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Advances in the Detection and Diagnosis of Oral Precancerous and Cancerous Lesions

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In the United States, an estimated 29,370 new cases of oral and pharyngeal cancer were diagnosed in 2005, with more than 7320 tumor-related deaths [1]. Oral cancer represents roughly 3% of total cancer cases in the United States and is the ninth most common form of malignancy among American men. Although the concept of ''early diagnosis leads to improved prognosis'' applies to oral cancer, most patients present with regional or distant (stage III or IV) disease, which is a problem especially notable among African Americans. The tendency for delayed or late diagnosis is reflected in an overall 5-year survival rate of approximately 59% for data pooled from 1995 through 2001. Although this figure represents a significant improvement for oral cancer survival for the first time in decades (up from 54% in 1974–1976), survival within the African-American population has remained comparatively lower (36% in 1974–1976, 40% in 1995–2001) [2]. Increased mortality from oral cancer is especially marked in African-American men, whose 5-year survival rate (34%) is substantially lower than that of their female counterparts (52%).

The most common form of oral cancer is primary mucosal squamous cell carcinoma (>90% of cases), although malignancies of salivary gland origin, sarcomas, lymphomas, melanoma, and metastatic disease also contribute to the total cancer burden. Because squamous cell carcinoma and its variants represent most oral cancer cases, this article focuses on the diagnosis and detection of this condition and its precursors. The ability to diagnosis precursor (precancerous) lesions is critical to the battle against oral cancer. With early detection, diagnosis, and treatment, noninvasive intraepithelial lesions (grades of epithelial dysplasia or carcinoma in situ [CIS]) can be conservatively managed with minimal surgical morbidity and 100% survival. In addition, advances in molecular diagnosis suggest that genetic or protein markers of precancerous change are likely detectable before clinically apparent mucosal lesions can be identified. If the promise of such ''prediagnosis'' can be realized, early detection of patients at increased risk for initial or recurrent disease would be possible and would hopefully lead to reduced patient morbidity and mortality.

Clinical features of oral precancerous and cancerous lesions

The signs and symptoms of precancerous lesions and even some early squamous cancers are often so subtle that they probably go unnoticed or ignored by patients and practitioners alike. Distinguishing lesional tissue from the surrounding mucosa, especially in the presence of complicating factors, such as local trauma or superimposed infection, can be difficult for even well-trained health care professionals. Together with estimates that only approximately half of the US adult population sees a dentist even once a year, it should probably not be surprising that most patients with oral cancer (60%) are diagnosed with stage III or IV disease.

Given that notable symptoms are typically a late-stage feature of oral cancer, early detection and diagnosis of oral precancerous and cancerous lesions clearly depend on patient participation in periodic (annual) oral examinations and the

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sensitivity and specificity of the oral examiner or examination procedure. Detecting the mucosal alterations that often precede the development of squamous cell carcinoma requires a sound knowledge of oral anatomy and anatomic variations as well as a thorough understanding of local and systemic factors or conditions that can mimic or obfuscate underlying precancerous change.

Leukoplakia

The term *leukoplakia* is defined as a white plaque or patch of oral mucosa that cannot be rubbed off or cannot be diagnosed as any other condition clinically, or subsequently, by microscopic evaluation. Leukoplakia is not a diagnosis; it is a descriptive term that encompasses a surprising variety of localized whitish areas of mucosal change that cannot be readily explained at the clinical level. The term has no diagnostic and, thus, no prognostic value. Written or imaging-based documentation of clinical features, including site, size, border, surface character, and presence of ulceration is a medicolegally sound practice that should always be performed as a standard part of patient examination. Inspection of the lesion border is of particular importance, because a well-defined sharply demarcated margin is suggestive of clonal (preneoplastic or neoplastic) growth (Fig. 1). Depending on the precise clinical setting, differential considerations, such as traumatic, reactive, or infectious conditions, can usually be addressed through local conservative measures and follow-up re-evaluation. Any leukoplakia that persists or progresses after 10 to 14 days despite appropriate conservative treatment should be considered a potentially premalignant condition.

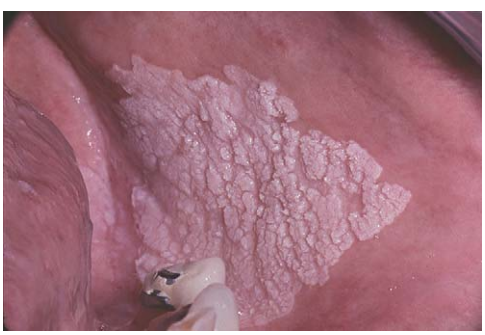

Fig. 1. Large leukokeratotic plaque (leukoplakia) of the left posterior and inferior buccal mucosa with a sharply defined border and surface irregularity, including plexiform fissuring. (Courtesy of C.M. Allen, DDS, MS, Columbus, OH.)

Leukoplakia is most commonly seen in older adult men, and more than 80% of patients have a history of smoking [3,4]. Although the buccal mucosa and gingiva are the most frequently affected sites (see Fig. 1), lesions that occur on the ventral tongue, floor of the mouth, and tonsillar pillars are more likely to demonstrate histologic evidence of dysplasia or carcinoma. These latter areas have been recognized for years as some of the oral anatomic regions at greatest risk for the development of squamous cell carcinoma (Fig. 2) [5]. For this reason, persistent leukoplakia in these areas should be considered as suspicious for carcinoma. Scalpel biopsy is warranted for any suspicious lesion and should be scheduled or performed as soon as conveniently possible. Use of diagnostic adjuncts, such as toluidine blue staining, may be helpful in guiding the biopsy procedure; however, heavily keratinized lesions are often negative with this vital stain. Cytologic methods, including brush cytology, are not advised for clinically suspicious lesions, because these tests can delay scalpel biopsy, definitive diagnosis, and appropriate therapy. Therefore, brush cytology would not be indicated for any persistent leukoplakia in the high-risk zone for oral cancer (see Fig. 2B). Even with small lesions (Fig. 3), excisional biopsy in these areas would be preferable for two reasons. First, complete removal of lesional tissue is more easily accomplished by scalpel biopsy and typically restores a normal background appearance to the mucosa at this site. Against this mucosal equivalent of a clean slate, the clinician's ability to detect signs of local recurrence is improved. Second, scalpel biopsy leads more directly to a final tissue diagnosis, decreasing the interval to appropriate treatment if needed. Finally, regardless of location, any leukoplakia that exhibits intralesional areas of reddish or erythematous change (eg, speckled leukoplakia, erythroleukoplakia) should also be viewed as a high-risk presentation (see the section on erythroplakia) that demands scalpel biopsy. With speckled leukoplakias, toluidine blue has been shown to stain the less heavily keratinized (reddish) areas suspicious for dysplasia or carcinoma and may be helpful in directing the biopsy procedure.

