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Lauren L. Patton, Joel B. Epstein and A. Ross Kerr

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Adjunctive techniques for oral cancer examination and lesion diagnosis

A systematic review of the literature

Lauren L. Patton, DDS, FDS RCSEd; Joel B. Epstein, DMD, MSD, FRCD(C), FDS RCSEd; A. Ross Kerr, DDS, MSD

In the past decades, adjunctive techniques have emerged with claims of enhancing oral mucosal examinations and facilitating the detection of and distinctions between oral benign and oral premalignant and malignant lesions (OPML). Clinicians who use these tools may be unaware of the state of the evidence supporting their effectiveness. Techniques that are promoted or assessed to improve earlier detection and diagnosis of oral malignancy include toluidine blue (TB), ViziLite Plus with TBlue (Zila Pharmaceuticals, Phoenix), ViziLite (Zila Pharmaceuticals), Microlux DL (AdDent, Danbury, Conn.), Orascoptic DK (Orascoptic, a Kerr Company, Middleton, Wis.), VELscope (LED Dental, White Rock, British Columbia, Canada) and OralCDx (Oral CDx Laboratories, Suffern, N.Y.) brush biopsy.

In developing countries such as India, where there is a high prevalence of disease, the focus is on downstaging oral cancer at diagnosis from advanced to earlier disease. In the United States, by contrast, these adjunctive techniques are marketed to facilitate the detection of premalignant disease. It is assumed that if a premalignant lesion is detected and treated, the lesion may not progress to cancer.

ABSTRACT

Background. Adjunctive techniques that may facilitate the early detection of oral premalignant and malignant lesions (OPML) have emerged in the past decades.

Methods. The authors undertook a systematic review of the English-language literature to evaluate the effectiveness of toluidine blue (TB), ViziLite Plus with TBlue (Zila Pharmaceuticals, Phoenix), ViziLite (Zila Pharmaceuticals), Microlux DL (AdDent, Danbury, Conn.), Orascoptic DK (Orascoptic, a Kerr Company, Middleton, Wis.), VELscope (LED Dental, White Rock, British Columbia, Canada) and OralCDx (Oral CDx Laboratories, Suffern, N.Y.) brush biopsy. They abstracted data relating to study design, sampling and characteristics of the study group, interventions, reported outcomes and diagnostic accuracy of adjunctive aids from 23 articles meeting inclusion and exclusion criteria, including availability of histologic outcomes.

Results. The largest evidence base was for TB. A limited number of studies was available for ViziLite, ViziLite Plus with TBlue and OralCDx. Studies of VELscope have been conducted primarily to assess the margins of lesions in known OPML. The authors identified no studies of Microlux DL or Orascoptic DK. Study designs had various limitations in applicability to the general practice setting, including use of higher-risk populations and expert examiners.

Conclusions. There is evidence that TB is effective as a diagnostic adjunct for use in high-risk populations and suspicious mucosal lesions. OralCDx is useful in assessment of dysplastic changes in clinically suspicious lesions; however, there are insufficient data meeting the inclusion criteria to assess usefulness in innocuous mucosal lesions. Overall, there is insufficient evidence to support or refute the use of visually based examination adjuncts.

Practical Implications. Given the lack of data on the effectiveness of adjunctive cancer detection techniques in general dental practice settings, clinicians must rely on a thorough oral mucosal examination supported by specialty referral and/or tissue biopsy for OPML diagnosis.

Key Words. Oral cancer; mouth neoplasm; dysplasia; diagnosis; early detection; brush biopsy; chemiluminescence; autofluoresence; fluorescence visualization; tolonium chloride; toluidine blue; OralCDx; VELscope; ViziLite.

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Dr. Patton is a professor, Department of Dental Ecology, and the director, General Practice Residency Program, School of Dentistry, University of North Carolina Chapel Hill, Room 431 Brauer Hall, Manning Drive and S. Columbia St., CB #7450, Chapel Hill, N.C. 27599-7450, e-mail "lauren_patton@dentistry.unc.edu". Address reprint requests to Dr. Patton.

Dr. Epstein is a professor, Department of Oral Medicine and Diagnostic Sciences, College of Dentistry, University of Illinois, Chicago, and the director, Interdisciplinary Program in Oral Cancer Biology, Prevention and Treatment, College of Medicine, Chicago Cancer Center, Chicago.

