COVER STORY

ARTICLE 1

IMPROVING DETECTION OF PRECANCEROUS AND CANCEROUS ORAL LESIONS

Computer-Assisted Analysis of the Oral Brush Biopsy

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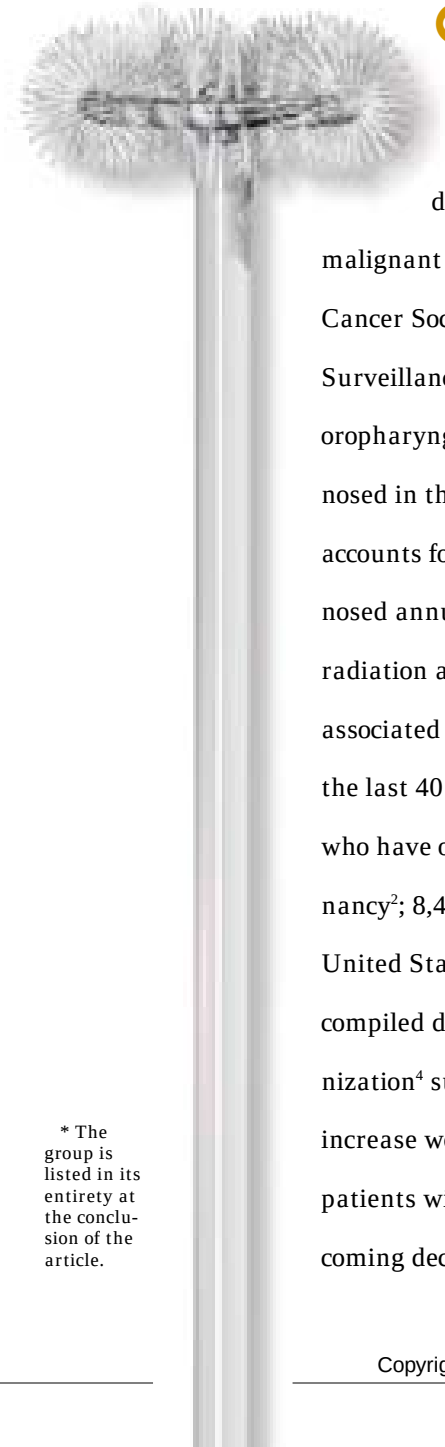


Cancer of the oropharynx is a major cause of cancer-related death in the United States, exceeding the annual death rates for cervical cancer and malignant melanoma.[1] According to the American Cancer Society's Department of Epidemiology and Surveillance,[2] an estimated 30,750 new cases of oropharyngeal cancer are expected to be diagnosed in the United States in 1999, a figure that accounts for about 3 percent of all cancers diagnosed annually. Despite advances in surgery, radiation and chemotherapy, the mortality rate associated with oral cancer has not improved in the last 40 years.[3] Ultimately, 50 percent of people who have oral cancer die as a result of the malignancy[2]; 8,440 such deaths are predicted in the United States this year alone.[2] A recent report of compiled databases from the World Health Organization[4] suggests that there will be a continuing increase worldwide in the absolute numbers of patients with oral cancer to be treated in the coming decades.

ABSTRACT

Background. A study group composed of researchers from across the United States undertook a study to evaluate the sensitivity and specificity of OralCDx (OralScan Laboratories Inc.), a computer-assisted method of analysis of the oral brush biopsy, in the detection of precancerous and cancerous lesions of the oral mucosa.

Methods. The study group conducted a multicenter double-blind study comparing results of OralCDx analysis with those of scalpel biopsy of suspicious oral lesions, as well as using OralCDx on oral lesions that appeared benign clinically.

Results. In 945 patients, OralCDx independently detected every case of histologically confirmed oral dysplasia and carcinoma (sensitivity = 100 percent, false-negative rate = 0 percent). Every OralCDx "positive" result was subsequently confirmed by histology as dysplasia or carcinoma. The specificity for the OralCDx "positive" result was 100 percent, while the specificity for the OralCDx "atypical" result was 92.9 percent. In 4.5 percent of clinically benign-appearing lesions that would not have received additional testing or attention other than clinical follow-up, OralCDx uncovered dysplasia or carcinoma (statistical sensitivity > 96 percent, $P < .05$, n = 131; statistical specificity for the OralCDx "positive" result > 97 percent and for the "atypical" result > 90 percent, $P < .05$, n = 196).

Conclusions. The authors propose that this multicenter trial demonstrates that OralCDx is a highly accurate method of detecting oral precancerous and cancerous lesions. OralCDx can aid in confirming the nature of apparently benign oral lesions and, more significantly, revealing those that are precancerous and cancerous when they are not clinically suspected of being so. All OralCDx "atypical" and "positive" results should be referred for scalpel biopsy and histology to completely characterize the lesion.

Clinical Implications. Given the difficulty in clinically differentiating premalignant and malignant lesions from benign lesions with a similar appearance, OralCDx appears to determine the significance of an oral lesion definitively and detect innocuous-appearing oral cancers at early, curable stages.

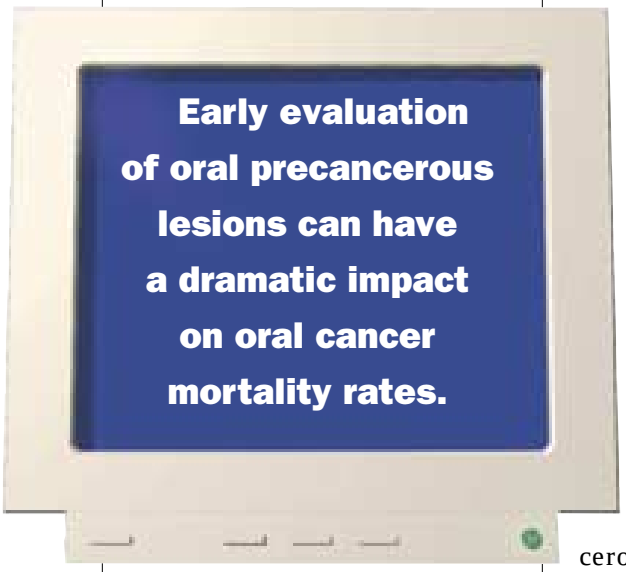
* The group is listed in its entirety at the conclusion of the article.

