactors that may affect the prognosis of established leukoplakia.

Leukoplakia and Smoking

Tobacco smoke is a powerful carcinogen and considered—along with chronic alcoholism—as the most important risk factor for developing head and neck cancers.[1,2] Thus, in a smoker with oral leukoplakia, it is necessary to differentiate between the so-called stomatitis nicotina or "smoker's palate," and true leukoplakia.

Stomatitis nicotina is a clinical picture characterized by whitish lesions on the surface of the soft palate or the floor of the mouth, in which tobacco smoke is the direct causal agent[11] (Table 1). This causal relationship is demonstrated by complete resolution of the lesions after smoking cessation for a period of 4 to 6 months. A typical feature of this clinical picture is a fingerprint-like or pumice-like pattern, in which a fine whitish striation on the surface resembles a fingerprint.[12] Typically, histological study reveals a "Christmas tree," "bell tower," or v-shaped keratinization pattern. These lesions have no malignant potential and result from the proliferative activity caused by tobacco smoke through the induction of epidermal growth factor receptor (EGFR), which subsequently activates cyclin D1.[12]

However, long-term exposure to this carcinogen may increase the frequency of mutations in the oral mucosa and, indirectly, cause genomic instability,[6] which is clinically manifest as loss of the fingerprint pattern and lack of resolution of the lesion after smoking cessation. In such cases, the plaque could be considered as leukoplakia according to the above definition.

Leukoplakia and *Candida*

There is much debate about whether *Candida* species are implicated in the etiology or progression of leukoplakias. Different types of nitrosamine-producing *Candida* species (other than the common *albicans* variant) have been isolated from clinically nonhomogeneous leukoplakias with histological dysplasia.[13,14] The lesion does not resolve on eradication of this surface mycosis, but eliminating the fungus does lead to transformation of the high-risk nonhomogeneous leukoplakia variant into a low-risk homogenous form. Fungal superinfection is therefore considered as a significant risk factor for oncogenesis.[15]

Leukoplakia and Papillomavirus

The possible implication of HPV in the etiology and potential for malignant transformation of oral precancerous lesions has been extensively studied, given the importance of this agent in cervical uterine cancer.[16-18]

However, it remains open to debate whether the virus acts as an etiological agent in the development of leukoplakia or simply represents a superinfection. The most studied HPV types, using in situ hybridization techniques, include types 6, 11, 16, 18, 31, 33, and 35. There is a well-established link between HPV-16 and HPV-18 infection and squamous cell carcinoma of the cervix.[19] It remains somewhat controversial however whether an association exists in the oral region. In a recent study, compared to the normal epithelium, a 2-to-3-fold higher incidence of HPV infection and 4-to-5 fold greater incidence of squamous cell carcinoma were detected in oral precancerous lesions.[19] High-risk HPV types are more frequently associated with squamous cell carcinoma than low-risk types; it can therefore be concluded that HPV-16 infection is a risk factor for malignant change in leukoplakia regardless of the harmful effects that tobacco smoke and alcohol might have on oral mucosa.

The proliferative verrucous variant has often been associated with HPV coinfection, in particular with serotypes 16 and 18, given the oncogenic potential of these HPV types in other mucosas and the rapid progression of this clinical variant. However, using polymerase chain reaction (PCR) techniques, Bagán et al[20] failed to detect HPV infection in a prospective study of 13 proliferative verrucous leukoplakias, placing in doubt the pathogenic relationship.

Leukoplakia and Epstein-Barr Virus

EBV has been implicated in a large number of cancers, and some studies have found it to be an etiologic factor in oral squamous cell carcinoma.

In a recent preliminary study, no evidence was found of a causal link between proliferative verrucous leukoplakia and EBV; however, the study was conducted in a small number of patients, and so an etiologic association cannot be ruled out.[21]

Pathogenesis of Leukoplakia

A better understanding of the molecular biology of cancer development is the only way to optimize our chances of predicting the oncogenic potential of leukoplakia. Recently, molecular biology studies have found that a variable percentage of oral leukoplakias are associated with molecular abnormalities also found in oral squamous cell carcinoma. These abnormalities reflect oncogenic potential regardless of histological atypia. In fact, the appearance of these cytogenetic abnormalities has been described in leukoplakias without cell atypia.

Loss of Heterozygosity or Microsatellite Instability

Loss of heterozygosity in a cell is the loss of normal function of the allele of a gene whose homologous allele was previously inactivated. This prior deactivation occurs in parental germ cells and is transmitted to their offspring to generate cells that are heterozygotic for the gene in question.

The development of this phenomenon in regions of the chromosome with tumor suppressing genes could be related to the process of malignant transformation.[22] The loss of heterozygosity in oral leukoplakia and its possible predictive value have been reviewed recently by Zhang and Rosin,[23] who establish that lesions with such loss limited to chromosomes 3p and/or 9p would form part of the group of leukoplakias of intermediate risk, with a 3.8-fold increase in risk of malignant transformation, whereas lesions with loss from 3p and/or 9p and loss from one or more of the 4q, 8p, 11q, 13q, and 17p chromosomes would be considered as high-risk leukoplakias, with a 33-fold greater risk of progression to cancer. Finally, low-risk leukoplakias are considered those that do not present any of the above losses of heterozygosity.

