Orcellex® Brush Biopsy and Liquid-Based Cytology – Assessment of a New Diagnostic Technique in Oral Potentially Malignant Disorder Management

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\textbf{Abstract}

Oral potentially malignant disorders (PMD) are recognizable mucosal conditions in which a high, but unpredictable, risk of transformation leads to invasive squamous cell carcinoma (SCC) development. Modern PMD management relies upon clinical recognition of suspicious lesions and incision biopsy to facilitate histopathology assessment and dysplasia grading, followed by interventional laser excision of ‘high risk’ lesions and long-term patient surveillance to monitor for further disease. Individual PMD lesion assessment and management remains highly subjective, however, and techniques which improve objectivity are required in contemporary clinical practice. Utilizing incision biopsy as the current ‘gold standard’, this paper reports on a clinical study undertaken to assess patient acceptability and diagnostic accuracy of Orcellex® brush biopsy and liquid-based cytology (LBC) in the assessment of 224 newly presenting patients in a specialist PMD clinic. The majority of oral lesions were leukoplakias (204) with the floor of the mouth and ventro-lateral tongue the most common site of origin (103). Patient acceptability was high (mean VAS satisfaction scores for brush biopsy 88.25, compared with 57.35 for incision biopsy) and 222 cytology samples (99%) were diagnostic. Cytology diagnoses included 62 hyperkeratoses and 102 dyskaryoses, whilst histopathology identified 101 hyperkeratoses (with or without lichenoid inflammation) and 83 dysplasias; agreement, however, was only seen in 44 cases (20%). Binary analysis (using a ‘normal’ or ‘abnormal’ score) showed no significant agreement between cytology and histopathology results (McNemar Test = 0; kappa agreement = -0.006), whilst ROC analysis showed no improvement in diagnostic accuracy using brush biopsy. Brush sensitivity was 47.6%, with a specificity of 51.7%, Orcellex® brush biopsy may have a role in the monitoring of PMD patients in specialist clinics, but is best regarded as an adjunctive technique which cannot replace incision biopsy and histopathological assessment.

\textbf{Citation:}


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1. Introduction

1.1 Oral potentially malignant disorders

Oral squamous cell carcinoma (SCC) is a lethal and deforming disease due to tumour invasion, oral-facial destruction, cervical lymph node metastasis and blood-borne dissemination. Oral cancers, however, are often preceded by potentially malignant disorders (PMD), recognizable mucosal diseases such as localized leukoplakia and erythroplakia, and sometimes more widespread conditions (van der Waal, 2009). Although the natural history of PMD remains poorly documented (Napier and Speight, 2008), a recent systematic review has estimated a 12% cancer rate over a mean transformation time of 4.3 years (Mehanna et al, 2009).

Despite the ability to identify PMD in patients, assessment techniques remain highly subjective and clinicians are unable to predict lesion behaviour or quantify the individual risk of malignant transformation. Contemporary PMD management is based upon incision biopsy for histological assessment followed by whole lesion excision for definitive histopathological diagnosis and treatment (Thomson and Wylie, 2002; van der Waal, 2009; Thomson, 2012).

Recently, a number of potentially useful non-invasive diagnostic adjunctive tools have been developed to improve the detection of the early signs of precancerous change within the oral cavity. These include optical imaging, vital staining techniques and brush biopsy cytology but, as most reports in the literature are based primarily upon anecdotal evidence, their true value in clinical practice is yet to be determined (Thomson and Goodson, 2012; Messadi, 2013).

1.2 Oral cytology

Cytodiagnosis refers to the process whereby individual cells are loosened from their tissue of origin and transferred to a cytological slide for microscopic evaluation to diagnose disease. Whilst effective sampling of exfoliated oral epithelial cells for cytological analysis has proved problematic in the past, a number of new cell collection devices, including a range of ‘cytobrushes’, have been developed recently to facilitate full-thickness sampling of stratified epithelium. Kujan et al. (2006) first reported the efficacy of oral brush biopsy and liquid-based cytology (LBC) in the collection and analysis of buccal mucosa and lateral tongue samples in healthy volunteers.

The Orcellex® brush (Rovers Medical Devices BV., the Netherlands) was developed as a novel oral cytobrush with a specially designed head comprising 5 segments of high-density fibres designed for optimal cell collection, storage and subsequent release of cell material from all oral epithelial layers (Figure 1A). The brush head is placed firmly against the mucosa and rotated 10 times (Figure 1B), then detached and transferred to BD Sure Path TMethanol-based preservative fluid for transfer to the laboratory. Highly cellular samples containing representative cells from basal and parabasal, intermediate and superficial epithelial layers are obtained. LBC, rather than conventional glass slide smears, is used and is seen to improve cell distribution, reduce cell clumping and assists in the production of thin layer preparations that can be used for accurate cytological evaluation (Figure 1C).