DETECTING ORAL CANCER

Detection of oral cancer in the early asymptomatic stage dramatically improves cure rates and patients' quality of life by minimizing extensive, debilitating treatments. The five-year survival rate for patients with early, localized disease approximates 80 percent; for those with distant metastases, it is 19 percent.[5] Unfortunately, more than 50 percent of patients with oral cancer display evidence of spread to regional lymph nodes and metastases at time of diagnosis, and approximately two-thirds of patients have apparent symptoms, a negative prognostic indicator.[6]

Although screening has been emphasized as a method of reducing the morbidity and mortality associated with oral cancers, the visual detection of oral cancer at an early stage is significantly hindered by the difficulty in clinically differentiating premalignant and malignant lesions from similar-looking benign lesions.[7,8] In contrast, visual inspection of the skin by dermatologists is a reliable screening method for detecting melanoma, with sensitivity and specificity rates of approximately 93 percent to 98 percent.[9,10] Early-stage oral cancers are asymptomatic. Furthermore, they often may appear innocuous, since the classic clinical characteristics associated with advanced oral cancers—including ulceration, induration, elevation, bleeding and cervical adenopathy—usually are absent in early-stage lesions.[11]

Sandler[12] emphasized the unreliability of the oral examination as a method of detecting early-stage oral cancer after studying 208 oral cancers and finding that approximately 25 percent of them appeared benign, lacking any clinical features of malignancy. Furthermore, the high prevalence of oral abnormalities discovered as a result of oral cancer screening programs, reported between 5 percent and 15 percent,[13-15] makes it impractical to subject every oral lesion to histologic evaluation.[16] Scalpel biopsy is an invasive procedure associated with potential morbidity. Thus, many oral lesions undergo biopsy only when they display either symptoms or clinical features typical of malignancy, while many innocuous-appearing early-stage oral cancerous lesions are merely observed clinically and left undiagnosed.[17] This may explain, in part, why more than 50 percent of oral cancers are diagnosed in the advanced stages.



Delays in biopsy and, thus, in recognition of early-stage oral cancers are well-documented and are common. One study demonstrated that one-third of patients eventually diagnosed with oral cancer received inappropriate therapy for incorrectly diagnosed conditions.[18] Given the limitations of the oral cavity examination in identifying oral cancer and the significant morbidity and mortality associated with advanced oral cancer and its treatment, the need for early detection of apparently innocuous oral cancers is compelling.

Early evaluation of oral precancerous lesions can have a dramatic impact on oral cancer mortality rates.[19] Erythroplakia, occurring as either an isolated lesion or as a component of leukoplakia (erythroleukoplakia), has been emphasized repeatedly as a marker of severe epithelial dysplasia or carcinoma in situ.[11,20] The significance of the leukoplakic lesion, the most common precursor of oral cancer (85 percent of all precancerous lesions are leukoplakic), also has important prognostic implications.[19] Like oral cancer, leukoplakia has a varied appearance, and although certain clinical features may indicate the lesions that have a greater risk of becoming malignant, leukoplakias that histologically display severe dysplasia, carcinoma in situ or frank carcinoma often are asymptomatic and appear totally harmless.[21] Moreover, lesions that are large and ominous-looking may prove to have no significant histologic abnormalities.

The clinical evaluation of

leukoplakia is further complicated by the fact that the appearance of the lesions changes over time.[22] The range of malignant transformation of leukoplakia varies considerably—from less than 4 percent[19,21] to more than 40 percent,[21,23] depending on the specific subtype studied. Since the malignant transformation of leukoplakia cannot be accurately predicted solely on the basis of clinical characteristics, histologic evaluation has been recommended for all suspicious lesions.[19] However, given the large number of patients with leukoplakia, estimated at 3 percent of the U.S. adult population,[14,23] it is not surprising that only 25 percent of leukoplakias ever are evaluated histologically.[24] Consequently, significant numbers of apparently innocuous premalignant lesions remain undiagnosed and may progress to oral cancer. Therefore, all cases of leukoplakia, erythroplakia and erythroleukoplakia require evaluation.

In light of the need for more precise methods of identifying oral cancer in its early stages, the U.S. Collaborative OralCDx Study Group undertook a study to evaluate the sensitivity and specificity of OralCDx (OralScan Laboratories, Inc.), a computer-assisted method of analysis of the oral brush biopsy, in the detection of precancerous and cancerous lesions of the oral mucosa.

MATERIALS AND METHODS

A prospective multicenter trial using OralCDx testing was conducted at 35 U.S. academic dental sites. Dentists specializing in oral and maxillofacial pathology, oral medicine and oral surgery obtained the specimens in the course of their routine clinical practice. During the study interval (1998-1999), all patients older than 18 years of age who had intraoral lesions displaying an epithelial component were eligible for enrollment. At investigator sites that required consent approved by an institutional review board, patients signed a consent form before participating. Oral lesions covered with clinically intact normal epithelium—such as mucoceles, fibromas and pigmented lesions—were not included in the study. Lesions of the vermilion border of the lips and cutaneous surfaces were also excluded.

