

Diagnostic Agreement Between Liquid-Based Oral Brush Cytology and Histopathology

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Research Article

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Abstract

Objectives

To assess agreement between oral brush cytology and histopathology in two cohorts and determine whether cytology identifies high-grade disease.

Methods

We analysed paired cytology and histopathology results from 32 patients in cohort I and 262 assessable pairs in cohort II. Original diagnoses were retained as separate categories, including cytological atypical squamous cells of undetermined significance (ASCUS) and histopathological carcinoma in situ. For the binary high-grade analysis, cytological HSIL or carcinoma was considered positive; severe dysplasia or carcinoma in situ on histopathology constituted a positive reference result. Cohorts were analysed separately.

Results

Exact category agreement was 20/32 (62.5%; 95% CI 43.7–78.9%; unweighted Cohen's κ = 0.11) in cohort I and 174/262 (66.4%; 95% CI 60.3–72.1%; κ = 0.11) in cohort II. Sensitivity for high-grade disease was 1/4 (25.0%; 95% CI 0.6–80.6%) in cohort I and 0/13 (0.0%; 95% CI 0.0–24.7%) in cohort II.

Conclusion

At the high-grade threshold used here, oral brush cytology missed most lesions with severe dysplasia or carcinoma in situ. It should not replace an indicated tissue biopsy.

Introduction

Oral potentially malignant disorders (OPMDs) comprise a heterogeneous group of mucosal conditions associated with an increased risk of oral squamous cell carcinoma [1, 2]. Oral leukoplakia is among the most common OPMDs, but its clinical course is variable, and malignant transformation cannot be predicted reliably in an individual patient [1, 2]. Histopathological assessment of surgically obtained tissue remains the reference standard for diagnosing epithelial dysplasia and invasive malignancy. Repeated surgical biopsies may, however, cause discomfort, require clinical resources and be poorly suited to frequent longitudinal surveillance.

Transepithelial brush sampling provides a minimally invasive means of collecting epithelial cells from visible oral lesions. Combined with liquid-based cytology, it can produce standardised cellular preparations for cytomorphological assessment and subsequent molecular analysis. Studies using

modified Bethesda classifications have reported encouraging diagnostic performance, although sampling devices, staining methods, thresholds and reference standards differ between studies [3–8].

Objective

To assess agreement between oral brush cytology and histopathology in paired samples from two cohorts, and to evaluate the diagnostic accuracy of brush cytology for detecting high-grade epithelial dysplasia or carcinoma. The findings were intended to inform whether brush cytology could reduce the need for incisional biopsy during follow-up of oral leukoplakia.

Materials and methods

Study design and cohorts

Cohort I

The source study included 42 adults with histopathological confirmed oral leukoplakia or erythroplakia. Thirty-two patients had an evaluable paired cytology and histopathology result from the same lesion; ten did not enter the diagnostic accuracy analysis because no evaluable pair was available. Among these 32 paired observations, biopsy histopathology showed mild-to-moderate epithelial dysplasia (SIN I–II) in 28 and severe epithelial dysplasia (SIN III) in four.

Cohort II

Cohort II was identified from the database of an ongoing five-year prospective study of patients with biopsy-confirmed oral leukoplakia, maintained in Research Electronic Data Capture (REDCap). The parent study has a planned sample size of 1,036 patients, allocated to either repeated transepithelial brush sampling at every follow-up visit ($n = 518$) or standard clinical follow-up, with repeat surgical biopsy at the discretion of the responsible specialist ($n = 518$).

Cohort II comprised 267 sample records: 25 from Dalarna, 90 from Östergötland, 76 from Gävleborg, and 76 from Uppsala. Paired, assessable cytology and histopathology results were available for 262 unique patients. These 262 paired observations were included in the present diagnostic analysis (Fig. 1). For each patient, the brush sample and surgical biopsy were obtained from the same lesion during the same examination visit. Brush sampling was performed first, followed immediately by surgical biopsy.

Histopathological carcinoma in situ was analysed as a separate category. One brush sample was classified cytologically as squamous cell carcinoma, whereas its paired biopsy showed low-to-moderate dysplasia; this discordant pair remained in the analysis. None of the paired biopsy reports recorded invasive carcinoma. The clinical label of cancer was therefore not treated as histopathological evidence of invasive carcinoma.