Study of these molecular alterations in resection margins complements histological information and can demonstrate the presence of molecular abnormalities at borders that are histologically free of disease—an observation which could explain recurrences and the development of oral squamous cell carcinoma.[24] In fact, molecular confirmation of complete resection of precancerous lesions was shown to be strongly correlated with a decreased risk of oral carcinoma in patients with high- or intermediate-risk leukoplakias, in contrast to low-risk forms where no such correlation was found.[25]

Aneuploidy

The DNA content, or DNA ploidy, provides information on the degree of genetic instability and aberrations in the genomic sequence. In the case of neoplasms, genetically stable diploid cells are replaced by unstable aneuploid cells. This abnormality has been studied using flow cytometry techniques in squamous cell carcinoma of the oral cavity. The studies performed by Sudbø et al[24] focussed on measuring the ploidy status in oral leukoplakias with histological features of dysplasia. Those authors found that aneuploidy in dysplastic leukoplakia was a prognostic marker for progression to carcinoma, regardless of whether the resection margins were free of disease. Thus, of the dysplastic lesions analyzed, 70% were diploid lesions considered to be of low risk (3% progressed to squamous cell carcinoma), 13% were tetraploid lesions

of intermediate risk (60% progressed to squamous cell carcinoma), and aneuploid lesions were considered the high-risk variant. The high-risk group accounted for 17% of all dysplastic lesions, with a rate of malignant transformation of 84%. Although the results obtained by Sudbø et al[24] have been called into question,[26] further studies are necessary to determine the prognostic value of this promising marker.

p53

Mutation in p53, a tumor suppressor gene, is the most common genetic abnormality in human cancers.[27] The physiological function of this gene is to prevent the accumulation of genetic damage in the cells by repair prior to cell division or induction of cell death through apoptosis. p53 plays a crucial role in DNA repair and cell-cycle regulation after carcinogen-induced damage to the DNA of the oral epithelium. The protein is activated by phosphorylation of its serine residues in response to carcinogen-induced cell stress, such that the level of active p53 protein within the cells determines response to carcinogen-induced DNA damage. With a low or intermediate cell content of p53, cells progress to detention of the cell cycle to allow DNA repair. However, if the cell content of p53 is high, cells enter apoptosis. When a p53 gene mutation occurs, the protective pathway of the cells fails, and malignant transformation may occur in the epithelium. In fact, it has been shown that in the presence of a p53 gene mutation, keratinocytes will enter apoptosis after irradiation with UV-B. However, when the p53 has accumulated 2 or more mutations, malignant transformation occurs.

Natural p53 has a short half-life and is often present within epidermal keratinocytes in such low quantities that they cannot be detected by immunohistochemical techniques. In contrast, the mutant form of p53 has a longer half-life, and so it can accumulate within epidermal cells, allowing them to be visualized with immunohistochemical stains.

The marked lack of specificity for the p53 mutation as a prognostic factor for malignancy probably arises because it is impossible to detect the p53 mutated variant selectively using immunohistochemical assays.[28,29] Thus, this increased positivity in immunohistochemical staining for p53 may represent an increase in nonmutated p53 in response to external aggressions. It would therefore be recommendable to combine immunohistochemical and molecular assays in order to establish the prognostic value of this mutation.

Poeta et al[30] analyzed the presence of p53 mutations in squamous cell carcinoma of the head and neck and found a prevalence of 53% in their patients. This study found a statistically significant association between p53-disrupting mutations and decreased survival after surgical removal of squamous cell carcinoma of the head and neck. The mutation was found to be an independent risk factor for poor prognosis.

Telomerase Activity in Leukoplakia

Telomerase is an enzyme with polymerase activity formed from a protein-RNA complex. It is produced in embryonic germline cells and its function is to lengthen the telomeres by copying the TTAGGG sequence. Telomerase plays an important role in the formation, maintenance, and renovation of telomeres, preventing cell apoptosis. It is suppressed by mature somatic cells after birth, allowing telomere shortening after each cell division. Overexpression of telomerase has been reported to be associated with a range of neoplastic diseases.[31]

The human telomerase reverse transcriptase (hTERT) gene encodes the catalytic subunit of telomerase and shows a positive correlation with telomerase activity in different molecular studies. Overexpression in leukoplakia, associated with increased telomerase activity, is an early phenomenon in the process of oral carcinogenesis and one that can be detected in precancerous stages. This phenomenon shows a marked positive correlation with the degree of atypia, showing severe dysplastic changes.[32]

Microarray Analysis

The development of microarray analysis allows the whole genome to be screened and this technique can be used for comparing dysplastic lesions with healthy tissue. Over-regulated genes in the dysplastic lesions include the genes related to inflammation (cyclooxygenase 2 [COX-2], decorin transcript variant A2, arachidonate 5-lipoxygenase, arachidonate 12-lipoxygenase, and prostaglandin E synthase), certain receptor genes (prostaglandin E receptors 3b1, a2),[33] and certain genetic markers associated with malignancy (psoriasin 1 and versican/CSPG2).[34]

Tissue Markers

Cell Surface Carbohydrates

Surface carbohydrates with blood group antigen activity are widely distributed in the different human tissues. These markers, which include antigens of the ABO, Lewis, and T/Tn systems, are present on the surface of epithelial cells of the oral squamous epithelium.[32]

During malignant tumor development, synthesis of these carbohydrates is altered by aberrant expression of glucosyltransferases.[33] Thus, in epithelial dysplasias, loss of histo-blood group antigens (blood group A or B) is detected in the horny layer. In addition, there is an increase in the number of cell layers staining for its molecular precursor (H-antigen), which is normally only expressed in parabasal cells. Some aberrant expression patterns appear in premalignant lesions without epithelial dysplasia, suggesting that changes in histo-blood antigen appear in the early stages of tumor development.

These changes in cell surface antigen expression appear in premalignant and malignant lesions. However, they are also detected in benign lesions, such as in wound healing processes,[35] thereby confounding interpretation of the results.

In certain tumors, such as cervical cancer, head and neck cancer, and carcinomas of the oral cavity, expression of these histo-blood group antigens in tumor cells has been shown to have prognostic implications. Thus, loss of expression of A or B blood antigens increases the tumor cell motility, matrigel invasion, and their tumor potential in syngeneic animals.[36,37]

Keratins

Keratins are proteins that make up the cytoskeleton of epithelial cells. Currently, 20 keratins are known, numbered from 1 to 20. In the oral squamous epithelium, a group of keratins can be detected in normal situations. However, during the process of malignant transformation, the type and distribution of these proteins change, and so they may represent another potential marker of dysplastic progression to squamous cell carcinoma.