Figure 1. The sterile brush biopsy instrument is specifically designed to provide an appropriate full-thickness sample of the lesion in question.

The investigators clinically characterized all oral lesions in the study either as innocuous or as causing suspicion of intraepithelial neoplasia. Suspicious lesions (categorized as Class I) were analyzed by use of both OralCDx and scalpel biopsy. Apparently innocuous lesions (categorized as Class II) that, in the investigators' opinion, required no further attention other than clinical follow-up were tested only by use of OralCDx. Patients with apparently innocuous lesions that produced abnormal OralCDx results, as defined below, subsequently were subjected to scalpel biopsy at the investigators' discretion.

OralCDx kits supplied to investigators consisted of an oral brush biopsy instrument, a precoded glass slide and matching coded test requisition form, an alcohol/polyethylene glycol fixative pouch and a preaddressed container in which to submit the contents. The test requisition form included demographic data such as the patient's age, sex and history of tobacco and alcohol use, as well as the location, clinical description and category (Class I or Class II) of the oral lesions.

All oral brush biopsies were performed using the supplied sterile instrument (Figure 1) that was specially designed to

TABLE 1

PROFILE OF STUDY PATIENTS (N = 945) AND CLINICAL CHARACTERISTICS OF ORAL LESIONS.

DEMOGRAPHIC INFORMATION	
Sex (n)	
Male	443
Female	502
Age (Years)	
Mean	55
Range	18-83
TOBACCO AND ALCOHOL USE	
Cigarette Use	**Percentage of Total**
None	63
< 1 pack cigarettes/day	4
≥ 1 pack cigarettes/day	33
Other Tobacco Use (snuff, pipes, cigars)	7
Alcohol Use	
None	51
Social use (less than 7 oz. per day)	43
Heavy use (7 oz. or more per day)	6

obtain a complete transepithelial specimen. Patients whose samples were considered inadequate for laboratory interpretation because they were incomplete transepithelial biopsy specimens—in other words, because they did not contain adequate representation of cells from all three epithelial layers of the oral mucosa (superficial, intermediate and basal)—were excluded from the study.

Depending on the lesion's intraoral location and accessibility, either the flat surface or circular border of the brush was placed against the surface of the lesion and, while firm pressure was maintained, rotated five to 10 times. Pinkness of tissue or pinpoint bleeding at the brush biopsy site was evidence of proper technique. Neither topical nor local anesthetic was used. The cellular material collected on the brush then was transferred to the bar-coded glass slide and rapidly flooded with the fixative to avoid air-drying. After approximately 15 minutes, the dry slide was placed in a plastic slide container and sent, with the bar-coded requisition form, in the preaddressed mailing container. The great majority of investigators had been trained in the oral brush biopsy and slide preparation technique at an investigators' meeting, and all were provided with written instructions.

All OralCDx specimens were analyzed at OralScan Laboratories in Suffern, N.Y., whereas oral and maxillofacial pathologists at the investigators' dental institutions histologically evaluated all scalpel biopsy specimens. Trial coordinators at OralScan Laboratories received all OralCDx slides and documents and entered demographic and clinical data retrieved from the requisition forms. The pathologist analyzing the OralCDx specimen was masked from all of the clinical and demographic data as well as histologic results.

All OralCDx slides were stained in accordance with a modified Papanicolau method. Stained slides then were scanned by the OralCDx computer system, which consists of a neural network-based image-processing system specifically designed to detect oral epithelial precancerous and cancerous cells. The OralCDx computer searches the brush biopsy specimen for a combination of abnormal cellular morphology and abnormal keratinization, which uniquely characterizes dysplasia and carcinoma of the oral epithelium. This image analysis process is performed using a specially designed and trained image processor that has been demonstrated to detect as few as two abnormal oral epithelial cells scattered among thousands of normal cells distributed on an oral brush biopsy specimen.

In addition, the OralCDx computer was adapted to complement existing oral cancer screening modalities that use vital dyes. Specifically, the significance of oral lesions stained with toluidine blue, a metachromatic vital dye that has been shown to increase the visual detection of oral cancers after a negative clinical examina-

tion,[25,26] can be determined with OralCDx testing.

Images of abnormal cells identified by the computer system are individually displayed on a high-resolution color video monitor for review by a pathologist specially trained in computer-assisted analysis of the oral brush biopsy specimen. The computer video microscope output is used by the pathologist in conjunction with a standard microscopic evaluation of each oral brush biopsy specimen. The computer does not provide a diagnosis of the brush biopsy specimen; rather, it assists in the search for and identification of abnormal cells, which are then visually assessed and interpreted by the pathologist, who renders a final diagnosis. The specimens were classified into one of the following four categories:

- "negative": no epithelial abnormality;
- "atypical": abnormal epithelial changes of uncertain diagnostic significance;
- "positive": definitive cellular evidence of epithelial dysplasia or carcinoma;
- "inadequate": incomplete transepithelial biopsy specimens (these specimens were excluded from the study).

In cases that the pathologist judged to be "atypical" or "positive" according to OralCDx, a summary screen containing representative cellular abnormalities was selected from the computer's video display, and these annotated images were printed and supplied to the dentist who submitted the oral brush biopsy specimen.

Statistical significance was determined using the normal approximation to the binomial distribution with the continuity correction for the normal test.

TABLE 2

CHARACTERISTICS AND INCIDENCE OF LESIONS.