Dr. Kerr is a clinical associate professor, Department of Oral and Maxillofacial Pathology, Radiology and Medicine, and the director, Oral Mucosal Disease Service, College of Dentistry, New York University, New York City.

THE DETECTION TECHNIQUES

Vital tissue staining. Tolonium chloride, more commonly referred to as TB, has been used for more than 40 years to aid in detection of mucosal abnormalities of the cervix and the oral cavity. TB is a metachromatic vital dye that may bind preferentially to tissues undergoing rapid cell division (such as inflammatory, regenerative and neoplastic tissue), to sites of DNA change associated with OPML or both. The binding results in the staining of abnormal tissue in contrast to adjacent normal mucosa. A prior meta-analysis summarized 12 studies conducted between 1964 and 1984 and reported an overall sensitivity of 93.5 percent and specificity of 73.3 percent.[1] These study groups had high prevalence rates (8-100 percent) of oral neoplasia, compared with the low 0.1 percent estimated prevalence rate in the general population.[1]

Visualization adjuncts. Several visualization adjuncts, described below, are intended for use as adjuncts to the standard visual and tactile oral examination under incandescent light. They function under the assumption that mucosal tissues undergoing abnormal metabolic or structural changes have different absorbance and reflectance profiles when exposed to various forms of light or energy.

- Described as a chemiluminescent light detection system, ViziLite was developed from predicate devices to detect cervical neoplasia. After receiving an application of acetic acid, sites of epithelial proliferation, having cells with altered nuclear structure, are purported to preferentially reflect the low energy blue-white light emitted by a device generating an "acetowhite" change. The ViziLite system no longer is available as a single product, but is a part of the ViziLite Plus with TBlue system.
- The Microlux DL system is a multiuse system developed from a blue-white light-emitting diode (LED) and a diffused fiber-optic light guide that generates a low-energy blue light.
- The Orascoptic DK system is sold as a three-in-one, battery-operated, hand-held LED instrument with an oral lesion screening instrument attachment that is used in concert with a mild acetic acid rinse promoted to improve visualization of oral lesions.
- The VELscope system is a multiuse device with a hand-held scope through which the clinician can scan the mucosa visually for changes in tissue fluorescence. The proposed mechanism of tissue fluorescence is that mucosal tissues have a reflective and absorptive pattern based on naturally occurring fluorophores in the tissue. Tissue fluorescence in the oral cavity is variable and is affected by structural changes, metabolic activity, the presence of hemoglobin in the tissue, vessel dilatation and, possibly, inflammation. This variability has not been defined. Exposure to blue light spectra (400-460 nanometers) may maximize a differential profile in areas undergoing neoplastic change in which a loss of fluorescence visualization is reported.

The gold-standard diagnostic test for oral mucosal lesions that are suggestive of premalignancy or malignancy remains tissue biopsy and histopathological examination.

Cytopathology. Cytopathology is the microscopic study of cell samples collected from mucosal surfaces (via smears, scrapings or lavage) or from internal sites via fine-needle aspiration. The OralCDx Brush Test system uses a specialized brush that collects transepithelial cellular samples composed of free cells and clusters. These samples are fixed onto a glass slide and sent to a laboratory where they are stained (via a modified Papanicolaou test), scanned and analyzed microscopically by means of a computer-based imaging system that can rank cells on the basis of degree of abnormal morphology. A cytopathologist interprets the computerized results. Results are reported as "negative or benign," "positive" or "atypical." Abnormal OralCDx diagnoses have included "positive" (defined as definitive cellular evidence of epithelial dysplasia or carcinoma) and "atypical" (defined as abnormal epithelial changes of uncertain diagnostic significance) results.

Biopsy. The gold-standard diagnostic test for oral mucosal lesions that are suggestive of premalignancy or malignancy remains tissue biopsy and histopathological examination.[2] The aim of our systematic review is to evaluate the evidence for effectiveness of existing adjunctive techniques for oral mucosal examination and lesion diagnosis,

ABBREVIATION KEY. CIS: Carcinoma in situ. **NPV:** Negative predictive value. **OPML:** Oral premalignant and malignant lesions. **PPV:** Positive predictive value. **SCCa:** Squamous cell carcinoma. **TB:** Toluidine blue.