The K5/K14 pair of keratins is found throughout the entire normal oral epithelium in the basement membrane, whereas K4/K13 and K1/K10 are present in the stratum spinosum of the keratinized and nonkeratinized epithelium, respectively.[36] A series of changes occur in the dysplastic epithelium: K5/14 is expressed in the parabasal and stratum spinosum, as well as in its normal location, probably reflecting hyperplasia of basal cells. In contrast, expression of K4/K13 and K1/K10 is reduced or even absent. These changes show a marked positive correlation with the degree of histological dysplasia.[37,38]

In normal epithelium, K8/K18 is expressed in the basal layer and suprabasal stratum spinosum of the simple epithelium. This pair is not usually detected by immunohistochemistry. However, in oral epithelial dysplasia, these keratins are detected by immunohistochemical staining in more than half the cases.

K19 is present naturally in the basal layer of nonkeratinized epithelium, but not in keratinized epithelium. In severe dysplasia, however, K19 is strongly expressed both in basal and suprabasal layers of keratinized and nonkeratinized epithelia. K19 may also be overexpressed in gingival inflammatory processes, which may limit its potential prognostic value in dysplastic epithelia.[39,40]

This loss of differential keratin expression in dysplastic lesions may correlate with a greater risk of transformation to squamous cell carcinoma, but this possibility has yet to be studied.

Integrins

Integrins are cell surface receptors that mediate cell-cell and cell-extracellular matrix signaling mechanisms. These receptors are composed of 22 subunits α and β, each with a ligand binding specificity. The normal basal epithelium expresses integrins α2β1 and α3β1. Expression of α3β6 is found in 90% of squamous cell carcinomas and 27% of dysplastic leukoplakias studied, but not in normal skin. This integrin was associated with transformation of dysplastic epithelial lesions. However, isolated expression of this integrin is not sufficient for progression of dysplasia to squamous cell carconima.[38]

The CD44 family of glycoproteins is also related to cell-cell and cell-matrix interactions. Expression of the CD44v7-8 isoform is decreased in squamous cell carcinoma compared to dysplastic leukoplakias. In turn, patients with tumors that express this isoform have a greater 5-year survival than those whose tumors are negative, suggesting that loss of expression is associated with worse prognosis.[39]

Granulocyte-Colony Stimulating Factor Receptor

Expression of granulocyte colony stimulating factor receptor is increased in dysplasia and in squamous cell carcinoma compared to normal and hyperplastic epithelium. This is probably a manifestation of an attempt to block the maturation defect, either in the initial precancerous phase or in established cancer.[40]

Growth Factor Receptors

Overexpression of transforming growth factor (TGF)-α, both in terms of area and intensity of staining, appears in parallel with the severity of the oral dysplasia. Similarly, EGFR staining increased in intensity in parallel with more extensive dysplasia.[41]

Cell Cycle Regulators

Loss of p16 expressed by the 9p21 gene—considered the first gene to be deactivated in squamous cell carcinoma of the head and neck—precedes the histological changes in the oral mucosa. In turn, a sequential increase was observed

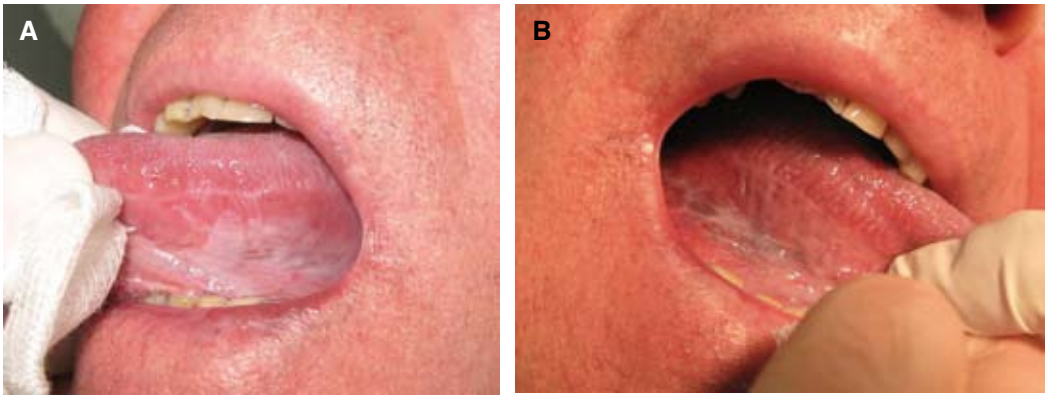

Figure 1.
A and B, Homogenous leukoplakia.

in the expression of cyclin D1 on progression from normal to hyperplastic and dysplastic tissue and finally squamous cell carcinoma.[42] It was also possible to show that the cyclin D1 gene was amplified in 70% of leukoplakias that progressed to squamous cell carcinoma.[43]

Matrix Metalloproteinases

The matrix metalloproteinases are a group of 20 proteolytic enzymes that play an essential role in tissue remodeling. Increased levels of metalloproteinases 1 and 9 have been detected in dysplasias that progress to squamous cell carcinoma compared to those that do not.[44]

Extracellular Matrix Metalloproteinase Inducer (CD147 and M6)

Expression of this inducer of metalloproteinase activity seems to be positively correlated with the degree of dysplasia. However, this expression decreases in squamous cell carcinoma cells.[45]

Vascular Endothelial Growth Factor

It has been observed that concomitant expression of metalloproteinase 11 and vascular endothelial growth factor is associated with risk of progression of dysplastic leukoplakias to squamous cell carcinoma.[46]

Clinical Characteristics

Clinical manifestations of oral leukoplakia can take different forms defined according to the clinical pattern (homogenous or nonhomogenous), distribution or spread of the lesion (focal or disseminated), and location within the oral cavity.[47]

Clinical Pattern
Homogenous Pattern

The homogenous pattern refers to lesions with a regular, smooth whitish surface and well-defined edges[48] (Figure 1). This clinical pattern shows a low risk of long-term malignant transformation (5%).[48-50]

Nonhomogenous Pattern

The nonhomogenous pattern includes leukoplakias that are associated with an erythematous component (erythroleukoplakia) or a nodular, erosive, ulcerated, or verrucous exophytic component. Malignization occurs in 25% of cases, and so it is considered high-risk.[15]