CHARACTERISTIC	INCIDENCE (n)
Predominant Color	
White	484
Red	127
Mixed	263
Mucosal-colored	20
Not specified	51
Morphology	
Flat	417
Plaquelike	310
Verrucous	59
Not specified	159
Ulcerated	
Yes	204
No	583
Not specified	158
Signs/Symptoms	
None	594
Pain	299
Bleeding	22
Not specified	30
Location	
Floor of mouth	54
Ventral tongue	58
Lateral tongue	143
Dorsal tongue	47
Attached gingiva	153
Buccal mucosa	243
Alveolar and labial mucosa	116
Hard palate	54
Soft palate	21
Oropharynx	3
Retromolar trigone	26
Not specified	27

RESULTS

A total of 945 patients were

TABLE 3

OVERVIEW OF ALL BRUSH AND SCALPEL BIOPSY RESULTS (N = 945).

BRUSH BIOPSY RESULTS	SCALPEL BIOPSY RESULTS			TOTAL
	Malignant or Dysplastic	Benign	Not Performed	
Positive	78	0	2	**80**
Atypical	53	14	99	**166**
Negative	0	182	517	**699**
Not Performed	0	0	0	**0**
TOTAL	**131**	**196**	**618**	**945**

TABLE 4

BRUSH AND SCALPEL BIOPSY RESULTS OF CLINICALLY SUSPICIOUS LESIONS: CLASS I (n = 298).

BRUSH BIOPSY RESULTS	SCALPEL BIOPSY RESULTS			TOTAL
	Malignant or Dysplastic	Benign	Not Performed	
Positive	64	0	0	**64**
Atypical	38	14	0	**52**
Negative	0	182	0	**182**
Not Performed	0	0	0	**0**
TOTAL	**102**	**196**	**0**	**298**

TABLE 5

BRUSH AND SCALPEL BIOPSY RESULTS OF CLINICALLY BENIGN LESIONS: CLASS II (n = 647).

BRUSH BIOPSY RESULTS	SCALPEL BIOPSY RESULTS			TOTAL
	Malignant or Dysplastic	Benign	Not Performed	
Positive	14	0	2	**16**
Atypical	15	0	99	**114**
Negative	0	0	517	**517**
Not Performed	0	0	0	**0**
TOTAL	**29**	**0**	**618**	**647**

enrolled during the study interval; 502 (53 percent) were women and 443 (47 percent) were men. The patients' ages ranged from 18 to 83 years. The demographic features of study patients are summarized in Table 1.

Brush biopsy specimens were obtained from oral lesions with diverse clinical features arising on mucosa from all regions of the oral cavity (Table 2).

Of 945 lesions, 298 were judged as clinically suspicious (Class I) and were evaluated by use of OralCDx and scalpel biopsy. The remaining 647 lesions tested by OralCDx were characterized clinically as Class II and, in the opinion of the investigators, did not require histologic evaluation. The results of all OralCDx and histopathologic tests are summarized in Table 3. These include 29 Class II specimens that had abnormal OralCDx results and subsequently were tested by scalpel biopsy. Of the 945 lesions, 131 revealed histopathologic evidence of dysplasia or carcinoma. OralCDx detected every one of these cases. Specifically, 78 oral lesions were identified as OralCDx "positive" with definitive cellular evidence of epithelial dysplasia or carcinoma, and 53 were reported as OralCDx "atypical," with both types warranting histologic analysis. The sensitivity rate, defined as a measure of the likelihood that a patient with dysplasia or carcinoma will have an abnormal OralCDx result, is 100 percent (131/131). Of the 131 cases, 29 initially were characterized as Class II lesions and ultimately were subjected to scalpel biopsy as a result of the OralCDx evaluation.

No features of dysplasia or carcinoma were evident in 196 of the oral lesions that were evaluated histologically (Table 4). Of these, OralCDx reported 182 as "negative," with no epithelial abnormalities, and 14 as "atypical." The specificity rates—defined as a measure of the likelihood that a patient with a lesion determined to be benign by histology will not have an abnormal OralCDx result—are 100 percent (196/196) for "positive" OralCDx results and 92.9 percent (182/196) for "atypical" OralCDx results.

Of the 647 apparently benign oral lesions characterized as Class II (innocuous-appearing lesions that in the investigators' opinion required no further attention other than clinical follow-up), 16 were reported by OralCDx as "positive" and 114 as "atypical" (Table 5). Fourteen of the "positive" and 15 of the "atypical" oral lesions subsequently were subjected to a scalpel biopsy. All 29 of the OralCDx "positive" and "atypical" Class II oral lesions proved histologically to be dysplastic or cancerous. Some of the patients with an abnormal OralCDx result who did not undergo a scalpel biopsy were lost to follow-up; in the majority of other instances, the investigators determined clinically that the oral lesion was benign. For instance, inflammatory conditions that were tested with OralCDx, such as pemphigus, lichen planus and geographic tongue, often are attended by cellular atypia and may result in OralCDx "atypical" reports. Overall, OralCDx uncovered 29 (4.5 percent) histologically confirmed, unsuspected oral precancers and cancers among the 647 apparently benign Class II oral lesions. Additionally, 15.3 percent (99/647) of the Class II lesions yielded an OralCDx "atypical" result but were not tested histologically.