Brush sampling and specimen processing

In cohort I, oral brush samples were obtained using a single-use Cytobrush® Plus, (Medscand Medical, CooperSurgical, Inc. CT, USA); in cohort II, the Orcellex® Brush (Rovers Medical Devices B.V., Oss, the Netherlands) was used. The brush was rotated firmly over the lesion for approximately 30 seconds, until slight pinpoint bleeding indicated transepithelial sampling. Collected cells were released into PreservCyt® Solution (Hologic, Inc., Marlborough, MA, USA) and sent to Unilabs Clinical Pathology and Cytology, Solna, Sweden. Slides were prepared using ThinPrep™ liquid-based cytology and stained using a regressive Papanicolaou method [9].

Senior cytology specialists examined the slides and assigned the cytopathological diagnoses.

Surgical biopsy and histopathology

All surgical biopsies were obtained under local anaesthesia using punch, incisional, or excisional techniques. Specimens were immediately placed in 4% formaldehyde solution (BiopSafe® biopsy container, 20 mL) and sent to the Department of Oral Pathology, Faculty of Odontology, Malmö University, Sweden, for histopathological examination. Tissue specimens were routinely processed, embedded in paraffin, sectioned, and stained with haematoxylin and eosin. Histopathological assessment was performed by a specialist in pathology according to the laboratory's routine procedures [10].

Cytological classification and histopathological grading Oral brush cytology was classified for analysis using a modified Bethesda classification: NILM (negative for intraepithelial lesion or malignancy), ASCUS (atypical squamous cells of undetermined significance), LSIL (low-grade squamous intraepithelial lesion), HSIL (high-grade squamous intraepithelial lesion), and cytological squamous cell carcinoma. ASCUS denotes atypia insufficient for a definite diagnosis of a squamous intraepithelial lesion and was not equated with histopathological low-grade dysplasia [3, 11].

Surgical biopsy findings were classified separately as no dysplasia (SIN 0), mild to moderate dysplasia (SIN I–II), severe dysplasia (SIN III), carcinoma in situ, or invasive carcinoma, and served as the reference standard. The matrices for both cohorts display ASCUS as a separate cytological category and carcinoma in situ and invasive carcinoma as separate histopathological categories, with zero counts where no cases were observed. The distinction between carcinoma in situ and invasive carcinoma was based on histopathological assessment.

Data and statistical analysis

Sensitivity, specificity, positive and negative predictive values, and overall binary agreement were calculated separately for each cohort using histopathology as the reference standard. Binary agreement beyond chance was assessed using Cohen's kappa, and discordance between paired binary

classifications was assessed using the exact McNemar test. Diagnostic accuracy results were reported in accordance with STARD 2015 [12].

For the binary high-grade analysis, HSIL, cytological carcinoma in situ, and cytological squamous cell carcinoma were classified as cytology positive; NILM, ASCUS, and LSIL were classified as cytology negative. Severe dysplasia (SIN III), histopathological carcinoma in situ, and invasive carcinoma were classified as reference-standard positive; no dysplasia (SIN 0) and mild to moderate dysplasia (SIN I–II) were classified as reference-standard negative.

For exact category agreement, cytological NILM was matched to no dysplasia (SIN 0), LSIL to mild or moderate dysplasia (SIN I–II), HSIL to severe dysplasia (SIN III), cytological carcinoma in situ to histopathological carcinoma in situ, and cytological squamous cell carcinoma to histopathological invasive carcinoma. ASCUS was retained as a separate cytological category without a directly corresponding histopathological grade. Pairs with ASCUS therefore remained in the denominator but were not counted as exact category matches in the primary analysis.

Exact category agreement and unweighted Cohen's kappa were calculated separately for each cohort. Linear and quadratic weighted Cohen's kappa were also calculated using aligned cytological and histopathological categories on a common ordered scale. For cohort II, ASCUS was positioned between NILM/no dysplasia and LSIL/mild to moderate dysplasia, and carcinoma in situ was positioned between HSIL/severe dysplasia and carcinoma/invasive carcinoma. The placement of ASCUS was an analytical convention for weighting and did not imply equivalence to a histopathological grade.

