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ORIGINAL ARTICLE

Diagnostic efficiency of differentiating small cancerous and precancerous lesions using mucosal brush smears of the oral cavity—a prospective and blinded study

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Abstract The aim of this study was to evaluate the diagnostic accuracy of oral brush biopsy to identify early malignancy. One hundred and eighty-six brush biopsies of suspicious mucosal lesions were obtained, haematoxilin and eosin (H&E)-stained and compared with the histology of conventional excision biopsies of the same site performed concomitantly. The sensitivity for identifying squamous cell carcinoma (SCC) was 88.5%. High-risk lesions including squamous intraepithelial neoplasia (SIN II, SIN III) and SCC were identified with a sensitivity of 86.4%, using a pap-analogous classification, which is considered to be carcinomatous, as well as moderate and severe dysplastic cells positive. Depending on the cytopathologic definition for malignancy and the tumour size, the test accuracy varied: Extending the cytopathologic criteria for malignancy by defining all dysplastic or malignant cytopathologic findings as positive, the sensitivity was increased to 95.2% at the expense of the specificity, which was reduced from 94.9% to 82.3%. Separately analysing SCCs of less than 20 mm, the sensitivity was reduced by 9.5% to 78%. Although small malignant lesions seem to be less reliable by the conventional oral brush biopsy, it is a useful screening instrument for early diagnosis of suspicious, epithelial lesions and could therefore contribute to improved cancer prognosis.

Keywords Brush biopsy · Prevention · Minimally invasive · Oral cancer · Diagnostic · Clinical study

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Introduction

Three percent of all cancer diseases in the EU are located in the oral cavity and the oropharynx, and 74,440 patients contract oral cancer [1]. Worldwide, 300,000 patients suffer from oral or oropharynx cancer [2]. When the tumour disease is beyond an advanced stage (Union Internationale Contre le Cancer (UICC) stage III or IV), the prognosis of surviving the following 5 years falls to 30–50%; whereas for diseases discovered, early the survival rate is 80% [3–5]. In addition, the treatment of advanced diseases often results in social stigmatisation, speech disabilities, or nutrition problems [6–9]. As a result, early discovery of oral cancer is an important objective. The failure of early diagnosis could be due to hesitation in taking excision biopsies in the general practitioner's setting. The oral brush biopsy could lower the threshold of sampling and should therefore be considered, as long as its limitations are made clear.

The diagnostic cytology of epithelial lesions was first established as a screening method to diagnose carcinoma of the uterine cervix in the 1950s. Since then, mucosa from

different localisations such as pharyngeal, cervical, or oral regions have been examined using this technique. In the 1950s and 1960s, the first smears were taken from suspicious oral lesions with a cotton tip [10, 11]. These results were very disappointing as only the superficial epithelium became ablated and obtained for analysis. By collecting cells from the deeper epithelial layers, the brush biopsy can distinguish simple leukoplakia, dysplastic lesions, and early carcinoma.

Studies evaluating the brush biopsy method have been performed with varied results. In these studies the sensitivity of the method has ranged from 71% [12] to 97.2% [13] due to variation of the sampling site, use of multiple examiners, and delay of cytopathologic evaluation.

Additional tools such as DNA image cytometry or computer assisted morphologic examinations have been developed and evaluated [9, 13–16]. In addition, biomolecular techniques such as loss-of-heterozygosity analysis or the detection of genomic mutations were used to improve the diagnostic accuracy of oral cytology [17, 18]. Protein markers such as laminin-5 or tenascin-C should help to identify malignant oral lesions immunhistochemically [19, 20]. Combining the conventional cytology and the tenascin-C immunostaining increased the sensitivity from 78% to 95% [19]. The diagnostic sensitivity of laminin-5 by immunoassay cytology was evaluated at 93% [20]. Recently, molecular biological techniques have also been published to characterise oral lesions by quantitative gene expression of *cytokeratin 17*, *GSTμ*, and *tenascin-C* [21–23].

The aim of this study was to evaluate the diagnostic accuracy of conventional brush smears taken from suspicious oral lesions in a blinded, prospective study design. In addition, the influences of the cytopathologic evaluation and the tumour size on the diagnostic accuracy were evaluated.