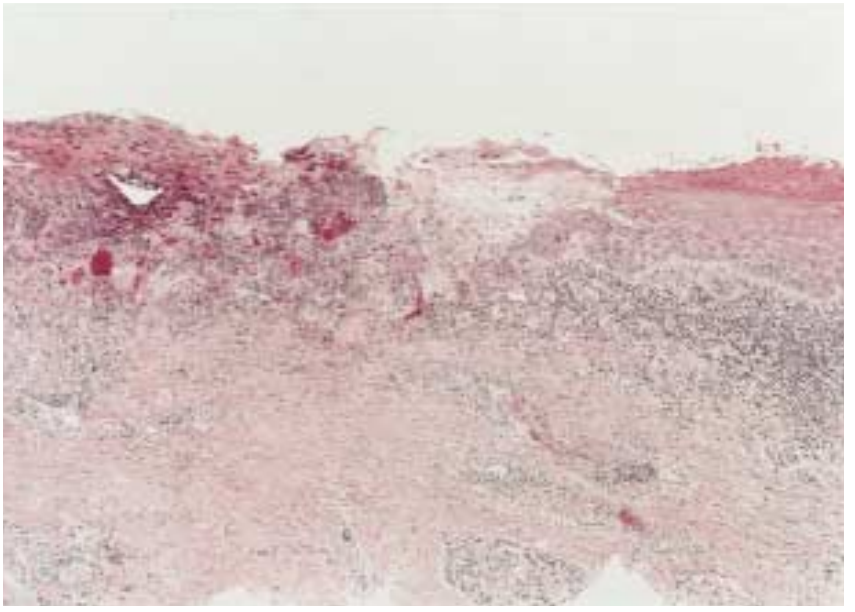
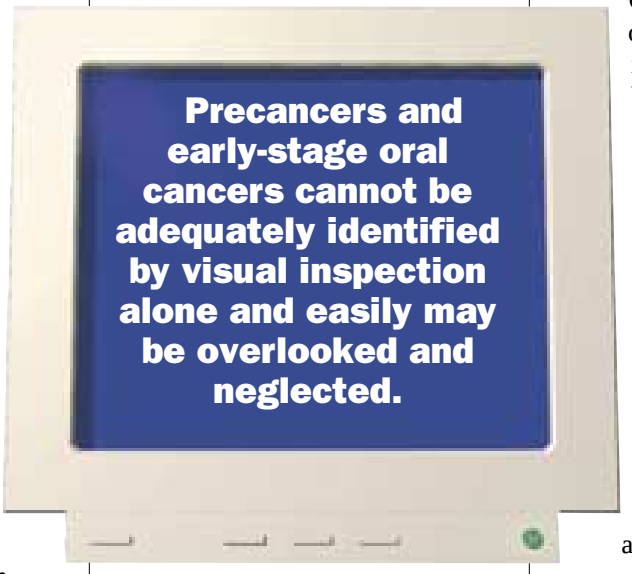


Figure 2. A photomicrograph of a specimen that underwent a scalpel biopsy ordered on the basis of a prior abnormal OralCDx brush biopsy result. Clearly, the defect created by the brush traverses the epithelium, terminating in an immediate subepithelial location.



The rate of OralCDx specimens lacking adequate biopsy representation of cells from all three epithelial layers from all investigator sites was 7 percent. Given the various levels of experience among the investigators with the brush biopsy technique, the median of 3.7 percent inadequate specimens (half the clinicians had inadequate rates that were lower and half higher than this figure) and the mode of 0 percent (16 of the 35 sites had no inadequate specimens) may be more representative of the "inadequate specimen" rate to be expected among experienced users.

DISCUSSION

Precancers and early-stage oral cancers cannot be adequately identified by visual inspection alone and easily may be overlooked and neglected, even by highly trained professionals