Proliferative verrucous leukoplakia (Figure 2) —currently considered as an independent entity from the set of leukoplakias—is associated with a high risk of malignization; as many as 80% may become malignant.[51,52] It is difficult to distinguish this lesion clinically from oral verrucous carcinoma, also known as oral florid papillomatosis or Ackerman tumor (Figure 3). Both entities appear in elderly women, usually in the gingival region although they progressively spread to most of the oral mucosa. They tend to affect large areas of mucosa and have well-defined borders. Clinically, they present as whitish verrucous lesions or in less keratinized polypoid forms. They are usually differentiated by histology. In verrucous carcinoma, unlike verrucous leukoplakia, marked exophytic and endophytic epithelial hyperplasia is present, invading the dermis and leading to destruction by compression. According to Slootweg and Müller,[53] the coexistence or subsequent development of squamous cell carcinoma, or epidermal dysplasia, was detected in 63% of a series of 27 patients with proliferative verrucous leukoplakia. Those authors concluded that this variant of leukoplakia is a clear precursor of carcinoma.

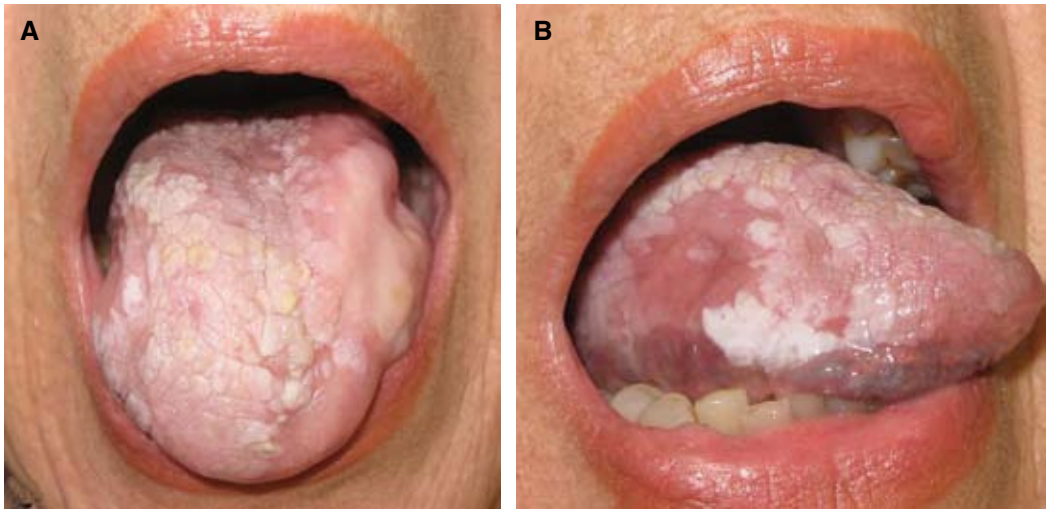

Figure 2. A and B, Nonhomogenous leukoplakia, verrucous exophytic variant.

Lesion Distribution

The distribution or extension of leukoplakia in the oral cavity is a prognostic factor for long-term malignization. Thus, focal leukoplakia is associated with good long-term prognosis whereas disseminated forms, which affect several points of the oral mucosa, are considered to indicate worse prognosis.[15]

Lesion Site

Leukoplakias located on the floor of the mouth and in the ventrolateral region of the tongue are associated with a greater risk of malignization, with an average rate of transformation of 43%.[54] This is attributed to the fact that these areas are more exposed to carcinogens in salivary secretions and that the epithelium is more permeable in this area, as indicated by experimental studies of oral mucosa.[55]

Other Characteristics

Lesions larger than 20 mm, rapid growth of leukoplakia, a history of squamous cell carcinoma, and regular consumption of alcohol or cigarettes—which are known carcinogens of the oropharyngeal mucosa—are other factors of poor prognosis to take into account when evaluating these patients.[11]

Histopathological Features

Moderate hyperkeratosis and epithelial hyperplasia without dysplasia are the most common histological findings reported for leukoplakia[11,15] (Figure 4).

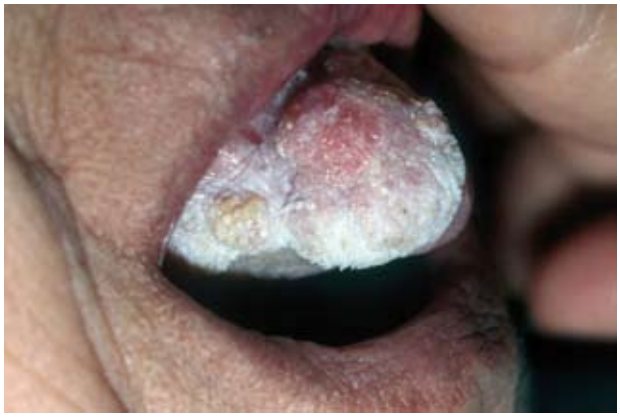

Figure 3. Verrucous carcinoma or Ackerman tumor.

Epithelial dysplasia, characterized by a nonspecific clinical picture but with definite histological findings[56] (Table 1), is the expression of a maturation disorder of the oral epithelium. It is present in 1% to 30% of cases,[57] and is universally accepted as one of the predictive factors of malignancy.[58-61] Thus, as many as 36% of patients who present oral leukoplakia with dysplastic changes develop squamous cell carcinoma[62] (Figure 5).

Currently, the prognosis and therapeutic approach to leukoplakia are based on the degree of histological dysplasia, which is considered mild, moderate, or severe according to the thickness occupied by the atypical infiltrate in the epithelium.[15] As demonstrated by several studies, this classification has the drawback of interobserver subjectivity.[63] For this reason, it is difficult to establish a strong correlation between the degree of dysplasia and the rate of transformation of leukoplakia. The classification is also subject to the following considerations. On the one hand, epithelial dysplasia does not necessarily progress to squamous cell carcinoma in all cases; in fact, in a small

Figure 4. A, Homogenous leukoplakia. B, Parakeratotic hyperkeratosis with acanthosis (hematoxylin-eosin, ×40). C, Marked acanthosis without dysplastic changes (hematoxylin-eosin, ×100).