Proliferative verrucous leukoplakia (PVL) is a more aggressive and often multifocal form of leukoplakia that frequently occurs in the absence of a significant smoking history [6,7]. Although it can affect any area of the oral cavity, the buccal

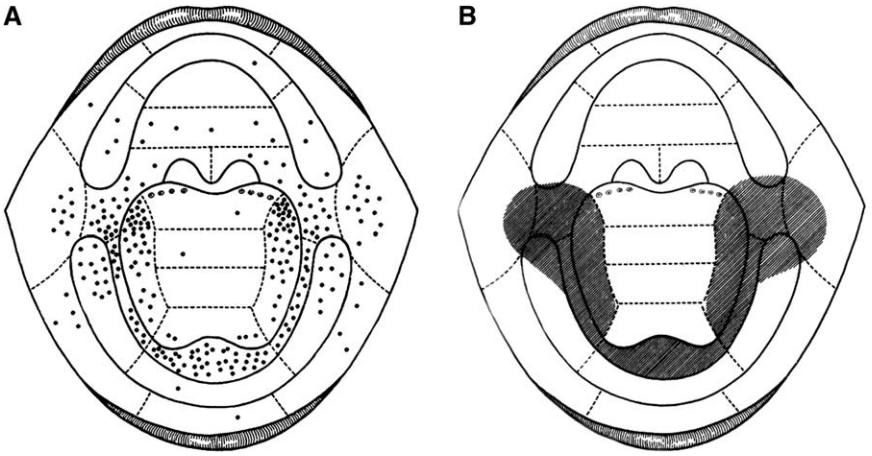

Fig. 2. (*A*) Site of origin of 209 consecutive cases of mouth cancer from the Memorial Hospital Head and Neck service between 1962 and 1965. (*B*) Cancer-prone crescent from which 75% of cancerous lesions originate. (*From* Moore C, Catlin D. Anatomic origins and locations of oral cancer. Am J Surg 1967;114(4):511; with permission.)

mucosa is a favored site among female patients, whereas the tongue is involved most often in male patients. In addition, female patients tend to be older (mean age of 65–70 years) than male patients (mean age of 49 years) at the time of diagnosis. Progression of PVL lesions to involve significant portions of the oral mucosa is often seen despite surgical treatment, and relatively rapid transformation to squamous cell carcinoma is a recognized complication.

Erythroplakia

As with its whitish counterpart, the term *erythroplakia* is used to describe a red macule or plaque that cannot be rubbed off or diagnosed clinically as any other condition. Although not a diagnosis, this presentation should always arouse clinical concern, because nearly 100% of true erythroplakias have been found on biopsy to represent severe dysplasia, CIS, or squamous cell carcinoma [8,9]. Not surprisingly, most erythroplakias arise in oral sites at the highest risk for squamous cell carcinoma: the floor of the mouth, ventrolateral surfaces of the tongue, tonsillar pillars, and soft palate (see Fig. 2B). Admixed areas of keratinization (speckled erythroplakia) may be seen. Depending on the precise clinical presentation, immediate scalpel biopsy of erythroplakia may be warranted even without conservative treatment or follow-up evaluation. Toluidine blue staining may be useful in biopsy site selection for cases of erythroplakia. As previously noted, the high index of suspicion for significant dysplasia or carcinoma in cases of erythroplakia would be a contraindication for cytologic methods.

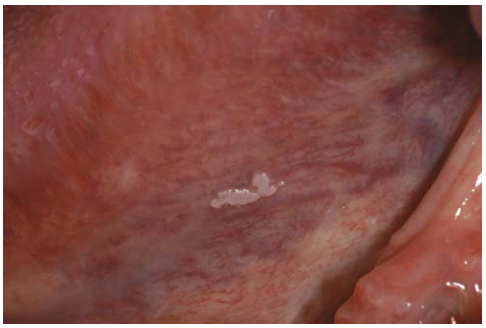

Fig. 3. Close-up view of small (0.8 cm × 0.3 cm), well-demarcated, asymptomatic leukoplakia of the right ventral tongue.

Squamous cell carcinoma

Most cases of oral squamous cell carcinoma present initially with clinical features of leukoplakia, erythroplakia, or both. Although any site can be affected, anatomic areas of increased risk for this disease have been recognized for years. In 1967, Moore and Catlin [5] presented scattergrams of oral cancer cases that provided a visual depiction of their distribution (see Fig. 2A). These plots were used to outline a "cancer-prone crescent" (see Fig. 2B), where more than 75% of the cancer cases were found, despite the fact that this region represented only 20% of the entire oral mucosa. Subsequently, the area of elevated

cancer risk has been extended by other authors to include the tonsillar pillar and soft palate complex [10]. As mentioned previously, the finding of any persistent mucosal alteration in this ''cancer risk zone'' should raise the clinician's index of suspicion and serve as a trigger for surgical biopsy.

The risk for oral cancer increases with age, and most patients are diagnosed after the age of 40 years. Men are more commonly affected than women, and, as mentioned previously, the risk is particularly high for African-American men. The major risk factor for oral squamous cell carcinoma is cigarette smoking, and roughly 80% of affected patients have a positive smoking history [3]. Alcohol consumption has a less well-defined association and may serve more as a cofactor, together with smoking. Smokeless forms of tobacco have also been considered as risk factors for oral cancer. Recent evidence, however, suggests that this historical view may need to be revised as several epidemiologic studies published during the past 10 years have failed to detect a significant association between the use of smokeless tobacco and the development of oral squamous cell carcinoma [11–20]. The only form of oral cancer not directly associated with smoking is cancer of the lip. This is related to sun exposure, and roughly 90% of such cases arise on the lower lip vermilion. It is also well recognized that patients can develop squamous cell carcinoma in the absence of any known risk factors. In patients less than 40 years of age, the most common site for this to occur is the ventrolateral aspect of the tongue. In older female patients, the gingiva is frequently affected.

Spread of oral squamous cell carcinoma is usually by local extension into and destruction of underlying tissues, including alveolar bone. Metastatic spread is commonly through the lymphatics to involve the ipsilateral cervical or submandibular lymph nodes.

Diagnostic adjuncts

A variety of aids or adjuncts to the diagnosis of oral precancerous and cancerous lesions have been developed over the years, several within the past decade. Although primarily developed for use by the general dental practitioner, data have been published to suggest possible utility in the hands of specialists as well. As with any test, proper case selection and correct performance of the test itself are critical to the sensitivity and specificity of its result.

Cytology

Oral exfoliative cytology has been an adjunct to oral diagnosis for many years; however, until recently, it has been primarily used to provide rapid and inexpensive identification of superficial infectious agents, such as fungi (using periodic acid–Schiff or KOH staining), or viruses (using Papanicolaou staining to permit visualization of the viral cytopathic effect in infected epithelial cells), such as herpes simplex virus (HSV; human herpesvirus [HHV]-1,2) and varicella zoster virus (VZV; HHV-3).

Use of oral cytology to test potentially precancerous epithelial lesions lost popularity for several decades after studies from the late 1960s through early 1970s had false-negative rates as high as 31% [21–23]. Given the significant margin of error, most practitioners abandoned this technique in the mid-1970s in favor of surgical biopsy analysis for potentially precancerous or cancerous lesions.