Material and methods

All patients attended the Maxillofacial Surgery Clinic at the University Hospital in Mainz, Germany and were examined between September 2005 and December 2007. To be included in the study, a mucosal lesion of the oral cavity was required that had been clinically diagnosed as squamous cell carcinoma (SCC) or suspicious epithelial lesions. Patients with clinically healthy mucosa were excluded. The geographically identical sampling site for cytology and conventional biopsy by excision was guaranteed by an immediate consecutive conventional biopsy by excision after brush biopsy by the same examiner. In each case, the most suspicious region of the lesion was sampled without prior disinfection. All samples were taken in the same way by the same person who did not know the cytologic or histopatholgic diagnosis at the time of sampling.

The cytologic samples were obtained using the Cytobrush®Plus GT (Medscan, Malmo, Sweden), a flexible sampler for single use. The brushes were obtained from the lesions in a rotating manner without local anaesthesia, so that petechial bleeding points occurred in the majority of cases. For the preparation of the cytologic specimen, the collected cells were immediately deposited onto a conventional glass microscope slide (SuperFrost®Plus Objektträger, Menzel GmbH & Co KG, Braunschweig, Germany) in a rotating manner, rapidly fixed by ethylalcohol (Merckofix®, Merck, Darmstadt, Germany) and later stained with H&E at the Department of Pathology.

Following the sampling for cytologic analysis, all oral lesions were examined by taking a conventional biopsy by excision after Ultracain® 1:200,000 (Aventis Pharma, Bad Soden, Germany) instillation or by performing a total resection under general anaesthesia immediately within hours by the same sampler. The obtained tissue was formalin-fixed, paraffin-embedded, and diagnosed at the Department of Pathology on H&E-stained 4-μm routine sections. The analysis of the cytologic and histopathologic samples was performed by different pathologists.

All cytologic smears were examined blindly, i.e. without information on the patient or the result of the histological examination, by an independent, experienced cytopathologist (S.B.). The complete smear was screened for tumour cells or suspicious cells respectively, following the cytopathologic criteria [24]. While the cytology was always analysed by the same cytopathologist, the histopathologic diagnosis was assessed by a team of two pathologists. In total, three different pathologists examined the histopathology samples.

The retrieval of the paraffin-embedded and cytologic samples was approved by the ethical committee of the Rheinland Pfalz Chamber of Physicians (Landesaerztekammer).

In order to identify epithelial lesions that require further diagnostic or therapeutic effort, the subgroups of benign hyperplasia or hyperkeratosis, mild, moderate or severe dysplasia, and SCC were used to classify the cytologic diagnoses. To provide sensitivity and specificity data, these subgroups were summarised into findings that demanded further diagnosis and were defined as positive, and findings that were harmless were defined as negative. Three classification systems were used to indicate positive results: (a) any dysplasia or carcinoma cell [12, 25], (b) moderate or severe dysplasia or carcinoma cells [26, 27], (c) severe dysplasia or carcinoma cells [28].

Correspondingly, the classification schema of squamous intraepithelial neoplasia (SIN) was used for the histological typing of the tissue samples to achieve a reference diagnosis. Two classification systems were used to indicate positive results of histopathology findings: (a) SCC and

carcinoma in situ (CiS), (b) high-risk lesions of SIN II/III and SCC.

Cross table provides sensitivity and specificity data with respect to the different cytologic classification systems and histologically high-risk or cancerous oral lesions.

In addition, the sensitivity and specificity of brush smears are evaluated by excluding all SCC measuring more than 20 mm in diameter.

Results

One hundred and thirty-five patients with oral lesions were enrolled in the study. The mean age of the patients was 62.8 (+/−18.3 SD) years, and the male–female ratio was approximately 2:1. In all cases, a definite histologic diagnosis was achieved by conventional excision biopsy. In 182 cases (98.4%), the cytologic smears offered a sufficient number of well-preserved cells, and thus sufficient technical quality to provide a cytologic diagnosis.

