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The sensitivity and specificity of the OralCDx technique: evaluation of 103 cases

Christian Scheifele*, Andrea-Maria Schmidt-Westhausen, Thomas Dietrich, Peter A. Reichart

Department of Oral Surgery and Dental Radiology, Zentrum für Zahnmedizin, Campus Virchow, Charité—Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany

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Summary In this study, we compared 103 OralCDx results with the histological findings of 96 clinical sites in 80 patients (33 females; 64.3 ± 13.7 years and 47 males; 53.2 ± 11.5 years). The histological findings were classified as follows: compatible with oral leukoplakia (OL; $n = 60$) or oral lichen planus (OLP; $n = 17$), both without dysplasia; dysplasia in OL or OLP ($n = 9$); and oral squamous cell carcinoma (OSCC; $n = 17$). There were seven (6.8%) specimens with an inadequate cell count. Overall, the sensitivity of the OralCDx technique to detect dysplasia and OSCC was 92.3% (95% CI: 74.9—99.1%), and the specificity was 94.3% (95% CI: 86.0—98.4%). The positive likelihood ratio (LR+) was 16.2 (95% CI: 6.2—42.1) and the negative likelihood ratio (LR−) was 0.08 (95% CI: 0.02—0.31). In conclusion, these figures are in agreement with previously published data and support the use of OralCDx as a screening tool of oral lesions, but further trials are still necessary.

Introduction

Standard exfoliative cytology of oral mucosal lesions, including oral precancer, has for years been criticised as not producing adequate and reliable results.[1] In recent years, new techniques, particularly the brush biopsy technique, have been developed. The computerised analysis of brush biopsies (OralCDx®, CDx Laboratories, Suffern, NY) has been introduced since 1999 for the evaluation of oral lesions that appear clinically benign and would otherwise not have received a scalpel biopsy.[2] Now, with more than 110,000 specimens tested,[3] it seems to represent a substantial progress of exfoliative cytology techniques.

The study by Sciubba[2] of 945 OralCDx biopsies reported a sensitivity of $\geqslant$96% and a specificity of $\geqslant$90% to detect cases of dysplasia or squamous cell carcinoma (OSCC). In a subsequent study[4] of 930 participants of a mass screening, 93 benign-appearing lesions not suspicious for oral precancer were identified by visual examination. OralCDx brush biopsies of these lesions were abnormal in seven cases. Out of these, six showed the OralCDx result "atypical", i.e. atypical epithelial cells, and

*Corresponding author. Tel.: +49-30-450-562-604; fax: +49-30-450-562-901.

E-mail address: christian.scheifele@charite.de (C. Scheifele).

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one was "positive", i.e. showed evidence of epithelial dysplasia or carcinoma.[2,4] In four of the seven lesions, scalpel biopsies were performed, of which three were histologically confirmed as dysplasias.[4] Another comparison of OralCDx results with histology in 298 cases revealed four false negative results, of which three demonstrated severe data discrepancies, and 150 false positive results.[5] In a letter to the editor, a rate of 84% false-positive OralCDx results in 100 cases with a specificity of 3.5% was reported.[6] Another study identified a rate of 3.5% false-negative OralCDx results in 115 cases and emphasized a possible delay of scalpel biopsies.[7]

The discussion whether to use the brush biopsy, and if so, when; and who should use it, has been addressed as a "Brush Controversy".[8] However, there is a constant need for reliable prediction of malignant changes in oral mucosal lesions.[6,8–14] Therefore, the aim of the present study was to further evaluate the diagnostic value of the OralCDx technique in patients with oral mucosal lesions.

Material and methods

The criteria to include a patient in the study were: (1) an OralCDx brush biopsy of a lesion with the clinical diagnosis oral leukoplakia (OL), oral lichen planus (OLP), or obvious oral squamous cell carcinoma (OSCC); and (2) a scalpel biopsy that had been performed within one month before or after the brush biopsy of the same lesion. Accordingly, we identified 103 OralCDx biopsies from 96 oral sites in 80 consecutive patients between July 2002 and September 2003. Informed consent was obtained prior to both procedures. The clinical diagnoses of these patients were OL ($n = 49$), OLP ($n = 18$), and OSCC ($n = 13$). Two distinct lesions were found in 11 of these patients with OL, one with OLP, and two with OSCC that showed field cancerisation. One patient with OL showed three lesions. Thus, histological findings of 96 lesions in 80 patients were available for the study.

Brush biopsies were taken according to instructions and sent to the OralCDx centre in Germany. Brush biopsy results were classified as negative; atypical epithelial cells; positive for dysplasia or OSCC; and inadequate cell count.[2] By definition, atypical and positive results then were summarized as positive results; inadequate results were excluded.[2] Histological results were classified as compatible with OL, compatible with OLP, any grade of dysplasia (in OL or OLP), and OSCC. Data collection and statistical analysis (Mann–Whitney-U test, contingency tables for $n = 96$) were performed with the SPSS 11 package.