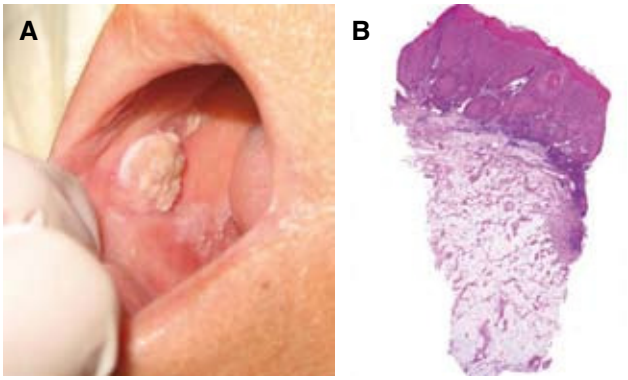

Figura 5. A, Squamous cell carcinoma on heterogenous leukoplakia. B, Malignant cell proliferation invading the papillary and superficial reticular dermis (hematoxylin-eosin, ×40). C, Neoplastic infiltrate of squamous cells with marked cellular atypia and architectural destructuring (hematoxylin-eosin, ×100)

number of cases, histological resolution of the dysplasia has been observed.[64-66] On the other, absence of histological dysplasia does not rule out the possibility of malignant transformation of leukoplakia. This observation is based on the fact that this variant without dysplasia shows a rate of malignant transformation of 15%.[57,67] It can therefore be concluded that presence of histological dysplasia—regardless of the histological grade—is considered an important predictor of malignant transformation of leukoplakia, although histological dysplasia is not indispensable for such a transformation to occur.

Differential Diagnosis

Diagnosis of oral leukoplakia is established after ruling out other well-defined entities that may present whitish plaques in the oral mucosa. Such conditions include: leukoedema; white sponge nevus or Cannon nevus (Figure 6); morsicatio buccarum or chronic biting (Figure 7); lesions caused by contact dermatitis, stomatitis nicotina, or smoker's palate; squamous cell carcinoma (Figures 5 and 8); verrucous carcinoma; oral hairy leukoplakia; linea alba (Figure 9); oral lesions of lupus erythematosus; oral lichen planus (Figure 10); and secondary syphilis. The most important clinical characteristics of these entities are shown in Table 2.[11]

Oral leukoplakia should also be differentiated from benign alveolar ridge keratosis, characterized by a whitish plaque in the gingival region of the maxillary or mandibular alveolar ridge and probably of traumatic or frictional origin. Histologically, marked hyperorthokeratosis is apparent, as well as pronounced hypergranulosis, with papillomatosis and acanthosis with fusion of the epidermal ridges without any accompanying infiltrate.[68,69]

Treatment

There is no consensus regarding the most appropriate treatment for oral leukoplakia. Among the many therapeutic options available (Table 3), eliminating risk factors (smoking, alcohol) is a preventative measure applicable to all patients with these lesions.[56]

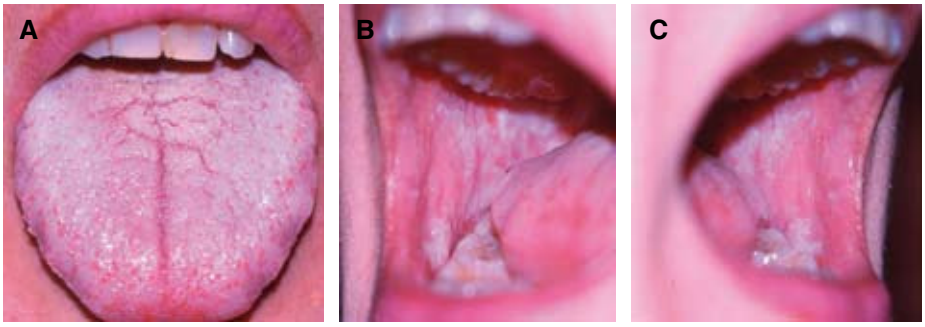

Figure 6. A, B, and C, White sponge nevus. (Courtesy of Dr. Enrique Gimeno Carpio, Hospital Arnau de Vilanova, Valencia, Spain).

Figure 7. Morsicatio buccarum.

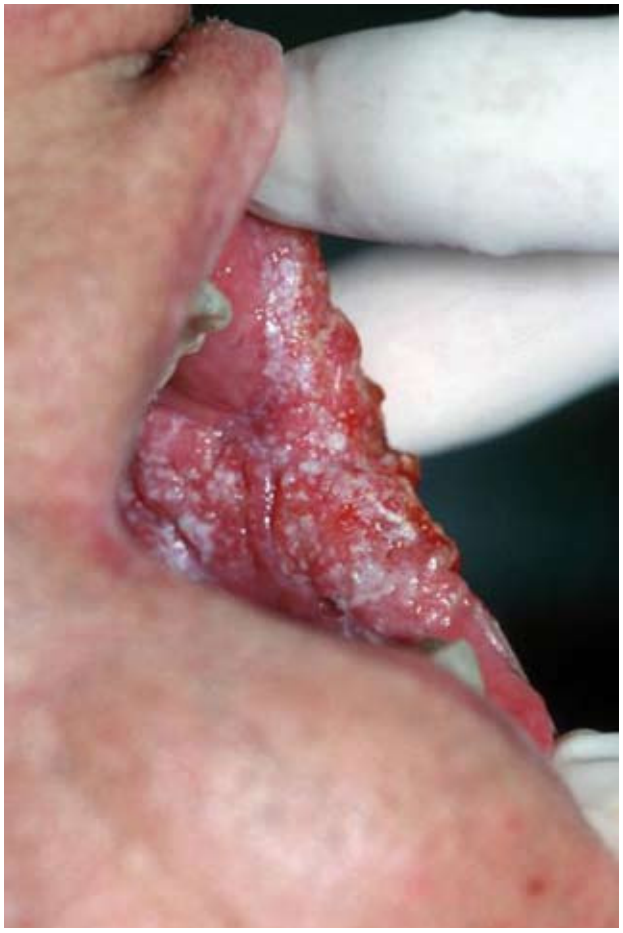

Figure 8. Squamous cell carcinoma.