In a secondary simplified agreement analysis, ASCUS was grouped with NILM, histopathological carcinoma in situ was grouped with severe dysplasia (SIN III), and cytological carcinoma in situ, if present, was grouped with HSIL. Cytological squamous cell carcinoma and histopathological invasive carcinoma were retained as the corresponding carcinoma category, separate from HSIL/severe dysplasia. Unweighted Cohen's kappa was recalculated after regrouping. The original diagnostic categories were retained in the full cross-tabulations, and the binary high-grade diagnostic accuracy analysis retained the threshold specified above.

Figure 1. Selection of paired cytology and histopathology observations. Cohort I included 32 of 42 patients; cohort II included 262 of 267 records from four regions. The five cohort II records without a complete, assessable pair were not included in the diagnostic analyses.

Results

Cohort I

Table 1
Cytology by histopathology in cohort I (n = 32).

Histopathology	NILM	ASCUS	LSIL	HSIL	CIS cytology	Cancer cytology	Total
No dysplasia	0	0	0	0	0	0	0
Low to moderate	9	0	19	0	0	0	28
Severe dysplasia	1	0	2	1	0	0	4
Carcinoma in situ	0	0	0	0	0	0	0
Invasive carcinoma	0	0	0	0	0	0	0
Total	10	0	21	1	0	0	32

Exact category agreement: 62.5% (20/32; 95% CI 43.7–78.9%). Unweighted kappa 0.11; linear weighted kappa 0.12; quadratic weighted kappa 0.13.

Table 2
High-grade binary classification in cohort I (n = 32).

High grade comparison	PAD positive	PAD negative	Total
Cytology positive	1	0	1
Cytology negative	3	28	31
Total	4	28	32

Table 3
Diagnostic measures in cohort I.

Diagnostic measure	Estimate
Sensitivity	25.0% (1/4; 95% CI 0.6–80.6%)
Specificity	100.0% (28/28; 95% CI 87.7–100.0%)
Positive predictive value	100.0% (1/1; 95% CI 2.5–100.0%)
Negative predictive value	90.3% (28/31; 95% CI 74.2–98.0%)
Binary agreement	90.6% (29/32; 95% CI 75.0–98.0%)
Binary Cohen kappa	0.37
Exact McNemar p	0.25

Cohort II

Table 4
Cytology by histopathology in cohort II (n = 262).

Histopathology	NILM	ASCUS	LSIL	HSIL	CIS cytology	Cancer cytology	Total
No dysplasia	166	24	3	1	0	0	194
Low to moderate	43	3	8	0	0	1	55
Severe dysplasia	3	0	0	0	0	0	3
Carcinoma in situ	5	4	1	0	0	0	10
Invasive carcinoma	0	0	0	0	0	0	0
Total	217	31	12	1	0	1	262

Of 267 records from the four selected regions, 262 complete, assessable pairs entered the diagnostic analyses (Fig. 1). The cytology–histopathology matrix totals 262 observations. Exact category agreement was 66.4% (174/262; 95% CI 60.3–72.1%); unweighted Cohen’s κ was 0.11, linear weighted κ was 0.12, and quadratic weighted κ was 0.15. The binary high-grade analysis classified 13 pairs as histopathology positive and two as cytology positive; none were positive by both methods (Table 5).

Table 5
High-grade binary classification in cohort II (n = 262).

High grade comparison	PAD positive	PAD negative	Total
Cytology positive	0	2	2
Cytology negative	13	247	260
Total	13	249	262

Table 6
Diagnostic measures in cohort II.

Diagnostic measure	Estimate
Sensitivity	0.0% (0/13; 95% CI 0.0%–24.7%)
Specificity	99.2% (247/249; 95% CI 97.1%–99.9%)
Positive predictive value	0.0% (0/2; 95% CI 0.0%–84.2%)
Negative predictive value	95.0% (247/260; 95% CI 91.6%–97.3%)
Binary agreement	94.3% (247/262; 95% CI 90.7%–96.8%)
Binary Cohen kappa	-0.01
Exact McNemar p	0.00739

Discussion

In cohort I, HSIL cytology identified one of four lesions with severe dysplasia. In cohort II, histopathology identified three cases of severe dysplasia and ten cases of carcinoma in situ. None of these 13 lesions had high-grade cytology. All three samples with severe dysplasia were classified as NILM; among the ten cases of carcinoma in situ, five were classified as NILM, four as ASCUS, and one as LSIL. A benign, indeterminate or low-grade brush result therefore did not exclude high-grade histopathological change in these observations.