Results

Table 1 shows the basic demographic data of the 33 females (41.3%) and 47 males (58.8%) with regard to the clinical diagnoses. On average, females were older (64.3 ± 13.7 years) than males (53.2 ± 11.5 years; $p = 0.002$). Table 2 shows the distribution of the 103 brush biopsy results with relation to the histological diagnoses. There were two false-negative brush biopsy results in two cases of OL. In both, the histological findings were reported as mild dysplasia. In addition, four cases of false-positive brush biopsy results were detected. The brush biopsy results were atypical in one case of OL and one case of OLP and "positive" in two cases of OL, all of them without dysplasia. An inadequate result was found in one case of OLP and four cases of OL. In one OL of these four, there were three subsequent OralCDx results from an identical site that were inadequate; the fourth was

Table 1 Gender and age distribution of subjects ($n = 80$), by clinical diagnoses

Clinical Diagnosis	Females			Males			Total		
	n	% of diagnosis	Age [years] (mean, SD)	*n*	% of diagnosis	Age [years] (mean, SD)	*n*	% of total	Age [years] (mean, SD)
OL[a]	17	34.7	66.9 ± 14.3	32	65.3	52.9 ± 11.9	49	61.3	58.3 ± 13.1
OLP[b]	11	61.1	62.7 ± 10.2	7	38.9	55.1 ± 12.0	18	22.5	59.7 ± 10.8
OSCC[c]	5	38.5	77.1 ± 9.6	8	61.5	51.0 ± 10.6	13	16.3	60.6 ± 16.3
Total	33	41.3	64.3 ± 13.7	47	58.8	53.2 ± 11.5	80	100.0	58.6 ± 13.1

[a] Oral leukoplakia.
[b] Oral lichen planus.
[c] Oral squamous cell in carcinoma.

Table 2 Distribution of brush biopsy results ($n = 103$), by histological diagnoses of incisional biopsy

Histological diagnosis	Result of brush biopsy									
	Inadequate		Negative		Atypical		Positive		Total	
	n	% of diagnosis	*n*	% of diagnosis	*n*	% of diagnosis	*n*	% of diagnosis	*n*	% of total
Compatible with OL[a]	6	10.0	51	85.0	1	1.7	2	3.3	60	58.3
Compatible with OLP[a]	1	5.9	15	88.2	1	5.9	—	—	17	16.5
Dysplasia[b]	—	—	2	22.2	5	55.6	2	22.2	9	8.7
OSCC	—	—	—	—	3	17.6	14	82.4	17	16.5
Total	7	6.8	68	66.0	10	9.7	18	17.5	103	100.0

[a] Without any dysplasia.
[b] Dysplasia found in OL or OLP.

Table 3 2×2 contingency table for the brush biopsy result "positive"

		Test: Brush biopsy result "positive"		
		+	–	Total
Criterion: Histologic diagnosis dysplasia or carcinoma	+	16	10	26
	–	2	68	70
	Total	18	78	96

Sensitivity: 61.5% (95% CI: 40.6—79.8%). Specificity: 97.1% (95% CI: 90.1—99.7%).

Table 4 2×2 contingency table for the brush biopsy results "positive" or "atypical"

		Test: Brush biopsy result "positive" or "atypical"		
		+	–	Total
Criterion: Histologic diagnosis dysplasia or carcinoma	+	24	2	26
	–	4	66	70
	Total	28	68	96

Sensitivity: 92.3% (95% CI: 74.9—99.1%). Specificity: 94.3% (95% CI: 86.0—98.4%).

negative. This OL was histologically characterised by hyperkeratosis. Overall, there were seven (6.8%) inadequate results.

Table 3 shows the 2×2 contingency table for the positive brush biopsy result and Table 4 for the summarized positive or atypical results. For the positive results alone, the sensitivity to detect dysplasia and OSCC was 61.5% (95% CI: 40.6—79.8%) and the specificity was 97.1% (95% CI: 90.1—99.7%). For the positive or atypical results, the sensitivity to detect dysplasia and OSCC was 92.3% (95% CI: 74.9—99.1%) and the specificity was 94.3% (95% CI: 86.0—98.4%).

Discussion

In the present study, we found two cases of dysplasia and no cases of OSCC, in which brush biopsy was false negative (cf. Table 2). In principle, there was evidence that specificity and sensitivity described in the initial study[2] could be confirmed. The major limitation of the present study, like in the majority of comparable studies,[4–7] is sampling bias. First, the hospital-based samples do not fully represent the patients of a general practitioner. However, this is exactly the target group of OralCDx and, second, the studies

are biased by the fact that only OralCDx results in cases that underwent a scalpel biopsy could be compared. Thus, the sensitivity in all these studies has to be interpreted with caution. Only an ethically approved study design that included *all* patients that underwent an OralCDx examination or, at least, a minute follow-up of these patients could avoid these general limitations.