BOX

Definitions of basic measures of accuracy of diagnostic tests and procedures.

SENSITIVITY
The probability that someone who has the target disease (an oral premalignant or malignant lesion [OPML]) will generate a positive result (an OPML as demonstrated by means of gold-standard tissue biopsy).

SPECIFICITY
The probability that someone who does not have an OPML will generate a negative test finding.

POSITIVE PREDICTIVE VALUE
The probability that a person with positive test results actually has an OPML.

NEGATIVE PREDICTIVE VALUE
The probability that a person with negative test results does not have the disease.

beyond the oral mucosal visual and tactile examination under incandescent light.

MATERIALS AND METHODS

We formulated four key questions to address the role of adjunctive tools in oral cancer early detection among adults during routine oral examination.

- What is the effectiveness of vital tissue staining with TB in detecting OPML?
- What is the effectiveness of visualization adjuncts (ViziLite, Microlux DL, Orascoptic DK, VELscope) in detecting OPML?
- What is the effectiveness of combining vital staining with visualization adjuncts (ViziLite Plus with TBlue) in detecting OPML?
- What is the effectiveness of cytopathology (OralCDx) in diagnosing OPML?

To address the questions, we conducted detailed automated searches of PubMed, ISI Web of Science and the Cochrane Library from Jan. 1, 1966, through Feb. 17, 2008. We searched review articles for additional articles missed in the automated searches. We included late-breaking reports that had been peer-reviewed and accepted for publication, where accessible. We excluded articles that were case reports and statements of expert opinion, as we did articles not published in English and not involving human subjects.

We included studies that reported histologic confirmation of lesions identified by using the adjunctive techniques and studies that specifically reported or presented data allowing calculation of the test's accuracy compared with the histologic gold standard of tissue biopsy. Because of their acceptance by public health practitioners as the basic measures of accuracy of diagnostic tests and procedures, we identified sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) where possible (Box). In studies in which investigators reported equivocal results with adjunctive techniques, we grouped those results with those of studies that had positive results and recalculated accuracy measures.

The search strategy and results are shown in the figure, which is available as supplemental data to the online version of this article (found at "http://jada.ada.org"). We assessed the articles as to whether they coincided with a priori inclusion and exclusion criteria that reduced to 23 the number of studies included in the analysis for the key questions. We abstracted from each article data relating to study design, sampling and characteristics of the study group, interventions and reported lesion diagnostic outcomes. We assessed information about the clinical setting of each study (whether a mucosal disease or cancer center clinic or a general practice) and the enrolled subjects' presumed oral cancer risk.

We gave each article a summary quality score by means of assessing a priori identified important attributes of the study that may have led to bias in interpretation of results, including research design, study protocol, data analysis, measurement and validity (the scale is available as supplemental data to the online version of this article, available at "http://jada.ada.org"). If actual study methodology was not reported, we downgraded the study. We summed the points awarded for each attribute totaling from zero to 20, and we rescaled them on a zero-to-100 scale for ease of presentation, with a score of 100 representing the best or highest possible quality. In developing the quality-rating item set, we were guided by the criteria advanced by Hadorn and colleagues.[3] Although customized to the question, many of the component items represent modifications of existing rating scales used by the Research Triangle Institute–University of North Carolina Evidence-based Practice Center.[4] Two of the authors (L.L.P., A.R.K.) rated all of the included studies, and they combined results to provide a single score with standard deviation for each publication.

RESULTS

Vital staining with TB. For the use of vital tissue staining with TB alone as an adjunct in

detection of OPML, 15 studies met inclusion and exclusion criteria[5-19] (Tables 1 and 2, available as supplemental data to the online version of this article [found at "http://jada.ada.org"]). Of the 15 studies, there were six studies[5,7-10,13] in which TB staining was assessed as an adjunctive technique in lower-risk populations. There was one study using TB staining to survey for mucosal changes in a population with prior treatment for upper aerodigestive cancer with no a priori knowledge of lesions.[17] Investigators in eight studies assessed TB as a diagnostic adjunct in subjects with suspicious lesions or histologically proven dysplasia or cancer in patients at higher risk.[6,11,12,14-16,18,19] Thirteen were prospective case series or convenience sample studies with variable study designs and enrollment criteria, and one was a prospective longitudinal study.[18] Two of the 15 were multicenter prospective studies.[13,17] A total of 2,400 lesions were stained, with the number of lesions in any given study varying from 18 to 1,030.