Among the nonsurgical therapeutic options, a Cochrane review concludes that interventions with topical bleomycin, systemic retinoids, and systemic lycopene may help resolve the dysplasia, but the supporting evidence is inadequate given the lack of long-term studies.[70]

Among the invasive therapeutic modalities, ablative procedures, which include cryosurgery, application of carbon dioxide laser light, and surgical resection, are the only options with an acceptable level of evidence for local short-term control of leukoplakia.[71] In the long term, the high therapeutic failure rate, local recurrence, and development of squamous cell carcinoma at the resection site have several explanations:

1. The evidence of molecular alterations (loss of heterozygosity) at the resection margins with no histological involvement suggests that truly disease-free margins are not often obtained.[24] In one study, Sudbø[24] showed that recurrence and malignancy occurred almost exclusively in leukoplakias with cell aneuploidy and not in the diploid or tetraploid forms. Despite complete resection, the aneuploid leukoplakic

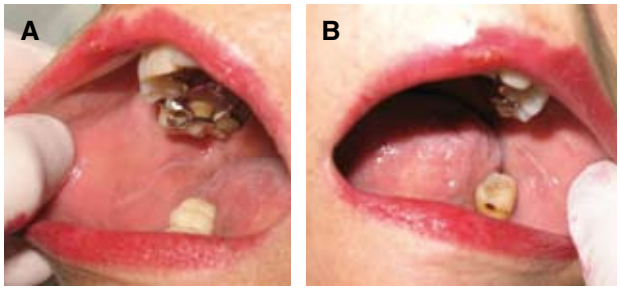

Figure 9. A and B, Linea alba.

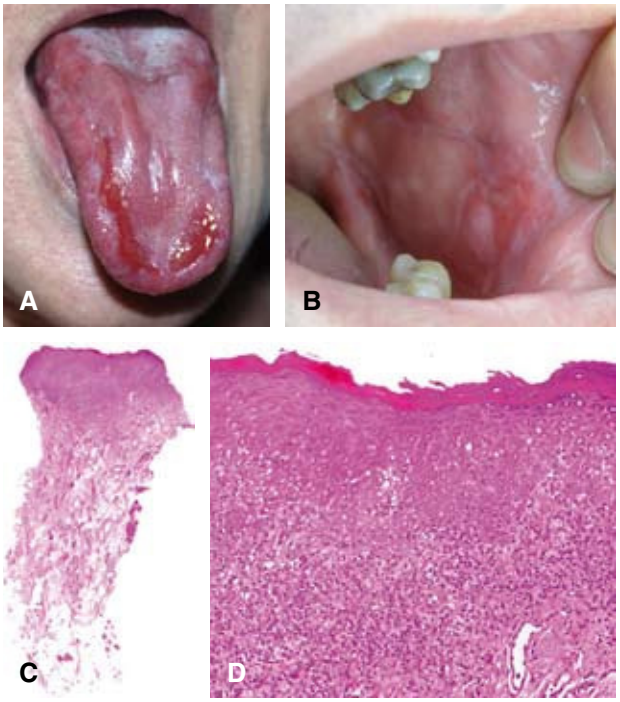

Figura 10. A and B, Erosive lichen planus symmetrically affecting the dorsum of the tongue and mucosa of the cheek. C, Inflammatory infiltrate occupying the papillary dermis and the superficial reticular dermis (hematoxylin-eosin, ×40). D, Lichenoid infiltrate composed mainly of lymphocytes (hematoxylin-eosin, ×100).

form had a risk of transformation of 70% at 3 years follow-up, a high risk of recurrence (30% at 3 years), and a high lethality (30% at 3 years), due to the tendency for subclinical involvement of the mucosa surrounding the lesion. Thus, combining histological and molecular analyses of the resection margins of leukoplakia can more accurately establish the risk of tumor recurrence.

2. The multifocal nature of oral carcinogenesis means that local surgical excision does not definitively eradicate of risk of developing carcinoma of the head and neck.[4,72-74] The most recent study showed that the cancer develops in the same place as the preceding leukoplakia in 79% of cases. In terms of cell ploidy, squamous cell carcinoma was located in the same place as the leukoplakia in 100% of the diploid forms, 81% of the tetraploid forms, and 73% of the aneuploid forms.[27] Thus, in cases with aneuploidy, a high percentage of squamous cell carcinoma (27%) developed at a different site to the preceding leukoplakia, with a mean distance between the 2 sites of 4.5 cm (3-8.5 cm).

Study of leukoplakia by molecular biology techniques therefore allows us to predict the risk of developing squamous cell carcinoma in initially unaffected areas of the oral cavity and to establish an individual follow-up schedule according to the characteristics of each patient. Currently, the therapeutic approach is still based on whether or not histological dysplasia (Figure 11) is present. Two risk groups are therefore defined:

1. Group with low risk of malignization, comprising: a) those leukoplakias lacking dysplasia, and b) those that show mild dysplasia located in low-risk areas or those with a thickness of less than 200 mm or that present clinically as homogenous leukoplakia. A range of therapeutic approaches can be taken in this group:

   – Regular patient follow-up. The interval between follow-up visits should not exceed 12 months in order to detect any change suggestive of malignant transformation.
   – Treatment of lesions with topical or oral retinoids. Experience in the use of this therapeutic option is rather unsatisfactory as lesions are not eradicated in the vast majority of patients.
   – Treatments using nonsurgical ablative techniques, such as cryotherapy and carbon dioxide laser ablation. Of these options, the use of laser light has shown better results in terms of controlling the lesions, and so is considered the treatment of choice in this low-risk group.

2. Group of high-risk of malignant transformation,[11] which comprises: a) those leukoplakias with mild dysplasia located in high-risk areas measuring more than 200 mm, or those associated with a nonhomogenous clinical form; b) leukoplakias with moderate or severe dysplasia; and c) verrucous leukoplakias.
   In this group, there is justification for aggressive surgical treatment, consisting of decortication of the entire thickness of the mucosa at the site of the leukoplakia.