Brush cytology (brush biopsy)

Brush cytology (brush biopsy; OralCDx; CDx Laboratories, Suffern, New York) was introduced in 1999 as an alternative to conventional exfoliative cytology for investigating persistent oral epithelial lesions not considered suspicious for carcinoma [24]. Using materials provided in a commercially available kit (Fig. 4), the technique differs from conventional exfoliative cytology in two significant ways. First, a small circular brush instrument is provided for use in a rotary fashion to collect a transepithelial specimen. The brush is continually rotated against lesional tissue until pinpoint bleeding is detected clinically, indicating penetration of the basement

Fig. 4. Fixative and brush instrument of the OralCDx brush biopsy system.

membrane and ensuring the likelihood of a full-thickness (transepithelial) sample. The instrument is then ''unloaded'' by rotating the brush against a glass slide to deposit and disperse the disaggregated epithelial cells. The sample is fixed with a solution provided by the company (see Fig. 4) and returned for interpretation. Automated computer-assisted specimen analysis initially determines specimen adequacy, and then identifies and stores cytologic abnormalities found within the specimen. These abnormal findings are subsequently reviewed by a pathologist trained in oral cytology, who provides a test result.

Results of brush cytology specimens are classified into one of four categories:

1. Inadequate: incomplete transepithelial specimen
2. Negative: no epithelial abnormality
3. Atypical: abnormal epithelial changes of uncertain diagnostic significance
4. Positive: definitive cellular evidence of epithelial dysplasia or carcinoma

For atypical or positive results, the company recommends that patients receive follow-up scalpel biopsy. This recommendation reflects the fact that the brush result is limited to reporting evidence of cellular abnormalities or atypia; it does not provide a final diagnosis. In the case of a negative result, clinical follow-up of persistent oral lesions is recommended.

Several studies have shown encouraging data with oral brush cytology for evaluation of oral precancerous and cancerous lesions. Sciubba [24] reported 100% sensitivity with 100% specificity for positive results and 92.9% specificity for atypical results in 945 patients. Unfortunately, biopsy confirmation of the brush result was not obtained for all atypical or negative cases, and the lack of such information has raised concerns that false-negative or false-positive results may have been left undetected [25]. In another study of 298 patients, the positive predictive value of an abnormal brush cytology finding resulting in a scalpel biopsy report of dysplasia or carcinoma was 38.3% [26]. A comparative study of brush cytology and scalpel biopsy in 80 patients reported the brush technique to have 92% sensitivity and 94% specificity for both positive and atypical results in detecting dysplasia and oral cancer [27]. For positive results alone, sensitivity was 62% and specificity was 97%. A positive likelihood ratio [sensitivity/(1 − specificity)] of 16.2 was also recorded for the brush technique, meaning that a positive or atypical result was 16.2 times more likely in a mucosal lesion with dysplasia or carcinoma than in a lesion without precancerous or cancerous change.

In contrast, results from a study of 112 patients reported a sensitivity of 71%, specificity of 32%, and positive predictive value of 44.1% with the oral brush system [28]. The authors were concerned that 6 of 15 lesions with a negative OralCDx result were found to have dysplasia or carcinoma on subsequent scalpel biopsy. Such a finding validates previous concerns with earlier studies for failing to provide follow-up scalpel biopsy findings on all cases, possibly resulting in an overestimation of sensitivity and specificity with the brush technique [25]. Finally, in a series of four cases of oral squamous cell carcinoma, the diagnosis of carcinoma was determined by scalpel biopsy despite negative brush biopsy results [29]. The time delay from the initial brush sampling to final diagnosis varied from 5 to 292 days (average = 117 days).

The brush system is easy to use, although its cost is not negligible. In addition to its application for innocuous-appearing but persistent mucosal lesions, it could be a useful alternative for assessing lesions in patients who refuse a scalpel biopsy. Brush cytology, especially in combination with vital staining, may also be useful for sampling multiple areas of a large lesion, cases of PVL, or in the follow-up of patients previously treated for dysplasia or squamous cell carcinoma.

Tissue fluorescence

Recently, a technique previously used as an adjunct to the examination of cervical mucosa (speculoscopy) has been adapted and approved for use in the oral cavity. Several different commercial products designed for this technique have been marketed, including: ViziLite (Zila, Phoenix, Arizona; now available as ViziLite Plus or ViziLite with TBlue marking system), MicroLux DL (AdDent Inc., Danbury, CT), and VELscope (LED Dental Inc., Vancouver, British Columbia, Canada) (visually enhanced lesion scope). With the ViziLite system (Fig. 5) and the MicroLux DL, the oral mucosa is first rinsed with mild acetic acid and then illuminated by an activated chemiluminescent (ViziLite) or battery-operated portable light source (MicroLux DL) with output in the blue-white spectrum. The acetic acid wash helps to remove surface debris and reportedly causes the epithelial cells to dehydrate

Fig. 5. ViziLite system components, including a disposable light source, acetic acid solution, and light holder. ViziLite Plus (ViziLite with TBlue marking system) kits also provide a toluidine blue (tolonium chloride) solution.

slightly, increasing the relative prominence of their nuclei. Under blue-white illumination, normal epithelium appears lightly bluish in color, whereas abnormal epithelium appears distinctly white. ViziLite Plus consists of the same device packaged together with a tolonium chloride solution (see section on toluidine blue). The tolonium chloride is intended for use as a marking dye to help highlight lesions identified with the light source. VELscope (Fig. 6) is an alternating current (AC)–powered, portable, reusable light source that provides a blue emission spectrum unique from the ViziLite or MicroLux DL system. With this device, areas of reduced autofluorescence are considered suspicious for abnormality or a positive finding.

In a survey study of 150 patients, the ViziLite system was visually shown to amplify areas of the mucosa where hyperkeratinization or chronic inflammation was identified [30]. Conditions like leukoedema, nonspecific ulcer, and fibroma were shown to be chemiluminescent-positive, together with two leukoplakias that were subsequently characterized as atypical by brush cytology or as hyperkeratosis and epithelial atypia by scalpel biopsy. No attempt was made by the authors to assess the sensitivity or specificity of the system. In a study of 40 Malaysian subjects, the sensitivity of the ViziLite test with follow-up scalpel biopsy was reportedly 100%, with a specificity of 14% [31]. The authors raised several concerns about the technique, including its cost and a high false-positive rate (19%). Finally, a published abstract has reported that the ViziLite test result was positive in 78% of all clinically suspicious lesions, including 66% of suspicious leukoplakias (61 of 92 cases) and 60% of erythroleukoplakias (6 of 10 cases) but only 25% of clinically suspicious erythroplakias (5 of 20 cases) [32]. In addition, 19% (12 of 58 cases) of the keratoses judged to be clinically innocuous were positive; however, additional histologic or diagnostic information was not provided.