Reported sensitivities for unaided visual examination were slightly lower than the sensitivities reported with the use of toluidine blue–aided examination.

The method of application of the vital dye was either mouthrinse or topical swab, using different preparations of TB. Some studies used a single application at baseline; others used a sequence of two applications, the second at a follow-up visit approximately two weeks after the first application, to reduce false-positive findings. Except for two studies,[7,17] all involved highly experienced examiners. A positive stained lesion generally was considered to be any that demonstrated uptake of blue dye, although some lesions reported as having equivocal, partial or speckled uptake were variably classified in the studies as either positive or negative or were assigned to a separate category. Some studies included and others excluded these equivocal results in the data analysis. Biopsy was not performed in all positive stained lesions in all studies. The prevalence of histopathological gold-standard diagnoses reported as positive ranged across studies (from 26 to 100 percent) and varied from either squamous cell carcinoma (SCCa) to dysplasia only or to any of dysplasia, carcinoma in situ (CIS), SCCa or other malignancies.

The quality ratings for several studies were low.[5-7,19] When we excluded these studies, the sensitivities of TB as a diagnostic adjunct varied from 38 to 98 percent (median, 85 percent) and specificities varied from 9 to 93 percent (median, 67 percent). The PPVs ranged from 33 to 93 percent (median, 85 percent) and the NPVs from 22 to 92 percent (median, 83 percent). Of note, Epstein and colleagues,[17] whose study was the only study using TB staining to survey oral mucosa in high-risk patients, reported the lowest specificity and NPVs, whereas Zhang and colleagues[18] reported the lowest sensitivity and PPVs. Investigators in three studies also reported accuracy data for the unaided visual examination as compared with histopathological findings.[8,14,17] Reported sensitivities for unaided visual examination were slightly lower than the sensitivities reported with the use of TB-aided examination, and they varied from 40 to 93 percent. Specificities varied from 50 to 75 percent. The PPVs ranged from 36 to 78 percent and the NPVs from 71 to 90 percent.[8,14,17] Although enhanced detection of dysplasias resulting from application of TB is more variable, detection of CIS and invasive SCCa was significantly improved in two of these studies[14,17] but not in the third.[8] A prospective longitudinal study[18] demonstrated that mucosal lesions retaining TB dye at baseline, even those categorized as presenting a lower risk (that is, those with little or no dysplasia at microscopic examination), were strongly predictive of risk of malignant transformation.

Visualization adjuncts. For the use of chemiluminescence or fluorescence visualization adjuncts ViziLite and VELscope in detection of OPML, five studies met our inclusion and exclusion criteria[19-23] (Table 2, available as supplemental data to the online version of this article [found at "http://jada.ada.org"]). No studies met our inclusion criteria for the Microlux DL or Orascoptic DK systems. None of the ViziLite and VELscope studies assessed these visualization adjuncts as examination adjuncts in a general population but, rather, assessed their performance as diagnostic adjuncts in patients who had oral lesions. Four used case series/convenience sample designs,[19-21,23] and one was a case-control study.[22] Two of the convenience samples were from a single center,[21,22] and one was a multicenter study.[23] The underlying population prevalence of dysplastic and neoplastic conditions was high, ranging from 18 to 88 percent. Three

studies assessed the ViziLite system[19,20,23] and two studies assessed the VELscope.[21,22]

The reported sensitivity of ViziLite was consistent across all three studies at 100 percent, because the studies involved only patients with previously visualized mucosal lesions. The other accuracy values were inconsistent: zero to 14 percent specificity, PPVs of 18 to 80 percent and NPVs of zero to 100 percent. The quality rating for the study by Ram and Siar[19] was low; when we eliminated this study from consideration, the results of the remaining studies (by Farah and McCullough[20] and Epstein and colleagues[23]) were consistent, demonstrating zero percent specificity, a low PPV (mean 20 percent) and zero percent NPV. The results of these studies suggested enhancement in visual parameters of the lesions in brightness, sharpness (margin delineation), surface texture and, in some cases, size of lesion compared with results of examination by means of standard illumination, although no previously unidentified lesions were reported. ViziLite no longer is available as a stand-alone device, but is available as a kit with TB swabs (see following section).