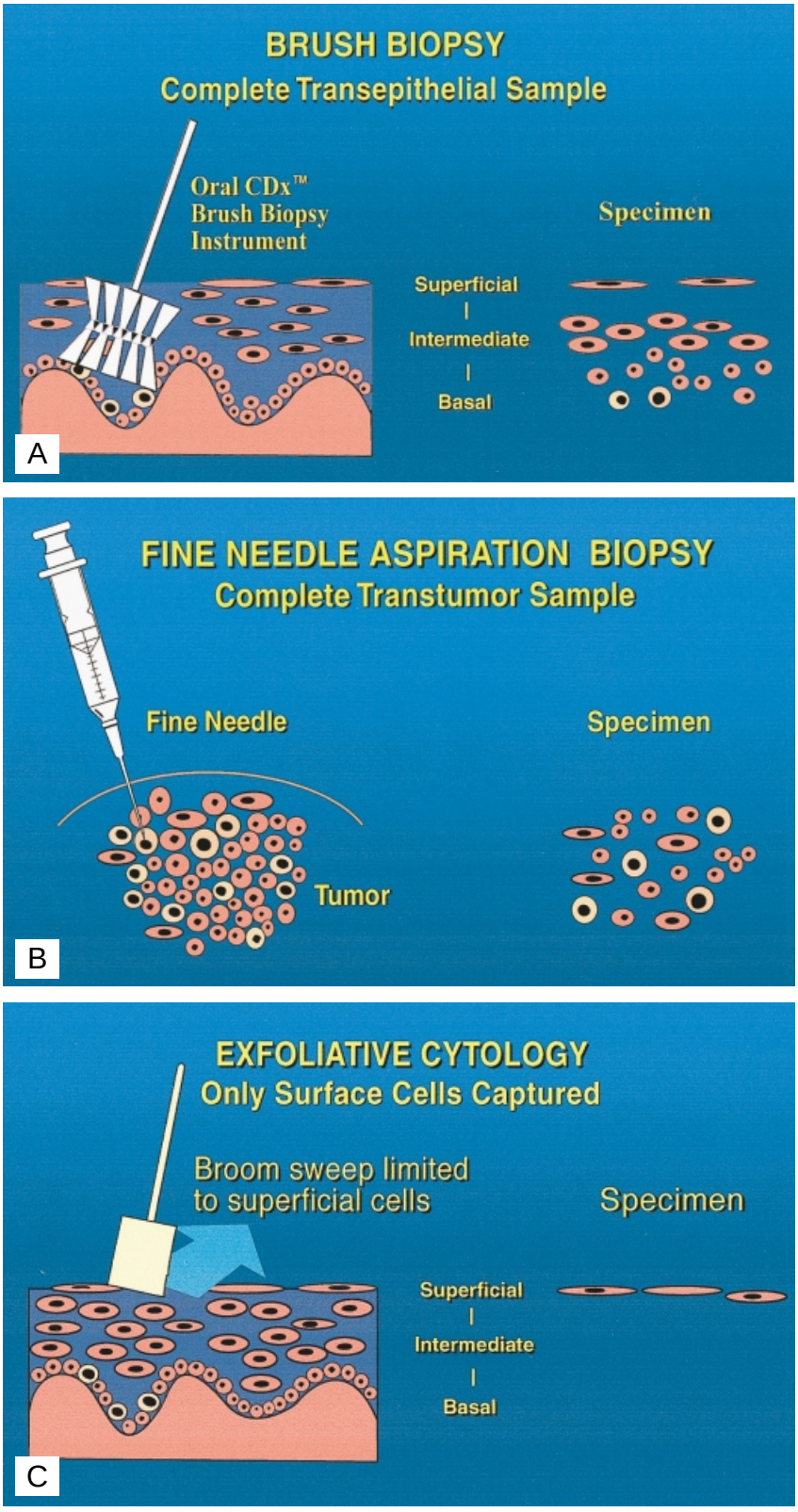


Figure 3. A. OralCDx biopsy provides a transepithelial specimen. B. Fine-needle–aspiration biopsy provides a transtumor specimen. C. Cytology provides information limited to the exfoliated superficial layer.

with broad experience.[7] Thus, a method of detection at early, curable stages is crucial and may lead to a reduction in the currently unacceptably high oral cancer morbidity and mortality rates. The results of this study demonstrate that OralCDx testing can be reliably used on oral lesions with epithelial abnormalities as a method of confirming their benign nature and, more importantly, revealing those that are precancerous and cancerous when they are not clinically suspected of being so.

Indeed, in this study, 4.5 percent of precancerous and cancerous lesions were deemed clinically benign by academic clinicians and would have remained undiagnosed at that time had they not been detected by OralCDx. All of these lesions proved to be precancerous or cancerous when undergoing subsequent histologic testing. An additional 15.3 percent of clinically benign lesions were diagnosed as "atypical" by use of the OralCDx technique. Although these lesions were not subjected to histologic evaluation, the results of this study suggest that additional precancers and cancers could be anticipated in this group.

On the basis of the results of this trial, it appears that OralCDx could provide invaluable assistance to clinicians in determining the significance of an oral lesion while examining the oral cavity. In this study, the brush biopsy was equivalent to a scalpel biopsy as a detection tool, since all precancers and cancers detected by scalpel biopsy also were detected by OralCDx. However, it should be emphasized that OralCDx does not substitute for a scalpel

biopsy; rather, it identifies oral lesions that require histologic evaluation. When this technique detects cellular morphologic abnormalities, histology is necessary to further assess the architecture of the lesion. Therefore, all OralCDx "atypical" and "positive" results should immediately indicate the need for a scalpel biopsy and histologic evaluation, to completely characterize (that is, to assign a stage and grade to) the lesion. Oral lesions with "negative" OralCDx results require the same careful clinical follow-up as negative histologically sampled lesions. Any patients whose samples are inadequate should have samples taken again to provide an optimal specimen for analysis.

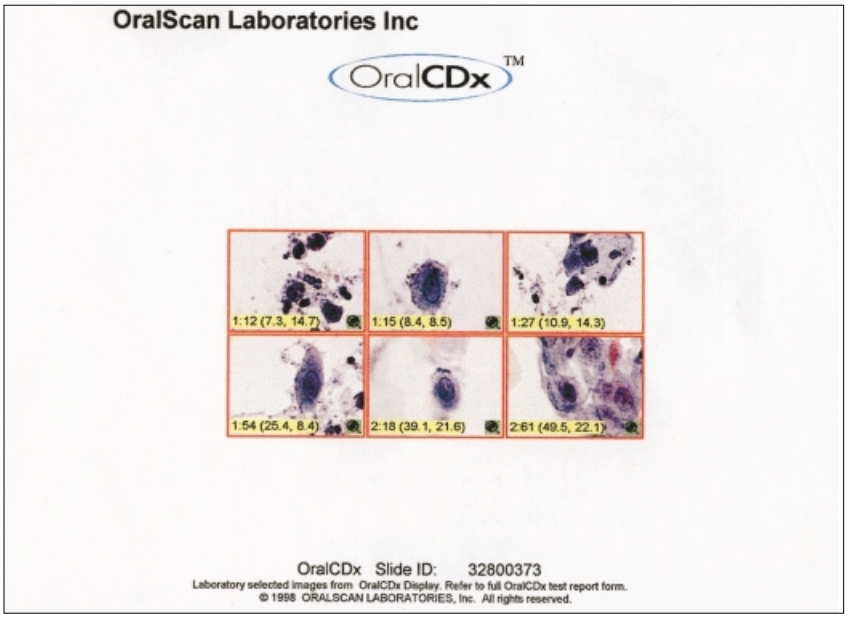
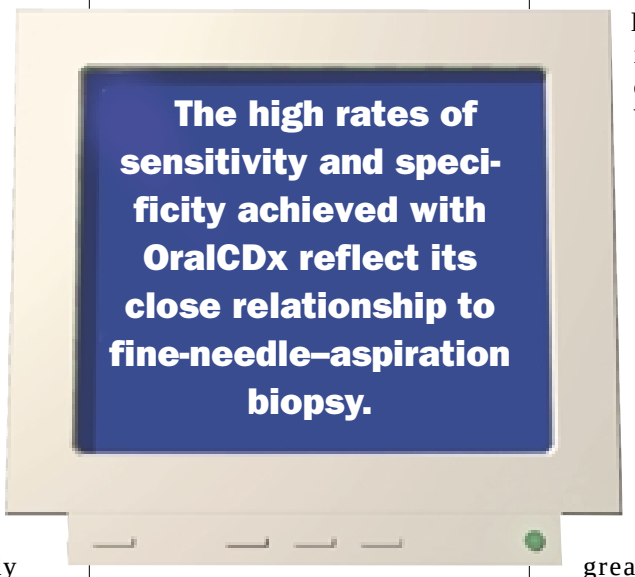


Figure 4. Color images of cellular abnormalities from OralCDx "atypical" and "positive" results with an accompanying explanation are mailed to the dentist. These images enable the dentist to demonstrate to the patient the abnormal test results.