Fig. 6. VELscope light source unit with viewing handpiece and fiber optic light guide.

Recently, investigators using an electrically powered fluorescent light source similar to the VELscope unit presented results from a pilot study involving 44 patients with a history of biopsy-confirmed dysplasia or squamous cell carcinoma [33]. The patients first received routine oral examinations under white light, followed by re-examination in a darkened room using the fluorescent unit. Compared with the uniform autofluorescence of normal mucosa, areas of reduced fluorescence (as compared with adjacent mucosa and mucosa from the contralateral anatomic site) were considered positive or suspicious. Next, the fluorescent results were correlated with microscopic features in 50 oral biopsies from the patient cohort. Of 7 biopsies from sites with normal autofluorescence, 6 exhibited normal surface epithelium, although 1 was diagnosed as severe dysplasia or CIS. Of the remaining 43 specimens obtained from sites with reduced autofluorescence, 10 showed severe dysplasia or CIS and 33 were diagnosed as squamous cell carcinoma. These data corresponded to a reported sensitivity of 98% and a specificity of 100%. The authors noted that the decision to perform a biopsy was not based on tissue autofluorescence but on standard clinical features (patient history, clinical appearance, and toluidine blue staining results). Unfortunately, the authors failed to correlate these features with tissue fluorescence, making it impossible to assess the added diagnostic value

of the fluorescent examination. A published abstract from the same group reported that a significantly higher proportion of oral premalignant lesions (n = 69) with reduced fluorescence were dysplastic (n = 42 [81%]) compared with lesions with normal fluorescence (n = 17 [41%]) [34]. In another abstract, 8 patients undergoing surgery for recently diagnosed T0 to T2 oral cancer were studied. In each case, the clinical lesions, areas of reduced tissue fluorescence (fluorescent-positive), and surgical margins were delineated, and punch biopsies (n = 18) were obtained from fluorescent-positive areas that extended beyond the margin of visibly abnormal tissue. Of these biopsies, 6 were diagnosed as carcinoma (33%), 4 as severe dysplasia (22%), 4 as mild to moderate dysplasia (22%), and 4 as hyperplasia or normal (22%). These results suggest that fluorescent examination may permit detection of precancerous lesions even when the oral mucosa appears clinically normal [35].

The ViziLite Plus test is simple to use; however, its cost is not negligible, and the light stick can only be activated once. Although the MicroLux DL provides a multiple-use light source, there is currently little evidence to suggest that either system improves detection of oral precancerous or cancerous lesions beyond visual inspection alone. The VELscope unit is a portable, multiuse, fluorescent device that is also simple to operate, but the unit is expensive and its durability has not been proven. Additional prospective studies are needed to evaluate the potential diagnostic benefit of tissue fluorescence for oral cavity examination.

Toluidine blue (tolonium chloride)

In 1964, Niebel and Chomet [36] first reported on the use of toluidine blue as a vital tissue stain to aid in the early detection of oral precancerous and malignant lesions. Also known by its chemical name of tolonium chloride, toluidine blue is a basic metachromatic stain that binds to DNA. Although not cancer specific, it has been reported to stain mitochondrial DNA, altered DNA in premalignant and malignant epithelial lesions, and cells with relatively increased amounts of DNA [37]. From 1964 to 1992, a number of studies showed toluidine blue to exhibit sensitivity that ranged from 86% to 100%, with a specificity ranging from 63% to 100%. A meta-analysis published in 1989 reported toluidine blue sensitivity as ranging from 93.5% to 97.8%, with a specificity ranging from 73.3% to 92.9% [38].

In 1996, Warnakulasuriya and Johnson [39] reported that all oral cancers (18 of 18 cases) tested were toluidine blue-positive; however, lower sensitivity (79.5%) and specificity (62%) were found with precancerous lesions, and a false-negative rate of 20.5% was observed. Problems with toluidine blue sensitivity, specificity, or both were noted in other studies of precancerous lesions in the middle to late 1990s and early 2000s [40,41]. In addition, false-positive rates as high as 35% were reported [41]. Variable study results over several decades probably explain why toluidine blue currently lacks widespread acceptance among generalists or specialists.

A series of recent reports may revive professional interest in this technique, however. Toluidine blue positivity was higher in oral premalignant lesions that showed loss of heterozygosity (LOH) at chromosome regions associated with the development of squamous cell carcinoma (3p, $P = .13$; 17p, $P = .049$) and was more likely seen with lesions that showed LOH in greater than two regions [42]. Importantly, the authors suggested that lesions with weak toluidine blue staining should be viewed suspiciously, because their molecular profiles were essentially identical to lesions that stained strongly. Similar molecular findings were reported in a study of 100 oral premalignant lesions that also examined clinical outcome, with an average follow-up time of 44 months [43]. Although only 5% (3 of 64 cases) of toluidine blue-negative lesions progressed to cancer, carcinomatous transformation was observed in 33% (12 of 36 cases) of the toluidine blue-positive lesions. This corresponded to a greater than sixfold elevation in cancer risk (relative risk = 6.67, 95% confidence interval [CI]: 1.87–23.70). Toluidine blue staining was associated with multiple LOH, especially including LOH at 3p or 9p, and this, in turn, was associated with a marked increased risk of carcinomatous transformation ($P = .0002$ or $P < .00001$). Of particular interest in this study, toluidine blue-positive lesions with minimal or no identifiable dysplasia on initial biopsy were almost fourfold more likely to transform to carcinoma than lesions found to be toluidine blue-negative (relative risk = 3.92, 95% CI: 0.92–16.80).

Use of tolonium chloride has also been of reported benefit in the follow-up of patients with previously treated upper aerodigestive cancer. In an examination of 96 biopsies performed in 81 patients, the sensitivity for detecting recurrent or secondary disease by clinical examination alone

was 40% compared with 97% with vital staining ($P = .0002$) [44]. Because the positive predictive values were similar for both arms of the study, the authors noted that the increased sensitivity with tolonium chloride did not come at the expense of unnecessary biopsies (false-positive results). In a separate report of 46 patients previously treated for oropharyngeal cancer, toluidine blue was used to direct subsequent follow-up punch biopsies of the stained tissue in these patients, together with nonstaining adjacent mucosa in 34 cases [37]. Evidence of equivalent LOH was noted in 25 of the 34 sample pair cases regardless of staining status, with discordant LOH in the remaining cases. Of these, 8 of the 9 cases showed a greater degree of LOH in the toluidine blue-positive sample compared with the unstained sample. In addition, the authors found that 59% of morphologically innocuous lesions initially thought to be false-positive results contained LOH, consistent with the hypothesis that toluidine blue staining may permit clinical detection of altered DNA even if the tissue appears microscopically benign. Most recently, a smaller study of 18 patients suggested that only dark toluidine blue staining should be viewed as a positive result [45]. The study was hampered by a high false-positive rate (31%) and the fact that all dark-stained lesions in their series were clinically ulcerated. Because this report stands in contrast to the earlier molecular-associated findings (similarly abnormal LOH patterns with dark- and light-stained lesions), confirmatory studies are needed.