Compared with the sensitivity of histopathological examination in patients with identified high-grade dysplastic lesions and SCCa, the reported sensitivities of tissue autofluorescence with the VELscope technology as an adjunct to visual examination were 98 percent and 100 percent; specificity was 100 percent and 78 percent; PPVs were 100 percent and 66 percent; and NPVs were 86 percent and 100 percent, respectively.[21,22] If we included low-grade dysplasias, the accuracy of the forerunner to the VELscope product increased in one study.[22] Both studies of the VELscope technology were conducted at the same center in patients with known oral dysplasia or SCCa confirmed by biopsy and did not involve use of the technology as an adjunct for detection or diagnosis of new lesions.

Visualization adjuncts combined with vital staining with TB. Two studies assessed the ViziLite system in combination with TB staining,[19,23] the latter by using the ViziLite Plus with TBlue kit in patients to assess lesions already identified on examination with incandescent light. The investigators found that ViziLite enhanced visual lesion characteristics in approximately 60 percent of lesions, identified all lesions previously identified with standard light and identified no additional lesions. The addition of TB application to the chemiluminescence-enhanced visual examination in the multicenter study improved the specificity and PPV and increased the NPV to 100 percent.[23]

Cytopathology. Four studies of the use of the OralCDx brush biopsy in detection or diagnosis of OPML met inclusion criteria[24-27] (Table 3, available as supplemental data to the online version of this article [found at "http://jada.ada.org"]). All were case series in which the investigators used convenience samples of subjects with oral lesions. Two were multicenter[24,25] and two were single-center studies.[26,27] One study was prospective[24] and the rest were retrospective. In one study, researchers performed concurrent brush and scalpel biopsies at baseline for a subset of the population studied who had lesions classified clinically as suspicious,[24] and in three studies, expert clinicians performed both the brush and scalpel biopsies.[24,26,27]

The prevalence of dysplasia or SCCa among subjects, as determined by means of scalpel biopsy, varied from 14 to 38 percent. Reported sensitivities varied from 71 to 100 percent, and specificities varied from 27 to 94 percent. The PPVs ranged from 38 to 88 percent and NPVs from 60 to 100 percent. In the study by Sciubba,[24] only a small percentage of subjects with clinically benign lesions who underwent brush biopsy had matching histopathology. For this reason, we based our accuracy calculations on the study sample of clinically suspicious lesions categorized as Class I. We did not include apparently innocuous lesions, categorized by Sciubba[24] as Class II, in outcome statistics.

DISCUSSION

Biopsy and histopathology. The current gold standard of diagnosis of OPMLs is scalpel or punch biopsy and histopathology. However, intraobserver and interobserver findings vary among pathologists in the diagnosis of the mild and moderate dysplasias that compose the largest proportion of premalignant disease[28,29] and in determining early-stage invasion of CIS or SCCa. Furthermore, a diagnosis of oral SCCa by means of histopathological examination depends on recognition and sufficient sampling of oral lesions because of variation in abnormal microscopic changes within the clinical lesion. In addition, histopathological changes may be present in areas in which there is no clinical evidence of an oral lesion on visual examination alone (for

instance, "field cancerization"),[30] and it now is known that molecular and genetic changes may precede both clinical and microscopic morphological changes and may be present in histologically benign tissue.

A diagnosis of high-grade dysplasia is an established predictive marker for malignant transformation. In contrast, the potential for progression from low-grade dysplasia to SCCa may be better predicted by a combination of histopathological and molecular changes. Given the large numbers of both visible and occult oral epithelial lesions with malignant potential encountered in clinical practice, most of which likely are benign, identification of those that are not benign is critical.