Brush biopsy vs. exfoliative cytology. The accuracy of computer-assisted analysis of the oral brush biopsy as determined in this multicenter trial sharply contrasts with the unreliable sensitivity of oral exfoliative cytology. A large number of studies were conducted in the mid-1960s examining exfoliative cytology as a method of potentially identifying precancerous and early cancerous oral lesions. The results of those studies were not encouraging. Oral exfoliative cytology was found to yield unreliable results, as evidenced by the 31 percent false-negative rate in 148 oral cancers in the study by Folsom and colleagues[27] and similarly high false negative-rates in other studies.[28,29]

Exfoliative oral cytology was unsuccessful because of its inherent limitations. The sensitivity of any cytologic evaluation depends on a tedious visual search for potentially rare abnormalities on the microscopic slide. Microscopic screening of a cytologic smear involves examination of hundreds of thousands of normal cells to identify abnormal cells that are sometimes few in number and small in size.[30]



Normal cells exfoliating in enormous numbers as a result of epithelial turnover outnumber abnormal cells exfoliating from a dysplastic or cancerous lesion and, therefore, impede recognition of cellular abnormalities. When exfoliative cytology is adapted to oral-mucosal abnormalities, the limitations are even greater and are exacerbated by additional factors. The total number of abnormal cells available for cytologic sampling is reduced by a keratin layer, and the high rate of epithelial turnover in the oral cavity results in greater exfoliation of normal cells, further diluting the number of abnormal cells on the smear.[27,31,32]

Brush vs. aspiration biopsy. In the past two decades, the field of aspiration biopsy has developed consider-

ORALCDx: REIMBURSEMENT AND FURTHER INFORMATION

Testing with OralCDx (OralScan Laboratories Inc.) is a potentially life-saving, reimbursable chairside service that can be integrated into any dental practice.

Patients undergoing OralCDx testing incur two fees.

- The dentist performing the brush biopsy charges for the procedure using the ADA or CPT billing codes supplied with the test kit acquired from the examining specialty laboratory. OralCDx test kits are provided to the dentist at no charge.
- The specialty laboratory bills the patient's insurance provider directly for a separate analysis fee comparable to charges for other routine anatomic pathology services.

Most dental and medical insurance plans, including Medicare, routinely reimburse for both of these fees.

Although the brush biopsy is not a difficult procedure, it is recommended that general dentists attend a brief instructional seminar to maximize proper use and application of the procedure.

These seminars are offered across the country at local, state, regional and national dental meetings, including a number of continuing education seminars scheduled for the fall and winter months of 1999. Still more of these seminars were being arranged at press time.

To learn about brush biopsy seminars offered in your area, call 1-800-560-4467. For information by e-mail, contact the company at "oralcdx@oralscan.com".

ably as a natural outgrowth of exfoliative cytology.[33,34] Fine-needle–aspiration biopsies from virtually all body sites provide cellular material from all layers of the lesion being analyzed. For example, fine-needle–aspiration biopsy of an enlarged lymph node that frequently accompanies tumors of the head and neck often provides the initial evidence of malignancy.[35] Aspiration biopsy specimens are composed of individual cells as well as of tissue fragments. The preparations obtained may be compared to a jumbled puzzle in which the components are fitted together by the pathologist to form a recognizable picture.[36] Histology offers intact architecture, which is crucial for tumor classification. However, as a disease detection tool, the aspiration biopsy offers similar evidence.[37,38] In fact, it has the advantage of offering significantly improved cytologic detail.

The high rates of sensitivity and specificity achieved with OralCDx reflect its close relationship to fine-needle–aspiration biopsy. The fine needle is used for sampling deep-seated lesions such as submucosal oral masses and enlarged lymph nodes.[39] The oral brush biopsy instrument is preferred for lesions of the exposed oral mucosal surface. OralCDx testing uses a brush biopsy instrument with a bristle shape and tangent modulus (that is, the bending force resulting from the bristle material and the relationship or angle of the individual bristles to the wire core of the OralCDx biopsy instrument) that is optimized to obtain a full transepithelial biopsy specimen (Figure 2) with minimal or no discomfort. As dysplastic and cancerous oral lesions frequently have an overlying keratin layer, cellular abnormalities in the deep basal layer of the epithelium are best sampled with this instrument.

Although not previously used in the oral cavity, the brush biopsy is a commonly used cancer detection technique in other body sites. Studies of the brush biopsy have validated its use as a diagnostic tool for a variety of upper gastrointestinal, endobronchial lung, biliary, pancreatic, rectal and other cancers.[40-44] The similarities between the two biopsy techniques, OralCDx and fine-needle aspiration, as well as the differences between these diagnostic tools and exfoliative cytology are schematically illustrated in Figure 3.