The apparently high binary agreement in cohort II (94.3%; 247/262) was driven mainly by the 247 pairs classified as below the high-grade threshold by both methods. Despite high overall binary agreement, chance-corrected agreement was negligible in cohort II ($\kappa = -0.01$), reflecting the predominance of concordant negative pairs and the absence of concordant positive pairs. Binary Cohen's κ was 0.37 in cohort I. The exact McNemar test ($p = 0.00739$) reflects 13 histopathology-positive/cytology-negative versus two cytology-positive/histopathology-negative discordant pairs. Overall agreement therefore obscures the failure to detect high-grade disease at this threshold. Comparisons between cohorts should account for their differing disease spectra and small numbers of high-grade cases.

In the secondary simplified category analysis, grouping ASCUS with NILM increased exact agreement in cohort II from 174/262 (66.4%) to 198/262 (75.6%), while unweighted kappa increased from 0.11 to 0.16. The 24 additional matches were ASCUS cytology paired with histopathological absence of dysplasia. Grouping carcinoma in situ with SIN III did not create additional matches because none of the ten carcinomas in situ lesions had high-grade cytology. The high-grade sensitivity therefore remained 0/13. This simplified analysis describes the effect of category grouping and does not alter the original diagnoses.

Cohort II included a large group without histopathological dysplasia, whereas this category was absent from the 32 paired observations in cohort I. Direct comparison of kappa or accuracy may therefore reflect differences in disease spectrum. Weighted kappa was calculated using different category scales in the two cohorts, which further limits direct comparison of these estimates. Weighted kappa in cohort II depends on the ordinal positioning of ASCUS and the separate carcinoma in situ category; the assigned category positions should not be interpreted as biological equivalence between cytological categories and histopathological grades.

Tomić et al. reported substantially higher agreement between transepithelial cytology and histopathology in a cohort of 57 patients that included benign lesions, potentially malignant disorders and oral squamous cell carcinoma ($\kappa = 0.791$; sensitivity 92.85% and specificity 100%) [7]. Their study therefore assessed a broader diagnostic spectrum than our cohorts, which contained no histopathological confirmed invasive carcinoma. The difference in case mix, particularly the inclusion of overt carcinoma, and differences in sampling and diagnostic categories limit direct comparison of kappa and accuracy estimates. In our data, grouping ASCUS with NILM and carcinoma in situ with severe dysplasia still yielded low chance-corrected agreement in cohort II ($\kappa = 0.16$), and none of its 13 high-

grade lesions were classified as high-grade by cytology. These findings suggest that performance in a cohort containing readily recognisable invasive cancers should not be assumed to apply to detection of severe dysplasia or carcinoma in situ during surveillance of oral potentially malignant disorders.

A distinction between cytological classification and histopathological grading is established in cervical screening. Cytology is used for screening and risk stratification; its sensitivity for high-grade disease varies with sampling, diagnostic threshold and population [13, 14]. Abnormal results can lead to colposcope evaluation and biopsy, while definitive grading of cervical intraepithelial neoplasia rests on tissue histopathology [13]. This distinction provides a conceptual framework for considering oral brush cytology as an adjunct to tissue biopsy, without assuming that the diagnostic performance of cervical and oral samples is the same.

Histopathology remains necessary when clinical findings raise suspicion of high-grade dysplasia or carcinoma, regardless of the brush cytology result. In cohort II, none of the 13 lesions with severe dysplasia or carcinoma in situ had high-grade cytology; repeat cytology alone could therefore delay tissue diagnosis in such cases. Brush cytology may still contribute to longitudinal assessment as an adjunct to clinical examination, but its role in directing biopsy decisions requires further evaluation. Molecular assays and artificial intelligence analysis of scanned cytology slides are being investigated as possible ways to improve risk assessment [15, 16]. These approaches have not been validated in the present cohorts and cannot be assumed to overcome the observed false-negative results.