These 182 cases were included for further sensitivity and specificity analyses. The results of the histologic and the cytologic examination of the remaining 182 cases are presented in Table 1. By histology, 102 (56.0%) cases were diagnosed as invasive SCC, two (1.1%) cases as CiS, 11 (6.0%) cases as dysplasia of various degrees (SIN I–III) and 67 (36.8%) cases as benign. The latter group included diagnoses of mucosal hyperkeratosis (n=36), of lichen planus (n=8), of unspecific inflammatory epithelial changes (n=16) and of lesions without squamous epithelial abnormalities, for example fibroma (n=10). According to the classification of the UICC, 39 (38.2%) of the invasive carcinomas were classified as T1, 36 (35.3%) as T2, 12 (11.8%) as T3, and 15 (14.7%) as T4 (Table 2).

Cytologically, SCC cells were diagnosed in 78 (42.6%) smears. Severe dysplasia cells were seen in 18 cases (9.9%), moderate dysplasia cells in five cases (2.7%) and slight dysplasia cells in 11 cases (6.0%). Seventy cases contained no dysplastic or neoplastic cells (38.5%). Figure 1 show typical cases of leukoplakia, early carcinoma, and invasive carcinoma. The results of the histologic and cytologic examination are presented in Table 1, which provides the statistical data for sensitivity and specificity evaluation of CiS and SCC diagnoses. The sensitivity and specificity change depending on the cytology classification being applied to define a benign or malign diagnosis. Cells of severe dysplasia or SCC were cytologically detected in 92 out of 104 cases of histologically proven invasive carcinoma or CiS (sensitivity, 88.5%; specificity, 95%). If the five cytologic specimens diagnosed as moderate dysplasia were also accepted as "high-risk lesions" according to the Ljubljana classification, analogous to the proposal of the Bethesda system in gynaecological cytology [26, 27], the rate of correctly classified carcinoma cases would be evaluated at a sensitivity of 86.4%. The Bethesda classification system defines all smears showing cells of moderate or severe dysplasia and carcinoma as positive. Further extension of the cytopathologic criteria for malignancy by defining all smears with dysplastic or malignant cells as positive would result in a higher sensitivity of 95.2% and a lower specificity of 94.5% (Table 3).

Table 1 Tumour size of SCC/CiS investigated

	Absolute	Relative (%)
CiS	2	
T1+CiS	39	38.2
T2	36	35.3
T3	12	11.8
T4	15	14.7

The sensitivity and specificity concerning high-risk lesions, defined as SIN II and SIN III, as well as carcinoma, would be detected with a sensitivity of 90% and a specificity of 95.9% using the Bethesda system for diagnostic evaluation. By extending the positive criteria for a high-risk lesion or carcinoma by also taking into account cytologies of mild dysplasia, the sensitivity increased and the specificity decreased (Table 4).

Focusing only on the diagnosis of the high-risk lesions of SIN II and SIN III, and excluding the histology diagnosis of SCC, the sensitivity was 62% using the Bethesda classification. Extending the positive criteria for a high-risk lesion by taking into account all cytologies with dysplastic cells, the sensitivity gained 23.5% (87.5%); the specificity, however, lost 8.4% (88.9%).

Understanding the brush biopsy as a tool for early cancer diagnosis, only the 41 small cancerous lesions (CiS and carcinomas ≤ 2 cm [pT1]) were considered as SCCs >2 cm in diameter and were excluded. Evaluating this collective, cells of severe dysplasia or SCC were cytologically present in 36 cases (sensitivity, 74.5%) (Table 3). Also including the six cases with moderate or severe dysplasia as indicative of a high-risk lesion, 38 cases could be correctly identified (sensitivity, 80.9%) (Table 4).

After the cytopathologist and the histopathologists assessed their diagnoses independently, all inconsistent cytology diagnoses were re-evaluated. In total, five SCC cases were cytologically diagnosed as benign lesions (2.7%); and in another three tumour cases, only cells of slight dysplasia were identified. As a second cytologic evaluation confirmed, these false-negative results were caused by a lack of malignant cells.