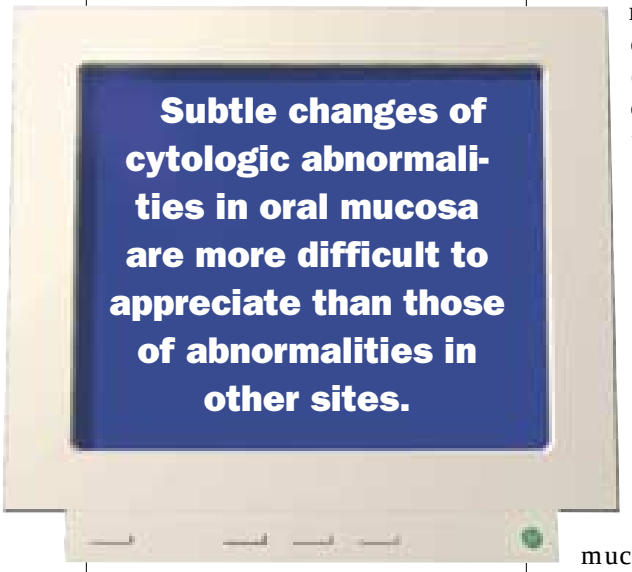
Advantages of the brush biopsy. The OralCDx oral brush biopsy is a rapidly conducted chairside procedure that results in minimal or no bleeding and requires no topical or local anesthetic. A transepithelial brush biopsy is not a difficult or demanding procedure to master, as shown by the relatively low number of inadequate specimens obtained in this study by clinicians experienced in this technique. The great majority of inadequate samples were obtained at the onset of the trial, as investiga-

tors were becoming familiar with using the brush.

In addition to precancer and cancer detection, OralCDx can provide morphologic evidence of a variety of benign oral processes. In this study, OralCDx uncovered epithelial abnormalities consistent with candidiasis, herpes simplex virus infection, human papillomavirus infection, pernicious anemia, radiation effects and pemphigus. The characteristic morphologic features of these diseases have been described elsewhere.[32,45]

Computer-assisted analysis. A critical component of OralCDx is the use of image analysis of the oral brush biopsy sample. Although automated cytology had been proposed in the late 1950s as a method of reducing false-negative findings, early attempts that relied on analysis by algorithmic computers were not successful. This limitation was finally overcome by the application of new, nonalgorithmic, neural network computers that were developed in the late 1980s for missile defense. In recent years, neural networks have been successfully applied to several medical diagnostic procedures, including cervical smear screening and interpretation of digital radiologic images such as chest radiographs and mammograms.[46-48] However, the diagnosis of oral squamous cell carcinomas using a neural network screening system developed to recognize abnormal cells in cervical smears resulted in an unacceptably high false-negative rate of 39 percent.[49]

By contrast, OralCDx uses an image analysis system that is adapted and optimized to detect epithelial abnormalities unique to oral brush biopsy samples, thereby enhancing its accuracy. In the current study, the results of OralCDx tests of Class I and Class II lesions showed excellent correlation with those obtained by scalpel biopsy. Therefore, although a majority of oral lesions in this study were categorized as Class II and not subjected to scalpel biopsy, it is likely that the correlation of OralCDx results with scalpel biopsy results for this group would be the same as that obtained from lesions tested by both methods.



The OralCDx neural network assists in the search of oral brush biopsy samples for potentially abnormal cells, which then are interpreted by the pathologist. The identification of these abnormal cells is labor-intensive, fatiguing and time-consuming; more importantly, however, abnormalities are easily overlooked. The OralCDx images of the neural network–selected cells presented to the pathologist for review identify cellular abnormalities that might otherwise have been missed with manual microscopic screening, optimizing the combination of human and computer capabilities.

Interpretation of the brush biopsy samples. The brush biopsy and use of neural network technology are interrelated with a third component of OralCDx, the specialized pathologic interpretation of the oral brush biopsy sample. The literature is replete with reports of failure of cytology laboratories to diagnose invasive cancer. The interpretation of oral brush biopsy samples is more problematic, given that there are few pathologists with expertise in this field and that there is not one U.S. undergraduate dental curriculum requirement for oral cytology.[50] Moreover, subtle changes of cytologic abnormalities in oral mucosa are more difficult to appreciate than those of abnormalities in other sites.[51] As a means of minimizing diagnostic error, OralCDx laboratory pathologists undergo specialty training in oral cytology. Furthermore, the laboratory that analyzes these specimens functions exclusively in the interpretation of oral brush biopsy samples. As emphasized by Hayes and colleagues,[32] Allegra and colleagues[51] and Morrison and Wu,[52] specialized training for pathologists interpreting oral cytology is mandatory.

OralCDx is easily integrated into conventional dental prac-

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tice. The dentist submitting the specimen receives a faxed report, usually within three days after the specimen arrives at the specialty laboratory. In addition, color images of cellular abnormalities from OralCDx specimens with "atypical" and "positive" results, with an accompanying explanation, are mailed to the dentist (Figure 4). These images enable the dentist to demonstrate to the patient the abnormal test results.

CONCLUSION

The most effective method of combating oral cancer is early detection, diagnosis and eradication of early-stage lesions and their precursors.[16] The results of this multicenter clinical trial suggest that by bridging the gap between clinical inspection and histologic evaluation of oral lesions with epithelial abnormalities, OralCDx could become instrumental in achieving this goal.

Since the oral cavity is the only region of the aerodigestive tract that can be effectively screened, dentists should continue to be encouraged to perform oral cancer examinations of all patients.[53] Public education that stresses the importance of yearly oral cancer examinations, identification of the warning signs of oral cancer, and recognition of the hazards associated with tobacco and alcohol use is necessary to reverse the high morbidity and mortality rates associated with this disease. The results of this multicenter trial demonstrate the potential value of OralCDx as an adjunct to the oral cavity examination in identifying precancerous and cancerous lesions at early stages, when curative therapies are most effective. ■

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