Diagnostic methods

Despite the growing number of adjuncts available to assist in the clinical evaluation of lesions with uncertain biologic potential, surgical biopsy remains by far the most popular means of obtaining a final tissue diagnosis. Once a diagnosis is established, additional studies (including imaging modalities) may be needed to determine the stage of disease and to guide treatment plan development. A variety of approaches have been used to obtain diagnostic tissue samples of suspicious oral lesions, and several are discussed here.

Punch biopsy

A punch biopsy is a soft tissue sampling instrument having a circular cutting edge of varying diameter. It is most frequently used by dermatologists to sample skin lesions but can be used on mucosal surfaces as well. Deep biopsies in areas like the palate can be relatively simple to obtain with a punch biopsy instrument; however, controlling the sample depth may be difficult, and subsequent use of scissors or a scalpel is often needed to free the specimen base from underlying tissues. For study purposes, an advantage of the punch instrument is its ability to provide reproducibly sized epithelial samples of lesion or control tissues.

Scalpel biopsy

The simplest form of surgical sampling may be the shave biopsy, where a shallow saucer-shaped or elliptically shaped specimen (including a thin layer of connective tissue) is removed using a scalpel or curved razor blade. As with the use of a punch biopsy, a shave biopsy is favored by dermatologists for the diagnosis of superficial lesions, such as actinic keratosis or early basal cell carcinoma, in which evaluation of deep margins is not considered essential. Because a determination of tissue invasion is critical to the distinction between intraepithelial neoplasia (dysplasia or CIS) and oral squamous cell carcinoma, use of a shave technique is typically not recommended for the diagnosis of suspicious intraoral lesions.

The final diagnosis for suspicious lesions of the oral cavity is usually made on the basis of an incisional or excisional scalpel biopsy. Excisional biopsy is most often reserved for clinically benign or, at worst, precancerous mucosal lesions that are less than 2 cm in diameter. In cases in which carcinoma is strongly expected, excision of lesional tissue should only be performed by the surgeon who is to be directly involved with definitive patient management. Otherwise, healing of the surface mucosa may obscure the precise location of the original lesion and hinder definitive treatment planning.

Most suspicious lesions of the oral cavity are diagnosed through an incisional biopsy, where a portion of the abnormal surface tissue is removed for histopathologic interpretation. As a rule, the tissue sample should include the most clinically suspicious portion of the lesion, including areas of erythroplakia, speckled leukoplakia, surface granularity, or ulceration. Careful application of toluidine blue staining may be useful in this setting by highlighting suspicious areas. For lesions greater than 3 cm in diameter, the use of multiple incisional biopsies and vital staining may

be warranted to help identify or exclude focal carcinomatous transformation.

With oral precancerous or dysplastic lesions, little correlation has been identified between grade of dysplasia (mild, moderate, or severe) and the risk of progression to cancer [46–48]. In the absence of reliable prognostic information associated with morphology, molecular approaches have been used to help identify genetic features that might better define the risk of progression for a given lesion. These are discussed in more detail in the section on cytochemical and molecular studies in this article.

In the case of squamous cell carcinoma, predicting tumor behavior based on its microscopic features has also been an ongoing challenge for the pathologist. Tumor grade, or degree of differentiation, has not been a satisfactory predictor of local recurrence or patient survival, especially compared with tumor stage (tumor extent). Although the thickness of early (T1) squamous cell carcinoma of the tongue has been strongly associated with the risk for regional node metastasis and survival, it does not predict the risk of local recurrence [49–51]. A multiparameter analysis of squamous cell carcinoma, incorporating variables like degree of keratinization, pattern of invasion, nuclear pleomorphism, mitotic rate, and lymphocytic response, has been advocated by a number of authors to help predict local recurrence and overall survival [52–57]. The Martinez-Gimeno scoring system, an analysis of six histologic criteria plus primary tumor size (T classification), was shown in a prospective study to have a sensitivity of 100% (95% CI: 98%–100%) and a specificity of 55% (95% CI: 44%–66%) with a positive predictive value of 59% (95% CI: 48%–70%) and a negative predictive value of 100% (95% CI: 98%–100%) for the risk of locoregional metastatic disease in cases of oral squamous cell carcinoma [58].

Recently, the concept of multiparameter analysis was examined and modified by Brandwein-Gensler and colleagues [59] to produce a histologic risk assessment system based on (1) perineural invasion greater than 1 mm involving nerves, (2) lymphocytic response, and (3) worst pattern of invasion (WPOI) (Table 1). In a study of 292 patients with cancer, the authors demonstrated that their three-tiered system of risk assignment was strongly predictive of local recurrence and overall survival (log rank: $P = .0004$ and $P < .0001$, respectively) across uniformly treated patients (Fig. 7). Margin status, however, was not significantly related to disease recurrence or survival. This system provides a logical basis for the recommendation of adjuvant radiotherapy or chemotherapy for patients with oral cancer, including the newly defined group with T1/T2 N0/N1 tumors and negative resection margins but a risk score of greater than 3

Table 1
Proposed histopathologic risk assessment system for oral squamous cell carcinoma

Point assignment for risk scoring			
Histologic variable	0	1	3
Perineural invasion	None	Small nerves	Large nerves
Lymphocytic infiltrate at interface	Continuous band	Large patches	Little or none
WPOI at interface	#1 or #2 or #3	#4	#5

Risk score (sum of all point assignments)	Risk for local recurrence	Overall survival probability	Adjuvant treatment recommendations
0	Low	Good	No local disease-free benefit seen for adjuvant RT
1 or 2	Intermediate	Intermediate	No local disease-free benefit seen for adjuvant RT
3–9	High	Poor	RT regardless of 5 mm margins

Abbreviations: RT, radiotherapy; WPOI, worst patternal invasion.

From Brandwein-Gensler L, Teixeira MS, Lewis CM, et al. Oral squamous cell carcinoma: histologic risk assessment, but not margin status, is strongly predictive of local disease-free and overall survival. Am J Surg Pathol 2005;29(2):175; with permission.

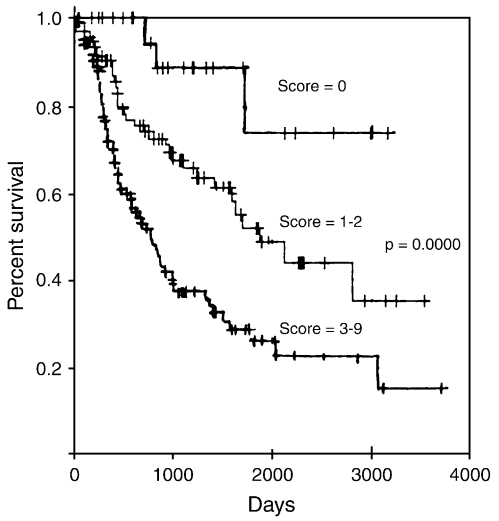

Fig. 7. Kaplan-Meier overall survival curves classified by risk assessment scoring system. (*From* Brandwein-Gensler M, Teixeira MS, Lewis CM, et al. Oral squamous cell carcinoma: histologic risk assessment, but not margin status, is strongly predictive of local disease-free and overall survival. Am J Surg Pathol 2005;29(2):175; with permission.)