Subsequently, a meticulous histological examination of the surgical piece is carried out to rule out existing areas of malignant transformation. In the event that regions of squamous cell carcinoma are detected in the excised piece, radical excision of the affected area should be done in conjunction with other complementary therapies (radiotherapy or chemotherapy).

The fact that current therapy cannot provide optimum control of many leukoplakias provides a rationale for the development of systemic therapy that acts on the entire oral mucosa: oral chemotherapy.

Table 2. Different Conditions That Give Rise to Whitish Lesions on the Oral Mucosa

Leukoedema	Grey-white benign lesion of the oral mucosa representative of spongiosis of the mucosa It is usually located on the mucosa of the cheek and characteristically disappears on stretching the surface
White sponge nevus; "Cannon nevus" (Figure 6)	Autosomal dominant genodermatosis of variable expression characterized by keratinization of the oral mucosa and, occasionally, of the anal and vaginal mucosas. It presents as a whitish spongy thickening with extensive involvement of the epithelial mucosa. Malignant transformation of this process has not been reported. It presents from birth and early childhood, and may be associated with other genodermatoses such as congenital pachyonychia or congenital dyskeratosis
Morsicatio buccarum or lesions from biting (Figure 7)	Whitish crushed aspect of oral or labial mucosa at the occlusion line, caused by chronic biting. Lesions are benign. The habit is most common in tense or anxious individuals
Contact allergy lesions	White plaques in areas of contact with dental prostheses or fillings, due to sensitization to different agents such as dental amalgam
Stomatitis nicotina or smoker's palate	Whitish-grey mucosa with umbilicated papules corresponding to the salivary glands, located in the palatine mucosa. This is usually caused by pipe smoking. Spontaneous resolution after cessation
Squamous cell carcinoma (Figures 5 and 8)	Whitish plaque that histologically shows a proliferation of pleomorphic keratinocytes spanning the dermal-epidermal junction
Verrucous carcinoma	Variant of squamous cell carcinoma characterized by a hypertrophic surface of cauliflower-like appearance and histologically by a digitiform or compressive invasion (bulldozing)
Oral hairy leukoplakia	A condition that appears in immunosuppressed patients associated with infection by herpes virus type 8. It is clinically characterized by whitish velvety plaques that symmetrically affect the sides of the tongue
Linea alba (Figure 9)	Horizontal ridge, usually hyperkeratotic, located bilaterally in the oral mucosa in the area between the teeth
Lupus erythematosus	Between 25% and 40% of patients with lupus erythematosus have oral lesions that particularly affect the palate and oral mucosa. The lesions begin as red patches and progress to irregular erosions and areas of atrophy with or without ulcers that often heal leaving a scar.
Oral lichen planus (Figure 10)	Plaques arranged to form a characteristic whitish network, at times with secondary ulceration, usually found in the cheek mucosa
Secondary syphilis	Broad-based well-defined plaques and nodules with associated areas of erosion. They are known as condyloma planum

Chemoprevention in Oral Oncology

Chemoprevention refers to the use of pharmacological or natural agents that inhibit the development of invasive cancer. This function can be achieved either by blocking the damage to DNA that initiates the carcinogenic process or by inducing apoptosis of premalignant cells in which damage has already occurred.[75] The possible targets of molecular therapy for preventing oral cancer currently under investigation include COX-2, EGFR, and peroxisome proliferation activated receptor (PPAR-γ).

Inhibition of Cyclooxygenase 2

COX-2 is selectively overexpressed in aneuploid dysplastic lesions, which are considered as the variants with greatest

Table 3. Current Therapeutic Options

Smoking cessation/regular follow-up
Surgical excision with or without grafting
Cryosurgery
Laser ablation
Antifungal therapy (Candida-associated leukoplakia)
Chemoprevention *Retinoids* *Vitamins A, C, and E* *Carotenes* *Lycopeneo*
Photodynamic therapya
Topical therapy *Bleomycin* *Vitamin A*