In four specimens with benign or reactive histological changes, dysplastic cells were seen cytologically. Two of these were even classified as moderate dysplasia. The

Table 2 Correlation matrix of histological and cytological findings in 189 cases of oral lesions

		Histology							
		Bening lesions	Mild dysplasia/SIN I	Moderate dysplasia/SIN II	SIN III		Invasive SCC		Σ
					Severe dysplasia	CiS	T1	≥T2	
Cytology	Benign epithelial cells	63	1	1	0	0	3	2	70
	Mild dysplasia	2	4	2	0	0	3	0	11
	Moderate dysplasia	1	0	0	0	0	3	1	5
	Severe dysplasia	1	0	1	2	0	6	8	18
	Tumour cells of an SCC	0	0	0	0	2	24	52	78
	Σ	67	5	4	2	2	39	63	182
					4 (SIN III)		102 (Σ T1, ≥T2)		

SIN squamous intraepithelial neoplasia, *SCC* squamous cell carcinoma

cytologic reevaluation of these cases showed histological inflammation coupled with cell-poor, hemorrhagic cytology with few severe dysplastic cells in one case. The other cytology, diagnosed histologically as moderate dysplasia, showed cells with large nucleoli and cytoplasmatic changes, possibly due to previous radiation therapy.

Discussion

This study focused on conventional cytology by evaluating the diagnostic accuracy of H&E-stained oral brush biopsy smears. In contrast to other studies, we evaluated the sensitivity and specificity with consideration of the cytopa-