(high-risk histologic features). Although prospective studies are needed to corroborate and extend these findings, the potential benefits of this simple yet elegant scoring system should be obvious to clinicians and pathologists alike.

Fine-needle aspiration cytology

Fine-needle aspiration (FNA) cytology is a valuable tool in the diagnosis of superficial masses of the head and neck region. Although most of these masses represent benign conditions, testing for cancerous lesions can include cervical or submandibular masses suspicious for metastatic nodal disease or conditions like primary salivary gland malignancy or lymphoma. A good discussion of this technique has been provided in a previous issue of *Oral and Maxillofacial Surgery Clinics of North America* [60]. More recently, FNA has been applied to the concept of sentinel node examination. Expertise in aspiration technique and cytologic interpretation of FNA specimens is essential for reliable results with this procedure. Although tumor sampling has been aided through guidance technology (ultrasound or CT), sampling errors or diagnostic challenges are reported with this technique and may necessitate subsequent open biopsy [61]. These limitations have been documented in a large (n = 6249) retrospective series of salivary gland lesions diagnosed by FNA, where a sensitivity of 73% and a specificity of 91% were recorded [62].

Sentinel node biopsy and cytology

Taken from its initial application with melanoma, the technique of investigating sentinel node tissue has recently been applied to oropharyngeal malignancies, such as squamous cell carcinoma. This procedure is intended to identify micrometastatic disease within a "sentinel" node considered most likely to drain the tumor bed and receive initial metastatic deposits from the primary malignancy. Sentinel node biopsy thus represents a less invasive means of providing staging information for the patient with oral cancer with an N0 neck.

The sentinel node technique uses lymphoscintigraphy, where the primary cancer site is initially injected with a radioactive tracer material, such as Tc 99m sulfur colloid. Different molecular weights of this material can be selected depending on the desired transit time for the study. Conventional radiography is then used to locate the sentinel node, and the patient is taken to the operating room. For open biopsy, the surgeon may inject a blue dye into the tumor bed to assist with visual identification of the node, supplemented by an intraoperative gamma detector. Use of a dye is not always recommended for head and neck tumors, because some authors claim that it can interfere with node identification or even tumor resection [63]. The node is then removed and examined histopathologically for micrometastatic disease, often aided by serial sections and the use of immunohistochemistry (IHC). Because only 6 of 10 occult metastases from primary squamous cell carcinoma of the oral cavity primary were reportedly detected using frozen sections, intraoperative evaluation of sentinel nodes does not seem to be sufficiently reliable for routine use [64].

A recent meta-analysis of this approach for squamous cell carcinoma of the oral cavity and oral pharynx reported a pooled sensitivity of 92.6% (95% CI: 0.852–0.964) [65]. In a study of 50 patients with oral, pharyngeal, or laryngeal cancer, 46 had identifiable sentinel nodes that were harvested by open biopsy [63]. All patients subsequently underwent neck dissection (39 unilateral and 21 bilateral). Occult metastases were found by open biopsy in 12 patients, and the authors noted that tumor detection required additional sectioning or IHC in three cases. For 9 of the patients, the sentinel

node was the only one to show micrometastatic disease, whereas multiple positive nodes were found in 3 patients. In addition, no patient with a negative sentinel node result was found to have tumor in other nonsentinel lymph nodes. Ultrasound-guided FNA cytology has also been used in an effort to provide an even more conservative approach to sentinel node assessment. Unfortunately, a lower sensitivity rate of 42% to 73% has been reported with ultrasound-guided specimens [66]. Some authors suggest an adjunctive role for FNA cytology in the evaluation of the patient with N0 neck cancer and have recommended that negative FNA results be followed by open biopsy of the sentinel node [65,66].

Another recent technology that has been used together with sentinel lymph node biopsy is positron emission tomography (PET) using ^{18}F-fluoro-2-deoxy-D-glucose (^{18}FDG). This imaging study is based on the increased metabolic activity of most cancer cells that results in preferential uptake of radiolabeled glucose by tumors, such as squamous cell carcinoma. In a prospective study involving resectable T1 to T3 lesions of oral and oropharyngeal squamous cell carcinoma, PET and CT were obtained in 62 patients [67]. A total of 38 patients with PET-negative findings were subsequently tested by sentinel node biopsy, including step-serial sections and IHC analysis. Five of these patients were found to have metastatic disease (PET false-negative results) and were treated with neck dissection. Although no significant differences were noted between PET and CT, negative neck sides were better predicted by PET. Only 41 (33%) of a possible 124 neck sides were treated after PET staging, positive sentinel node biopsy, or intraoperative evaluation of tumor extension. In contrast, standard treatment guidelines and CT examination would reportedly have resulted in 100 neck side procedures (81%). Importantly, none of the 41 patients diagnosed as PET-negative had evidence of clinical relapse, with a median follow-up of 33 months (range: 10–52 months). The authors proposed a staging ladder for clinically N0 patients based on the high specificity of prerequisite PET examination followed by the high sensitivity of sentinel node biopsy, which may result in fewer unnecessary neck dissection procedures. Finally, some authors suggest that use of combined (fused) PET and CT imaging could provide an additional advantage in patient staging over either modality alone or MRI [68–70].

Complicating factors with sentinel node biopsy and cytology include the rich lymphatic system of the head and neck that can produce bilateral drainage patterns by lymphoscintigraphy as well as the complex anatomy that can make precise localization and identification of suspicious nodal tissue quite difficult. The finding of multiple radioactive nodes can hinder determination of the true sentinel or "first echelon" node, with some authors favoring harvest or sampling of the three most strongly radioactive nodes [63,66,71].

Cytochemical and molecular studies

The diagnosis of oral precancerous and cancerous lesions continues to be made almost exclusively on the basis of routine morphologic evaluation of formalin-fixed paraffin-embedded tissue sections of scalpel biopsy specimens. Well-recognized cytologic and architectural changes associated with premalignant oral epithelial lesions are used to determine the presence and degree of epithelial dysplasia. This time-honored system represents the "gold standard" for identification of oral premalignancies and is used, at least broadly, to predict biologic behavior or risk of malignant transformation for a given precancerous lesion. Unfortunately, the earliest morphologic signs of dysplasia can be mimicked by a host of reactive conditions, and numerous studies have documented significant variability (interobserver and intraobserver) in the diagnosis of oral epithelial dysplasia. The predictive value of increasing degrees of dysplasia for the risk of malignant transformation is also unreliable. At the same time, although the histologic diagnosis of squamous cell carcinoma is less susceptible to variability among pathologists, studies relating its morphologic features to biologic behavior and prognosis have only recently been reported.