General limitations in study design. Studies differed in the types of lesions they included, ranging from known SCCa only to lesions with serious pathology—that is, CIS, SCCa, severe dysplasia or all of these—to all grades of dysplasia and malignancy. Investigators in some studies combined all grades of dysplasia as positive, whereas others considered only high-grade dysplasia or CIS/SCCa to be positive. In studies that combined all dysplasias, there was no breakdown by grade, making it difficult to interpret the technique's performance in lesions with a high risk of malignant transformation versus lesions with lower risk.

Many studies involved a small sample size and were single-center studies. There are few multicenter trials in this area for adjuncts other than TB.

Most studies lacked information about the clinical appearance of lesions or described what constituted a "suspicious" lesion. Most studies were conducted by experts in the clinical diagnosis of oral mucosal lesions; this lowered their quality score on our scale, which was designed to critique studies with the nonexpert in mind. Because many of the studies of the effectiveness of these adjuncts are sponsored by the manufacturers of the products, one cannot rule out conflict of interest on the part of the research organizers and authors; however, blinded prospective multicenter studies do address potential conflict of interest. Most of the studies we reviewed did not compare oral lesion detection rates achieved with standard mucosal examination under incandescent light with rates achieved with examination augmented with use of the adjunctive device. Therefore, the findings of these studies are limited in terms of applicability to general dental practice settings.

TB studies. The West Midlands Regional Evaluation Panel in England did not recommend TB dye as an adjunct to oral cancer screening in primary care.[31] In a systematic review of community-based oral cancer screening programs, Patton[32] concluded that although the evidence supporting use of TB as an aid in diagnosis of oral cancer is fair, there is insufficient evidence to determine whether the use of this or other adjunctive techniques will increase the detection of oral malignancies in community screening programs. Despite the fact that the published studies do not assess TB's role as a diagnostic adjunct in lower-risk populations such as patients in general dental practices, the results of the 1989 meta-analysis by Rosenberg and Cretin[1] and our systematic review support a recommendation for the use of TB as a surveillance or diagnostic adjunct in populations at higher risk. Nevertheless, these studies generated variable and sometimes conflicting results, the nuances of which are worthy of discussion.

Molecular and genetic changes may precede both clinical and microscopic morphological changes and may be present in histologically benign tissue.

Performance based on disease severity. Given the variability in study design and the fact that all levels of disease are reported in some studies, it is difficult to tease out the nuances of the effectiveness of TB staining in identifying dysplastic lesions. Accuracy data from the studies reviewed in this article indicate that TB is effective in the identification of SCCa and CIS. A recent prospective longitudinal study showed that TB staining (and allelic loss) is predictive of malignant transformation, even in benign lesions or those with low-grade dysplasia, and is important support for further study and use of TB.[18]

Populations. The only surveillance study in which subjects with a history of undergoing cancer therapy were enrolled with no a priori lesion was the multicenter study by Epstein and colleagues[17]; mucosal tissue changes related to the prior oral cancer treatment may explain, in part, the high percentage of false-positive findings reported. Also, the investigators assessed a

subset of 30 patients in this trial for allelic loss (loss of heterozygosity). They saw molecular abnormalities associated with risk of progression to cancer even in histologically benign lesions that had positive TB results. Therefore, many of the false-positive TB results, recorded on the basis of benign histologic examination results, were thought to represent true molecularly positive lesions.[33] Most of the studies either were conducted in referral specialty clinics or involved surveillance or higher-risk populations (for example, patients in Department of Veterans Affairs facilities). Therefore, the underlying population prevalence of OPML in these studies was higher than that expected in a general dental practice with a low-risk population. This observation makes the PPVs and NPVs difficult to interpret, because predictive values vary on the basis of the underlying OPML prevalence in the population studied.

Methods to reduce false-positive findings. Mashberg[8-10] used a two-week waiting period for lesions seen at baseline before staining to show persistence and demonstrated high specificities compared with results from other similar studies. Onofre and colleagues[15] used a similar protocol and, despite a low prevalence of OPML, showed a similar specificity to those in studies with a higher prevalence of OPML. However, the study by Epstein and colleagues,[17] which also used a waiting period, reported a low specificity (9 percent) on the basis of histopathological findings, although this could be accounted for by the study's cancer survivor population, in whom false-positive results are more common because of mucosal sequelae after radiation therapy.