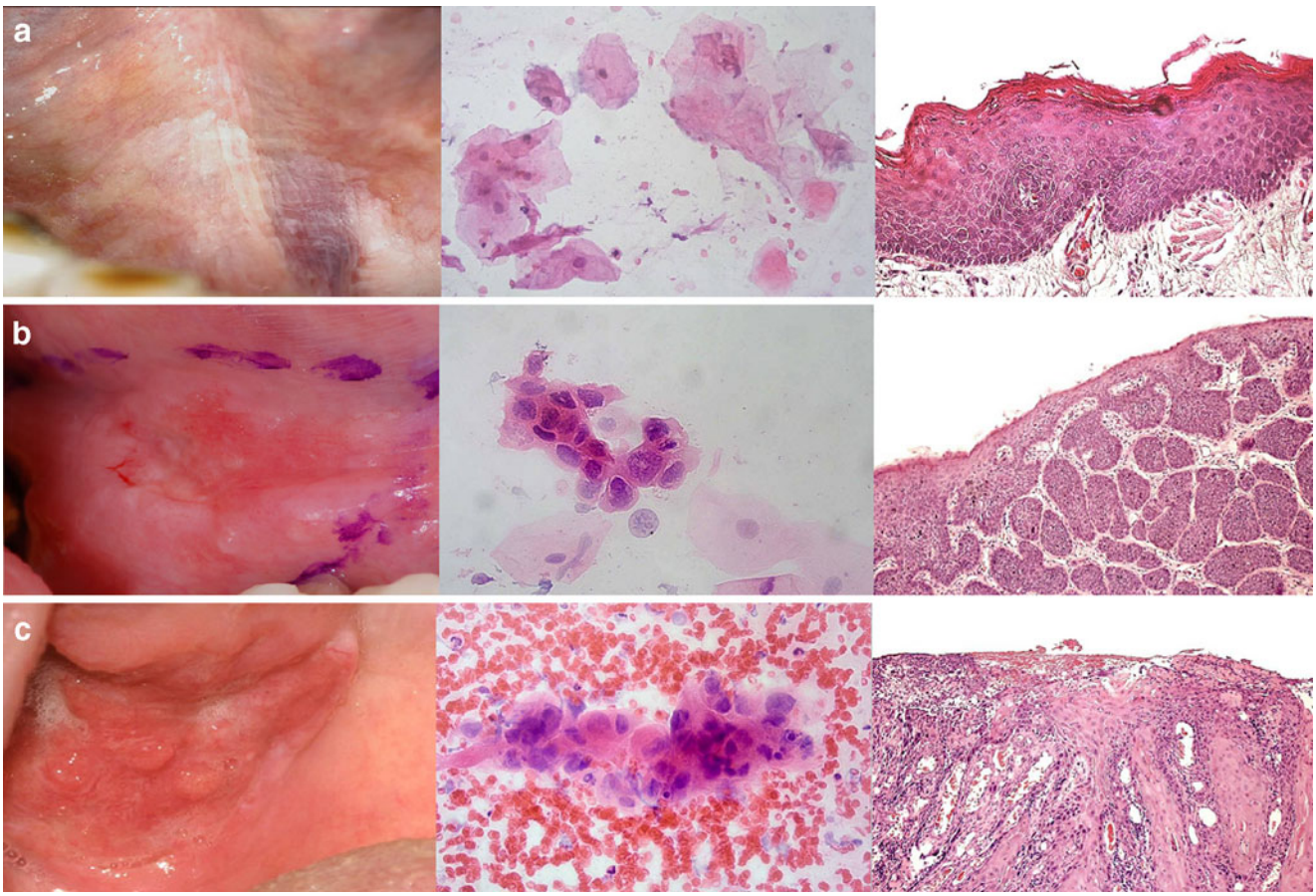

Fig. 1 **a** Clinical view, cytology and histology of simple leukoplakia. **b** Clinical view, cytology and histology early carcinoma (T1). **c** Clinical view, cytology and histology of Squamous Cell Carcinoma (SCC) (T3)

Table 3 Diagnostic accuracy of oral brush biopsy to diagnose SCC, described by sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV), according to different diagnostic systems and tumour size

	Diagnostic system	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
All lesions	Any dysplasia/carcinoma	95.2	83.3	88.4	92.9
	Bethesda	92.3	93.6	95.0	90.1
	Pap-analogous	88.5	94.9	95.8	86.0
All lesions excluding SCC>20 mm	Any dysplasia/carcinoma	92.7	92.3	74.5	95.6
	Bethesda	85.4	92.4	87.5	92.4
	Pap-analogous	78.0	95.0	88.8	88.1

thologic classification as well as the tumour size. In order to improve prognosis, the oral brush biopsy screening method must be able to identify small, malignant mucosal lesions.

Taking into consideration all cytologies with cells of severe dysplastic or malignant cells and comparing these with the histological gold standard, the conventional brush biopsy could identify 88.5% of all SCC.

Using also a pap-analogous classification, which considers all smears with severe dysplastic or malignant cells to be positive [28, 29], Remmerbach et al., however, found a sensitivity of 91% and a specificity of 96% [13]. Maraki et al. evaluated identification of malignant oral lesions using brush smears at a sensitivity of 100% and a specificity of 97.4% [30].

Others defined all cytologies with malignant or dysplastic cells of any degree as positive. Driemel et al. described a sensitivity of 78% and a specificity of 96% [19, 25]. Poate et al. evaluated the accuracy of computer assisted cytologic diagnosis using Papanicoulaou-stained smears and histological reference diagnosis; a sensitivity of 71% and a specificity of 32% were found [12]. These results are in contrast with the findings by Sciubba, who also used computer assisted cytologic evaluation and Papanicolaou staining; he reported a sensitivity of 100% when identifying oral cancer using brush biopsy [15]. Recent studies using the computer assisted cytological diagnostic technique of Oral CDx found low PPVs, high false-positive rates and high sensitivity values for the diagnosis of oral dysplastic lesions and malignancy [31, 32].

Expanding the cytologic criteria for malignancy, the data of the presented study suggested a sensitivity of 95.2% and a specificity of 83.3% to identify malignant lesions through oral brush biopsy. Compared to the pap-analogous classification, the sensitivity and the negative predictive values were increased (95.2% vs. 88.5%, 92.9% vs. 86.2%), and the specificity and the positive predictive values were decreased (82.3% vs. 95%, 87.6% vs. 95.8%). Obviously, the diagnostic system used to find malignant lesions has a great influence on the diagnostic results of oral brush biopsy, but this could not explain the inconsistent data referenced above.