Although most experts agree that cellular alterations at the DNA level almost certainly precede microscopic morphologic changes that can be recognized by even the most experienced pathologist, a consensus has yet to be reached as to what parameter(s) might be most useful in the diagnosis and management of oral lesions. In this section, some of the chemical, IHC, and molecular markers that have been used in the early characterization of oral epithelial dysplasias and squamous cell carcinomas are presented.

Nucleolar organizing regions

Nuclear organizing regions (NORs) are loops of ribosomal DNA loops located on the short

arms of chromosomes 13, 14, 15, 21, and 22 and are associated with acidic nonhistonic proteins that can be visualized by silver-staining techniques (argyrophilic) [72]. Because the number or size of argyrophilic NORs (AgNORs) correlates positively with cellular proliferation, they have been used to study a variety of neoplastic conditions, including dysplastic and malignant oral epithelial lesions, as has been previously discussed in an earlier issue of *Oral and Maxillofacial Surgery Clinics of North America* [60].

Mean AgNOR counts were shown in a recent report to be differ significantly between nondysplastic (2.14) and dysplastic (2.65) clinical leukoplakias (95% CI: 0.670–0.936), with a sensitivity of 75% and specificity of 83%, with a cutoff mean AgNOR value of 2.37 [73]. Using this cutoff value, a subsequent report compared AgNOR counts with the gold standard of histopathologic diagnosis in 52 archival biopsy specimens. The test sensitivity was 67%, and the specificity was 59%, whereas the false-positive and false-negative rates were 41% and 33%, respectively [74]. The authors noted that mean AgNOR count had little correlation to the diagnosis of dysplasia and suggested lowering the cutoff value to reduce the high false-negative rate. Even more recently, mean AgNOR number, size, and percentage of total nuclear area were significantly increased in 12 cases of squamous cell carcinoma compared with corresponding normal patient tissue ($P < .01$), although significant case-to-case variability was noted [75]. For example, although AgNOR size was significantly larger in 11 of the 12 carcinoma cases, AgNOR number differed in only 8 of 12 cases, and the percentage area of the nucleus occupied by AgNORs varied in only 6 of 12 cases.

In two separate studies, one research group has combined brush cytology sampling with AgNOR counts. Using image analysis technology, a combined sensitivity of 98.2%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 99.5% were reported for the detection of cancer cells in 251 samples from 181 patients [72]. As discussed previously, however, these findings are confounded by the fact that only 63% of the brush cytology–AgNOR results were confirmed by scalpel biopsy, including only 57% (47of 83 lesions) described as "leukoplakia." In a follow-up paper using manual AgNOR counts, a sensitivity of 92.5%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 84.6% for the detection of squamous cell carcinoma were reported in 337 samples from 75 patients [76]. Once again, corresponding biopsy results were obtained in only 64 (19%) of 337 cases.

Limitations with the technique include the time and effort required to perform the study manually as well as staining variability and counting subjectivity. Although computer-assisted image analysis has the potential to overcome some of these problems, such hardware adds to the overall cost and maintenance requirements.

Abnormal DNA segregation (DNA aneuploidy)

It has been recognized for many years that abnormal chromosomal segregation resulting in aneuploidy can be a marker of neoplastic transformation. In the view of some authors, errors in DNA segregation may be one of the many causes of cancer and not merely a result [77]. Abnormal chromosomal content is thought to be the most common characteristic of solid tumors in human beings. Discussion of abnormal DNA content was provided in a previous issue of *Oral and Maxillofacial Surgery Clinics of North America* in an article on flow cytometric analysis [60]. Since then, studies using flow and image cytometry have provided additional information regarding the diagnostic usefulness of this technique [72,78–84]. In a series of 25 resected oral squamous cell carcinomas, all tumors were found by image analysis to be aneuploid and multiploidy was seen in 15 cases (60%); however, aneuploidy alone did not seem to be related to clinical progression [78]. A total of 29 mucosal lesions in 21 patients that progressed to carcinoma were compared with 29 control lesions that did not progress (mean follow-up of 112 months). The lesions were matched for location and level of dysplasia [79]. Using quantitative image analysis, the progressive lesions exhibited significantly greater levels of DNA aberration than controls ($P = .0096$). Of clinical importance, 3 lesions initially judged to be reactive by histopathologic examination but found to be nondiploid or aneuploid by DNA analysis all progressed to carcinoma. Comparison of flow cytometric and image cytometric results was performed in another study involving 32 cases of oral squamous cell carcinoma [80]. Image cytometry of stained sections was found to be more sensitive in detecting abnormal cell DNA content, and the presence of aneuploidy had prognostic implications. Nine of the 32 patients died within 5 years of initial treatment, and the tumors had greater than 10% abnormal cellular DNA in all 9 cases. To test the prognostic value of this

finding, a multivariate analysis was performed on 195 patients with oral cancer. In descending order of importance, the three independent variables found to have a statistical association with survival were (1) abnormal DNA content, (2) clinical stage, and (3) growth pattern (endophytic versus exophytic).

Combining brush cytology with DNA cytometry has been used to provide a less invasive means of patient sample analysis [72,76,82]. This combination was compared with incisional biopsy in a report of 98 patients, with a sensitivity of 100% and specificity of 97.4% [82]. Aneuploidy was found in 1 of 21 leukoplakia cases, 3 of 3 erythroplakia cases, and 15 of 15 squamous cell carcinoma cases. The findings of Remmerbach and colleagues [76] indicated that DNA aneuploidy was detectable in brush cytology specimens from 1 to 15 months before histologic evidence could confirm the presence of malignancy. In contrast, flow cytometry results were recently reported in 67 cases of oral squamous cell carcinoma [83]. Although 27% of the tumors were aneuploid, the authors found no relation between ploidy status and local recurrence, distant metastases, or survival.

Despite promising results, there has been recent public acknowledgment of significant scientific fraud by one of the most active proponents of aneuploidy analysis in the study of oral cancer [84,85]. This disclosure makes it impossible to summarize our current knowledge with any certainty until the evidence presented in several reports is re-examined and the conclusions are revised as necessary. Because of the ongoing controversy, the future of aneuploidy in the diagnosis and management of oral precancerous and cancerous lesions is unclear. Readers are advised to stay abreast of the related scientific literature as the story unfolds.

DNA alteration (loss of heterozygosity (LOH))

As mentioned previously, the progression of oral epithelium from a benign to malignant process begins at the genetic (DNA) level and is ultimately expressed at the cellular and clinical levels. For some lesions, such as a true leukoplakia with no observable dysplasia on biopsy, a clinical lesion may even precede the pathologist's ability to detect histomorphologic evidence of premalignancy. It is further recognized that carcinogenesis does not result from a single area of DNA damage but is a multistep process that requires an accumulation of several DNA alterations collectively resulting in uncontrolled neoplastic growth.