Unless a lesion has features mandating immediate biopsy, the elimination of potential causes (such as minimizing frictional sources) and reexamination in 10 to 14 days, assuming patients return for follow-up, will reduce false-positive findings secondary to inflammatory conditions in a general dental population.

TB types and staining protocols. The generally accepted protocol is application of 1 percent acetic acid solution before and after TB application; however, methods and materials varied in the studies we evaluated. It was difficult to assess if there were any significant differences when TB was used as a rinse or a local swab application and when and whether it was prepared from pharmaceutical-grade TB. Although a 1 percent TB solution can be prepared from nonpharmaceutical (laboratory) -grade TB, the pharmaceutical-grade TB (which meets higher standards) is available in the United States only as part of the combination ViziLite Plus with TBlue kit.

Staining intensity. There was variability in the reporting of TB staining patterns. Some studies reported only a royal-blue intense stain as positive, whereas others reported any staining as positive. Variable staining intensity may complicate clinical decision making. We recalculated accuracy data, where possible, to combine positive and equivocal staining. This procedure may have led to an underestimation of accuracy results. However, it appears that any staining should elevate the index of suspicion, and intense staining may be even more sensitive.

Visualization adjuncts. We found no published studies of the Microlux DL or Orascoptic DK systems identified by means of the search methodology, so this discussion relates only to ViziLite and VELscope.

Visible parameters that may lead to enhanced visualization were reported in approximately 60 percent of visible lesions with chemiluminescence. Enhanced visualization associated with ViziLite does not necessarily mean the enhancement is restricted to suspicious lesions; it may include a variety of suspicious and nonsuspicious lesions, thus reducing specificity. The utility of enhanced visual findings in low-risk populations is not known.

VELscope reportedly is useful in assessing lesion margins in patients with OPML and, therefore, may be useful in surgical management. No published studies have assessed the VELscope system as a diagnostic adjunct in lower-risk populations or in patients seen by primary care providers. Given the variability of fluorescence of the oral mucosa and the high prevalence of benign lesions that may show loss of fluorescence, additional studies are needed.

Cytopathology. There are inconsistencies in specificities and PPVs of OralCDx test results across studies. The study by Svirsky and colleagues[25] did not provide sufficient information to determine the technique's utility in a clinical setting. They did not provide the original clinical lesion diagnosis before scalpel biopsy for each of the 298 lesions. Lesion inclusion in the study was based on the availability of prior brush biopsy results. A weakness of the study by Poate and colleagues[26] is its lack of gold-standard histopathological examination performed on all suspicious lesions. This omission resulted in sta-

tistics involving only 112 of 120 lesions. Thus, the reported sensitivity, specificity and PPV may be biased. OralCDx samples reported as "inadequate" generally were excluded from data analysis, whereas in an "intent to diagnose" trial design, inclusion of these results would have reduced the utility of the test.

When used in a low-risk population, in whom oral malignancy is rare and in whom the test is applied to benign-appearing oral epithelial lesions, the accuracy of the OralCDx test was reduced and the rate of false-positive findings increased.[24,34] This outcome was observed in the American Dental Association's health screening events in 1999 and 2000 involving 930 dentists and dental hygienists reported by Christian,[34] and in the apparently innocuous Class II lesion group (defined as having an innocuous clinical appearance) of the study by Sciubba.[24] We did not include in this review the study by Christian[34] or the data on the apparently innocuous Class II lesion group in the study by Sciubba[24] owing to lack of histologic confirmation of negative results with the brush test. Given the paucity of positive brush test results, it appears that the accuracy of the OralCDx brush test is linked more to the agreement of an atypical result and histopathological findings when used on already identified clinically suspicious Class I lesions. It is important to recognize that atypical results may be associated with false-positive rates related to inflammatory lesions.

Approaches to augment the brush test's specificity could greatly bolster the test's utility. It appears that the accuracy of this test can be improved by sampling lesions that are present 10 to 14 days after removal of any suspected etiological agent. Obvious long-standing developmental or submucosal lesions are not appropriate for use of this adjunct. Similarly, lesions highly suggestive of malignancy should undergo prompt tissue biopsy for determination of histopathological diagnosis.