Not only the different cytology classifications, but also the different staining methods should be checked for possible effects on the diagnostic accuracy of oral brush biopsy. Comparing the studies by Sciubba and Poate et al., who both used the same method of Papanicolaou staining, there were great differences with respect to sensitivity and specificity; as a result, the staining method does not explain the different results.

Another explanation for these differing results may be the different ways of obtaining a reference diagnosis. Many of the studies referred to did not use histology as a reference tool, but checked the cytologic diagnosis through follow-up clinical examinations, as Sciubba did [15]. A test method cannot reach a higher quality level than the reference method itself, and since identification of carcinoma by clinical examination has a reported sensitivity of 74% and specificity of 43%, the sensitivity of clinically

Table 4 Diagnostic accuracy of oral brush biopsy to diagnose high-risk lesions and SCC, described by sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV), according to different diagnostic systems and tumour size

	Diagnostic system	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
All lesions	Any dysplasia/carcinoma	94.5	88.9	92.8	94.1
	Bethesda	90.0	97.2	98.0	88.6
	Pap-analogous	86.4	98.6	99.0	85.5
All lesions excluding SCC>20 mm	Any dysplasia/carcinoma	91.5	88.9	84.3	94.1
	Bethesda	80.9	97.2	97.5	88.6
	Pap-analogous	74.5	98.6	97.2	85.7

controlled cytological diagnoses cannot reliably exceed 74% [33, 34]. Thus, the evaluation has to rely on a histological reference.

In a self-critical manner, all cytologies of false-negative results were re-evaluated in the study presented. In these specimens, an oral brush biopsy was not sufficient to collect carcinoma cells for the correct diagnosis. Possibly, stronger bristles would have enabled the brush to collect cells also from the deeper tissue layers and supply sufficient diagnostic material. On the other hand, this diagnostic method would be more invasive and painful. The false-positive results had been due to reactive, inflammatory cellular changes and hints at a limited validity of cytologic diagnosis in inflamed lesions. In 1.6% of all cytologies, the specimen quality was not adequate for diagnosis. In these cases a second sample could be taken easily.

Another striking point is the differing study populations that often consisted of advanced malignant lesions. In the study conducted by Remmerbach et al., the fraction of carcinoma with a diameter of more than 20 mm amounted to 75% of all examined carcinoma. Just 4% of the examined lesions were CiS [13]. Maraki et al. also included only 4% precursor lesions in her study [30].

We analysed the sensitivity and specificity of oral brush biopsy separately for small lesions, and focused on all carcinoma with less than 20 mm in order to compare these results with those that are found by including all tumour sizes. The fraction of invasive carcinoma was reduced to 39 cases compared to 102 cases. In addition, the sensitivity to identify malignancy (SCC or CiS) changed from 88.5% to 78%. Also, the sensitivity to diagnose high-risk lesions was lowered, measured at 74.5% compared to 86.4%, when all lesions of all tumour stages were considered. These results show the influence of the study population on the sensitivity of oral brush biopsy. The accurate evaluation of small, oral lesions is crucial if oral brush biopsy is considered to be a screening instrument that helps to distinguish malignancy and high-risk lesions from benign squamous cell hyperplasia or reactive, inflammatory processes. Therefore, it could be useful to expand the cytologic definition for malignancy by filtering all malignant or dysplastic cells in favour of a higher sensitivity and further histological evaluation.

Particularly as a tool to check for suspicious oral lesions and diagnose progression to a high-risk lesion, a high sensitivity is required. Therapy can then be started earlier and hopefully lead to a better prognosis.

If brush biopsy, however, is required to support a therapeutic decision, a test method with high specificity is needed. Then the pap-analogous classification modified by Böcking is recommended to characterise an oral lesion, attesting a test specificity of 95% [28].

Conclusion

Our results suggest that there is limited accuracy of the conventional oral brush biopsy in finding a definitive diagnosis of precursor and related lesions, particularly early SCC less than 20 mm in diameter. Conventional brush biopsy could be indicated as an additional diagnostic tool for oral lesions, which are not highly suspicious for malignancy, and therefore do not demand an immediate histological diagnosis.

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Conflict of interest The authors declare that they have no conflict of interest.

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