The small numbers of high-grade lesions resulted in imprecise sensitivity estimates. Furthermore, neither cohort included histopathologically confirmed invasive carcinoma, so sensitivity for invasive oral squamous cell carcinoma could not be assessed. These limitations restrict generalisation beyond the disease spectrum represented in the present cohorts.

Conclusion

In these paired samples, high-grade cytology identified one of four lesions with severe dysplasia in cohort I and none of 13 lesions with severe dysplasia or carcinoma in situ in cohort II. Sensitivity estimates are imprecise because few high-grade lesions were available. A cytology result below HSIL should therefore not be used alone to rule out high-grade histopathological disease or defer a clinically indicated tissue biopsy.

Ethics approval

The study was conducted in accordance with the 1964 Declaration of Helsinki. Ethics approval: Cohort I Ethical Review Board Stockholm 2015-1213-31 and Cohort II Ethical Review Board Linköping 2022-01545-01-300736.

Declarations

All participants consented to participate in the study.

Conflict of interest

The authors declare no conflict of interest.

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Author contribution

C.R.S. and J-M.H. contributed to the conceptualisation of the study and wrote the manuscript with tables and figures. C.R.S., M.K., B.Z. and M.S.A. contributed to sample collection and registration. All authors reviewed the manuscript.

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Figures

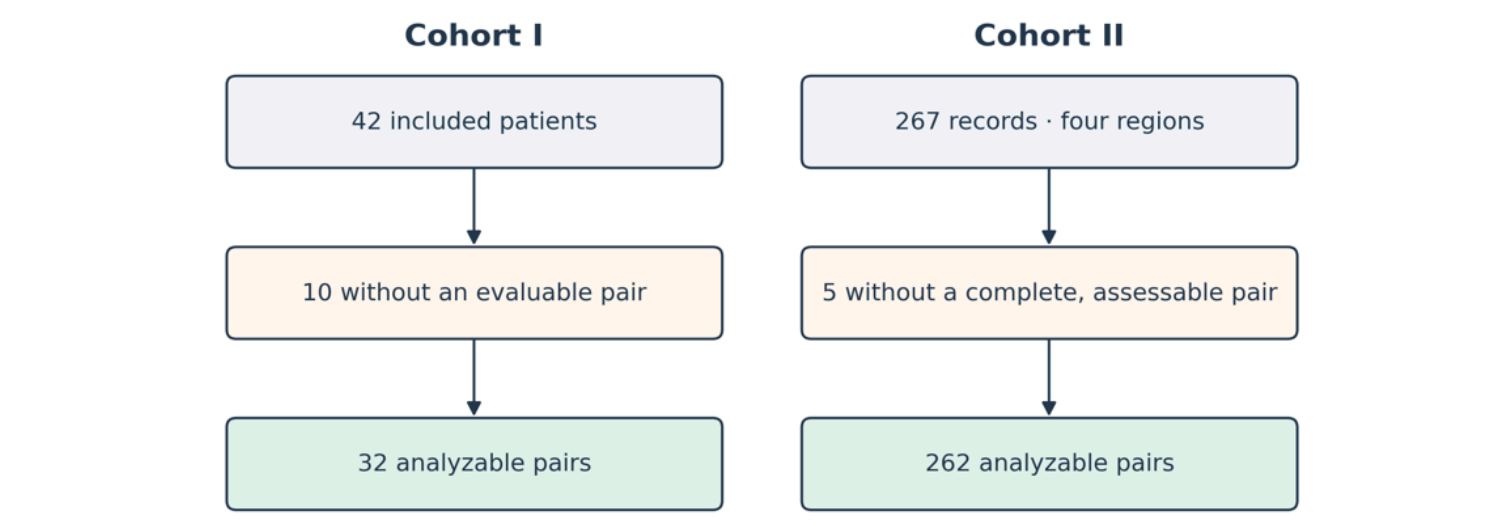


Figure 1

Study material and analysis flow

Figure 1. Selection of paired cytology and histopathology observations. Cohort I included 32 of 42 patients; cohort II included 262 of 267 records from four regions. The five cohort II records without a complete, assessable pair were not included in the diagnostic analyses.

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