Recent work has supported the concept that premalignant oral lesions may have an identifiable genetic profile associated with the risk for malignant transformation. Using microsatellite analysis to detect areas of loss of allelic balance or LOH, early DNA changes in precancerous oral lesions have been found in the 3p and 9p chromosomal regions [86]. For a given lesion, the risk for progression to cancer was low if no LOH was found, intermediate if LOH at 3p and 9p was found, and high if LOH at 3p and 9p was seen together with additional areas of genetic damage [86,87]. Overall, lesions with LOH at 3p and 9p plus other defined chromosomal areas had a 33-fold increased risk for progression to squamous cell carcinoma compared with lesions without LOH [86,88]. Others have reported that only 2% of low-risk lesions by LOH analysis are likely to progress to cancer over a 5-year period compared with 50% of high-risk lesions [16,87,89]. In examination of the known "high-risk zones" for oral cancer (see Fig. 2B), genetic analysis showed that leukoplakia from sites of high risk (71 cases) possessed a greater degree of LOH than similar lesions in low-risk sites (56 cases) [90].

In addition to permitting insight into the risk of progression for a given lesion, the discovery that clinically and microscopically "normal" margins can harbor genetic damage significantly alters the concept of a clear or negative excisional margin with oral precancerous conditions. In a study of 66 dysplastic lesions designed to assess the treatment impact on patient outcome, such clinical features as sex (male versus female), history of smoking (nonsmoker versus ever smoker), location (high-risk versus low-risk site), and appearance (homogeneous versus nonhomogeneous) were not associated with lesion progression or recurrence [87]. Likewise, the histologic grade of dysplasia (mild or moderate) was not related to progression. Using LOH analysis to assign low-, intermediate-, and high-risk patterns, the authors found that although lesion treatment (surgical removal of clinically abnormal tissue) reduced progression to cancer for lesions of all LOH patterns, the reduction was not statistically significant. To further examine this finding, repeat biopsy was performed on 19 patients at the site of the original excision. In 17 patients, LOH patterns observed in the repeat biopsy indicated incomplete removal of the initial lesion.

Importantly, in 8 of the 17 cases, there was no clinical evidence of mucosal abnormality at the time of the second biopsy. When the treatment impact was reassessed by combining molecular and clinical criteria (evidence of residual clones, completeness of surgical removal, or clinical evidence of recurrence), the risk of progression risk was significantly reduced in cases with intermediate- and high-risk LOH patterns.

LOH is also seen in virtually all cases of squamous cell carcinoma and may be useful in predicting biologic behavior. Allelic imbalance has been reported at several chromosome arms, including 3p, 4q, 5q, 7q, 8p, 10q, 11q, 13q, 18q, 20q, and 22q [91]. In addition, accumulation of multiple LOH seems to be related to the risk of tumor recurrence. In a study of 68 patients with previously treated oral cancer, biopsies of subsequent leukoplakic lesions that did or did not progress to a second oral malignancy were performed [92]. Progressing lesions were 26 times more likely to exhibit LOH at 3p or 9p than nonprogressive lesions. In contrast, histopathologic evidence of dysplasia was not associated with increased risk of a second malignancy. Such data affirm the idea that molecular evaluation of lesional tissue and margin status may be more informative than routine histopathologic evaluation in the management of oral squamous cell carcinoma and precancerous disease.

The future

Despite the clinical promise shown by molecular techniques in areas ranging from early diagnosis to the follow-up of previously treated patients with oral cancer, such technology is primarily used at a handful of major clinical research centers in North America and is not routinely available to most oral and maxillofacial surgeons. Limiting factors include the added costs and requirement for additional equipment, the complexity of the tests themselves, and the need to calibrate for those who interpret test results. The natural reluctance of practitioners to adopt new techniques or protocols, even in the face of compelling evidence, also makes the standard of care for precancerous and cancerous lesions slow to change.

Ideally, diagnostic adjuncts or tests should be relatively affordable, should be simple to perform, and should use easily obtainable patient samples or specimens. As has been done with AgNOR counting, the combination of brush cytology sampling with thin-film slide preparation and molecular nucleic acid analysis may provide optimal sample material for studies of LOH or other genetic abnormalities using specimens obtained directly from clinically normal or abnormal mucosa.

It has also been shown that tumor markers or associated biomarkers can be detected in the serum and, more recently, the saliva of patients with cancer [93–95]. Saliva, especially, has many ideal characteristics for future diagnostic applications that range from routine screening to post-treatment follow-up of the patient with oral cancer. Saliva is easily accessible, is collectible by noninvasive means, and has previously been shown to contain identifiable DNA abnormalities in patients with oral squamous cell carcinoma [96,97]. Recent evidence using the reverse transcriptase polymerase chain reaction (RT-PCR) has also revealed quantifiable levels of mRNA in saliva [95]. Using microarray analysis, Li and colleagues [95] examined the saliva from 10 patients with recently diagnosed oral cancer in comparison to age and sex-matched controls with comparable smoking histories. From a total of 10,316 transcripts, 1679 were shown to differ significantly (up- and downregulated) between patient and control samples. Using more stringent selection criteria, the authors presented a total of seven salivary mRNAs as candidate cancer-related biomarkers, including interleukin (IL)-8, IL-1B, DUSP1, HA3, OAZ1, S100P, and SAT. Together, these biomarkers gave a sensitivity of 91% and specificity of 91% for distinguishing patients with oral cancer from controls. Of additional interest, these researchers have recently reported that salivary IL-8 mRNA and protein levels, as measured by quantitative RT-PCR and ELISA, respectively, were significantly higher in patients with oral cancer compared with matched controls [98]. Serum IL-6 mRNA and protein levels were also elevated in these same patients with cancer. The combination of salivary IL-8 and serum IL-6 gave a sensitivity of 99% and a specificity of 90% for detecting oral squamous cell carcinoma.

Finally, serum analysis by matrix-assisted laser desorption and ionization time-of-flight mass spectrometry was recently used to evaluate specimens from 57 patients with oral cancer and 29 control patients [99]. In this technique, chip-based arrays are used to bind various proteins through a number of interactions, including hydrophobic and/or hydrophilic, anionic and/or cationic, and metal-binding properties. The technology permits large numbers of specimens to be screened simultaneously, but it is expensive and requires

additional characterization work for proteins of interest. Of several proteins initially identified, the authors reported that a C-terminal fragment of the fibrinogen α-chain was the most highly predictive marker for cancer, with a sensitivity of 100% and specificity of 96.6%. Elevated tissue fibrinogen levels have previously been reported in association with breast cancer, small cell carcinoma of the lungs, and melanoma.

Technology is poised to play an increasingly active role in the diagnosis and management of patients with oral precancerous and cancerous lesions. Until more laboratories, including those based in smaller community hospitals, acquire the needed molecular technologies to perform new more diagnostically robust sample analyses, routine histopathologic examination is likely to remain the standard of care for most patients with oral precancerous and cancerous disease. Careful prospective examination of the recently proposed scoring system for oral squamous cell carcinoma (see Table 1) may represent an important step toward bridging the gap between current clinical and laboratory practice and the molecular diagnostics of the future.

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