Using the literature toward an evidence-based approach to use of these adjuncts. An evidence-based approach to reviewing the literature invariably leads to the elucidation of study methodological flaws and biases. While it was refreshing to see the evolution in study methodology across time, there remains a need for well-designed multicenter prospective studies to add to our knowledge in this field. On the basis of the studies meeting our a priori inclusion criteria (that is, those with histologic endpoints), our quality rating of the studies and our systematic review with its noted limitations, there is evidence of the following:

- TB is effective as a diagnostic adjunct for use in high-risk populations, such as patients seen in mucosal disease clinics or cancer centers, patients with a history of an OPML and patients with known risk factors. TB also is effective as a diagnostic adjunct in assessing high-risk mucosal lesions, which are epithelial lesions that clinically are suggestive of being an OPML.
- OralCDx's cytologic test has utility in detecting dysplastic changes in high-risk mucosal lesions. Data are insufficient to assess the test's utility in low-risk populations or clinically innocuous lesions, given the lack of data meeting our inclusion criteria. This test has been promoted to assess lesions the practitioner might not investigate further. It is not recommended for assessment of clinically suspicious lesions in which the practitioner normally would perform a scalpel biopsy.
- There is insufficient evidence to support or refute the use of visually based examination adjuncts in dental practice. ViziLite may increase the visibility of mucosal lesions, but only one study assessing the combined use with TB had histologic endpoints. The effect of chemiluminesence alone on detection of premalignant mucosal lesions is unknown.

There remains uncertainty as to whether the use of adjuncts for identifying and assessing oral mucosal abnormalities results in a meaningful reduction in morbidity and mortality.

There remains uncertainty as to whether the use of adjuncts for identifying and assessing oral mucosal abnormalities results in a meaningful reduction in morbidity and mortality through increased attention, on the part of both provider and patient, to the early detection and diagnosis of OPMLs. A formal consensus conference is needed to further define appropriate use of these adjunctive techniques on the basis of current research.

Need for research to aid general dentists in early-detection efforts. Multicenter studies

are needed to explore the utility of current adjunctive techniques as used by primary care providers. Studies should be conducted in patient populations at lower risk of experiencing OPML—that is, populations more similar to those found in private dental practices. None of the diagnostic adjuncts for OPML have been assessed in community dental and medical practice settings. Nearly all studies have been conducted in high-risk patients by specialists. Therefore, we recommend studies in general practice settings to assess these adjunctive techniques' utility in the detection and diagnosis of OPML, as well as biopsy site selection and margin determination.

Future studies should include comparison of findings of visual and tactile oral mucosal examination with adjunct-assisted examination. Numbers, types and locations of identified lesions should be compared for each method. Future studies should be prospective, have consecutive enrollment of subjects, use defined and accepted criteria for definition of suspicious epithelial lesions, use an "intent to diagnose" design that includes inadequate samples and indeterminate results, and demonstrate persistence of the lesion after removal of putative causes. Clinical lesions should be assessed for agreement regarding lesion inclusion by two examiners who have undergone interexaminer technique calibration. The adjunctive method or diagnostic technique should be well-described and acceptable to patients and providers, and examiners should be trained in its use. Scalpel or punch biopsy technique and site selection methodology should be clear and acceptable for histopathological diagnosis. Categorization of results should be based on clearly defined criteria, with description of handling of equivocal results. Histopathological examination results should be categorized and reported according to standard severity grading (mild, moderate or severe dysplasia; CIS; SCCa) and defined combinations of histologic diagnoses. Use of a central laboratory or consensus of two or more pathologists who are blinded to clinical lesion identification is recommended. Appropriate statistical methods should be used and results should include false-positive and false-negative findings. Longitudinal data collection and multicenter studies are encouraged to assess the effectiveness of the adjunctive technique in contributing to early identification of dysplastic lesions that later undergo malignant transformation.

CONCLUSION

Molecular and genetic analysis is not a routine procedure for oral lesions in which biopsy is performed in daily practice. New methods under investigation that involve examination of molecular markers in exfoliated cells and in oral fluids and new imaging methodologies may contribute to future advances in diagnosis, evaluation of response to treatment interventions and determination of prognosis of oral mucosal lesions. The future is promising for further development and evolution of oral-cancer diagnostic aids to enhance the quality of patient care provided by all clinicians. ▪

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