Inadequate results

Inadequate results with an insufficient cell count have been described as between 2% and 7%, with a median of 3.7%.[2,6] Our rate of 6.8% is comparatively high and may be explained by the initial phase of getting acquainted with the technique. Inadequate results are also possible in cases of heavily hyperkeratinised OL, in which the transepithelial brush biopsy cannot collect a sufficient count of basal cells.[12,13] In the present study, the case of OL, in which the brush biopsy had to be repeated three times, could be such an example. It should be remembered, however, that these cases actually represent a contraindication for OralCDx.[2] Therefore, proper identification of eligible oral lesions is mandatory.[12,13]

Sensitivity

It has been criticised that the results of the first study[2] could be of limited significance with regard to clinically benign appearing lesions.[6,7] As quoted in detail, in this part of the study only 26.1% of the negative and 22.3% of the positive OralCDx results had been examined by a subsequent scalpel biopsy.[6,7] Two studies and a letter to the editor were found that investigated false-negative rates.[5–7] The sensitivity calculated in these studies amounted to 95.9%[5] and 90.9%.[6] Hence, it seems of particular importance to test for false-negative results. In the present study, the sensitivity of 92.3% was between these values.

False-negative rates from 1% to 4.1% have been reported.[5–7] These comparable results have been interpreted in two ways: One study concluded that these rates—which reflect a sensitivity of >90%—were not acceptable.[7] It is of importance that at least the first of the four false-negative cases reported in this study definitely showed no indication for brush biopsy.[7] Advanced OSCC, as in this case, are often necrotic and/or super-infected. Therefore, a transepithelial approach is impossible.[13] In addition, there was a reported delay of 75—292 days between brush and scalpel biopsy in the other three cases.[7] Other studies have identified three groups of potential causes, besides the possibility of a true false-negative result: (1) topographic error between the site of brush and scalpel biopsy; (2) time delay between both; and (3) known and inevitable intra- and interobserver variabilities in the histologic assessment of oral premalignant lesions.[5,10,15,16] In the present study, there was no time delay in the two cases of false-negative dysplasias that resulted in a false-negative rate of 7.7%. It might be concluded, therefore, that detailed recordings, namely photographs of a lesion and the brushed sites, are crucial for taking adequate brush biopsies.

Specificity

The specificity of 94.3% in the present study is consistent with that of the multicentre brush biopsy study.[2] It might be of interest to consider why the specificity of 3.5—25.4% in subsequently published results was extremely low.[5,6] From the beginning, it was emphasized that false positive results of the OralCDx technique are possible in other oral lesions with a certain grade of epithelial atypia, e.g. inflammatory conditions.[2,5] If the oral brush biopsy would be used regardless of the clinical aspect of lesions, the rate of false positive results should markedly increase. Therefore, specificity of brush biopsies seems to be dependant on the clinicians experience to identify eligible lesions. In consequence, there is a demand that only fully trained clinicians should use brush biopsies.[13] However, this also is an indicator that the clinical examination is an inseparable part of a brush biopsy. In addition, OSCC arising in clinically healthy appearing mucosa cannot be detected.[10]

Likelihood ratios

It seems difficult to give objective interpretations of reported figures of sensitivity and specificity. To summarize both, likelihood ratios have been proposed to delineate the abilities of a test.[17] The likelihood ratio of a positive test (LR+) is defined as the ratio of the true-positive rate to the false-positive rate [sensitivity/(1-specificity)].[17] In this study, the brush biopsy with a sensitivity of 92.3% and a specificity of 94.3% has a positive likelihood ratio of $0.923/(1-0.943)$, or 16.2 (95% CI: 6.2—42.1). Thus, a "positive" result is 16.2 times more likely in a lesion with dysplasia or OSCC than in a lesion without. The likelihood ratio of a negative brush biopsy (LR−) was 0.08 (95% CI: 0.02—0.31). There are accepted threshold values of likelihood ratios (i.e. >10.0 for the positive and

<0.1 for the negative likelihood ratios) to recommend a test for clinical use.[17,18] Consequently, according to these likelihood ratios the brush biopsy can be recommended, but data still need to be confirmed by larger controlled trials.

In addition, it should be mentioned that there are other advances in the field of oral exfoliative cytology. A combination of exfoliative cytology and subsequent DNA content analysis by image cytometry has shown very promising results.[14,19,20] Recently, a Cochrane review of screening programmes for oral cancer included only one study as acceptable out of 100 studies that were reviewed. Given the limited evidence, it was concluded that there is no robust evidence to support or refute *any* method of screening for oral cancer.[9] The results of the present study confirm that the OralCDx technique meets all basic criteria for a diagnostic test of clinically benign appearing oral lesions. However, the use of any diagnostic test of these lesions is dependent on further criteria: First, it has to be assured that patients at risk actually see their dentist; second, that these general practitioners are actually able to identify eligible lesions; and third, the outcome of OSCC identified by the test should demonstrate a significant benefit, compared to conventional screening methods. At least evidence of the latter should be provided to justify use and additional costs of the method.

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