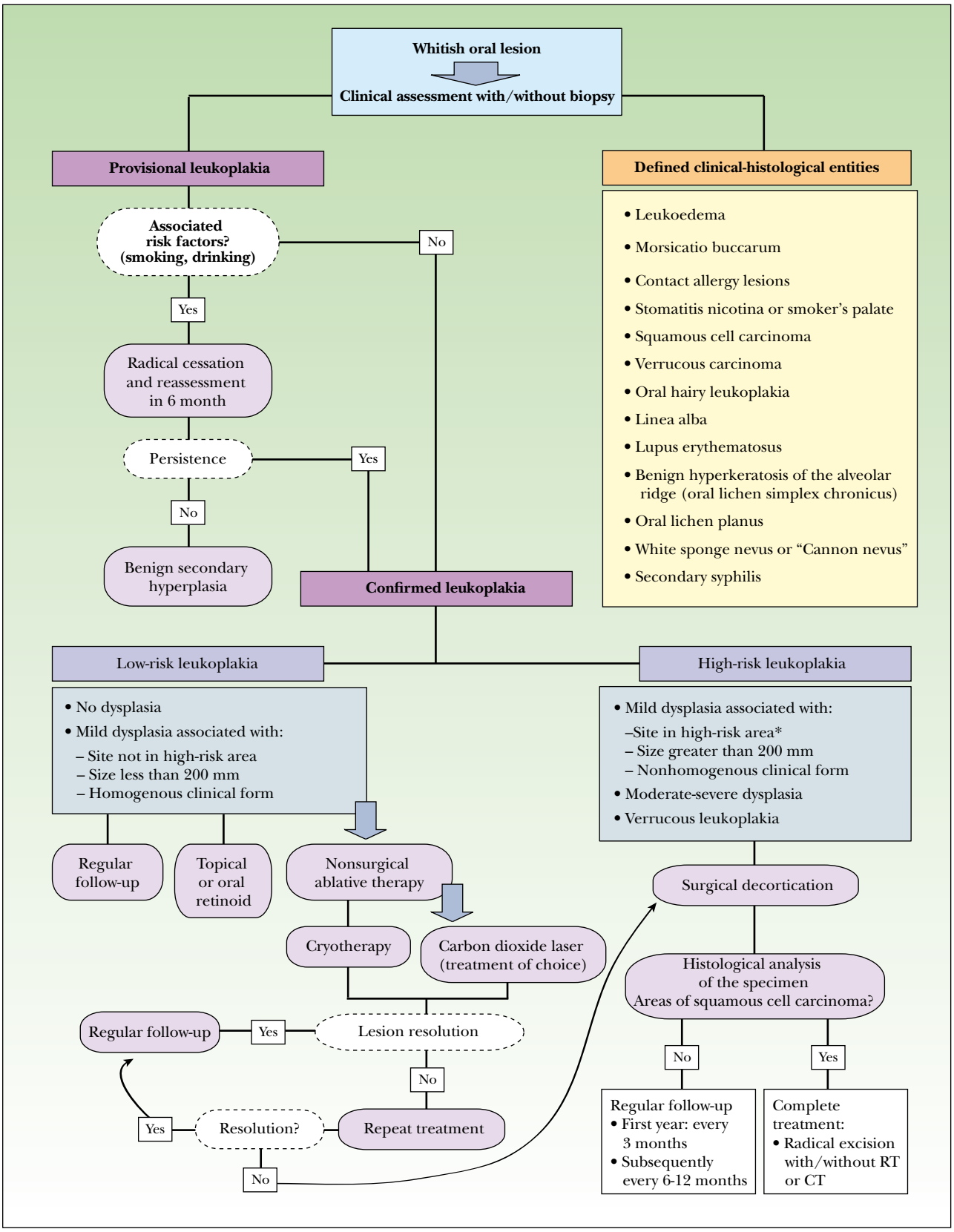

Figure 11. ADiagnostic-therapeutic algorithm for leukoplakia. CT indicates chemotherapy; RT, systemic radiotherapy. *Areas of high risk: floor of the mouth and ventrolateral area of the tongue.

malignant potential.[76] This phenomenon occurs during malignant transformation of the oral mucosa, which is somehow related to the presence of genomic instability.[77]

Inhibition of the Epidermal Growth Factor Receptor

Tyrosine-kinase specific inhibitors for EGFR are promising therapeutic targets in the control of head and neck carcinoma.[78] EGFR is overexpressed in most premalignant and malignant oral lesions, and is associated with an advanced stage and decreased survival.[79-81] Human epidermal growth factor receptor 2 (HER2 or erbB-2), a member of the EGFR family, is also overexpressed in oral carcinogenesis. In fact, it has been observed that combination therapy of paclitaxel with PKI199 (an irreversible inhibitor of the tyrosine-kinase domain of EGFR) prolongs survival in tongue cancer by increasing programmed cell death.[82]

Combination Therapies of COX-2 and EGFR Inhibitors

Torrance et al[83] reported a greater level of cancer prevention by combining agents against 2 molecular targets: COX-2 and EGFR. Specifically, EKB-569 (an irreversible inhibitor of the tyrosine-kinase domain of EGFR) combined with sulindac (a nonselective COX inhibitor) showed greater activity in animal models of intestinal neoplasm.

Studies of cell cultures from aneuploid leukoplakias have found that COX-2 and EGFR signaling pathways are interlinked,[6] suggesting that blockade of both pathways is necessary to effectively prevent the development of oral cancer through a synergistic effect on the control of leukoplakia. This double blockade would also be useful for controlling the harmful effects on oral mucosa produced by cigarette smoke, an oncogenic agent that induces activation of EGFR leading to increased COX-2 levels.[84] Therefore, combined inhibition of EGFR and COX-2 is a promising strategy in the prevention and treatment of head and neck cancer.

PPAR-γ Agonists: Nonsteroidal Antiinflammatory Drugs

Epidemiological studies have shown the anticarcinogenic efficacy of nonsteroidal antiinflammatory drugs (NSAIDs) mainly in colorectal, gastric, and esophageal cancers.[84-88] Their anticancer activity is mediated by an agonist effect on PPAR-γ, thereby inducing apoptosis through caspase activation.[89] The NSAIDs studied include indomethacin, ketoprofen, and ibuprofen, which are able to act as PPAR-γ agonists.[90]

Control and Follow-up

In view of the high risk of recurrence after surgery and of the risk of developing squamous cell carcinoma of the head and neck at a site distant from the primary leukoplakic site, these patients should undergo follow-up for the rest of their lives at regular intervals. These intervals may range from 3 to 6 months in patients considered at high risk and from 6 to 12 months in those patients considered at low risk.

Conclusions

Currently, each year, more than 300 000 cases of oral squamous cell carcinoma are diagnosed worldwide. This aggressive epithelial cancer is associated with high mortality and substantial morbidity in those patients who survive, despite progress in surgery, radiotherapy, and chemotherapy. This dire prognosis has not improved significantly in the last 4 decades. Treatment failure is due to the development of second primary tumors in up to 20% of the patients with early-stage squamous cell carcinoma, and local recurrence and metastasis in those with locally advanced disease, who account for two-thirds of the cases at the time of diagnosis. Prior treatment before cancer develops can reduce the annual incidence of this aggressive cancer.

Leukoplakia, considered as the most common precancerous lesion in the general population, should therefore be perfectly characterized in order to define the "high risk" variant with greatest potential for malignant transformation. Traditional prognostic markers of leukoplakias, such as clinical characteristics and the grade of oral epithelial dysplasia, are of limited prognostic value due to the lack of interobserver reproducibility. In the future, routine study of different molecular biological variables, such as the loss of heterozygosity, cell ploidy, and the presence of p53 mutations, should help to more accurately identify high-risk leukoplakias and allow a more aggressive therapeutic approach to be taken in these cases.

Among the different current therapeutic options, the future incorporation of histological and molecular study of the surgical margins, as well as systemic therapies that act against different molecular targets, will help provide better local and distant control of the disease.

Conflicts of Interest

The authors declare no conflicts of interest.

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