Figure 1: (A) Close-up view of the Orcellex® brush head, which comprises 5 segments of high-density fibres to optimize cell collection (B) brush biopsy in use to collect cell samples from floor of mouth mucosa and (C) appearance of a Papanicolaou stained thin-layer liquid-based cytology preparation seen under microscopic examination x40
The accuracy of Orcellex® brush biopsy cytology has not been formally assessed as an objective tool in PMD management. Since the brush can potentially harvest cells from an entire mucosal lesion rather than just examining a small incision biopsy sample, the technique may improve diagnostic accuracy and ultimately guide the management of individual potentially malignant lesions.

2. Objectives of Research

The aim of this paper was to establish the accuracy of the novel diagnostic adjunctive technique, Orcellex® brush biopsy, in comparison to standard incision biopsy histopathology diagnosis and to determine its acceptability to patients as an investigative technique.

3. Materials and Methods

3.1 Background to the study

The PMD service at the Newcastle upon Tyne Hospitals NHS Foundation Trust in North-East England is run by a consultant oral & maxillofacial surgeon with a special interest in PMD management (PJT), and deals with approximately 1200 patient attendances a year. New patients with suspicious mucosal lesions are assessed by clinical examination, provisional incision biopsy diagnosis, risk factor modification and, for cases designated ‘high risk’, treated by interventional laser surgery (Thomson and Wylie, 2002). Long term patient follow up and active surveillance facilitates early recognition of further disease and reduces the risk of malignant transformation (Goodson and Thomson, 2011; Thomson, 2012).

3.2 Clinic procedures

Following standardized clinical examination procedures and the identification of a lesion requiring incision biopsy diagnosis, patients underwent Orcellex® brush biopsy using the standardized technique described and illustrated previously in Figure 1; this was followed within 2 weeks by conventional, incision biopsy sampling. The first 50 participating patients were also asked to complete a simple 100mm visual analogue scale (VAS) questionnaire to record their satisfaction rating for both brush and incision biopsy procedures from 0, ‘not at all satisfied’, to 100, ‘very satisfied’. Additional ‘free text’ patient comments and feedback was encouraged.

3.3 Laboratory procedures

Cytology specimens were all processed and stained using the BD Prep Stain TM automated process in which, following density gradient centrifugation to enrich the cellular sample and remove obscuring non-diagnostic debris, discretely stained, thin-layer slide preparations were produced. Reporting of specimens was carried out by 2 experienced cyologists (VW and SJJ), applying the Bethesda guidelines for liquid-based squamous cellularity (Solomon 2002); diagnosis and consensus grading was carried out as required. Having confirmed an adequate cellular sample, cytology analysis described cells as normal, hyperkeratosis, exhibiting cellatypia or mild, moderate or severe dyskaryosis or suggestive of SCC; the presence of inflammatory cells and candida were noted as appropriate.

All incision biopsy specimens underwent standardized histopathology examination by 2 experienced oral pathologists (PS and CMR) working to agreed diagnostic criteria and using the World Health Organization (WHO) classification to grade PMD specimens into mild, moderate or severe dysplasia categories (Gale et al., 2005); both pathologists independently assessed the biopsy material and discordant grading was resolved by review and consensus.

3.4 Justification for research

We are not aware of any other publication that has examined the accuracy of Orcellex® brush cytology as an oral diagnostic technique during PMD assessment and management.

3.5 Statistical analysis

Analyses were performed using SPSS, version 19.0 (Statistical; Package for the Social Sciences, Chicago, IL, USA). Kappa agreement between mean VAS satisfaction scores for brush and incision biopsies was calculated, whilst McNemar’s test was used to compare paired data for brush biopsy cytology and incision biopsy histopathology results. Receiver Operating Characteristic (ROC) space analysis was used to determine the efficacy of brush cytology as a diagnostic technique. Calculations of sensitivity (proportion of correctly identified positive results), specificity (proportion of correctly identified negative results), positive predictive value (positive results correctly diagnosed) and negative predictive value (negative results correctly diagnosed) for the Orcellex® brush were also determined.

4. Results

4.1 Patients and oral lesions

224 patients were recruited to the study, comprising 128 male and 96 female (with a mean
age of 59.5 years); no specific age or sex influences were observed in this cohort. Clinically, 204 lesions presented as leukoplakia, 17 erythroplakia and 3 erythroleukoplakia, with the majority arising on the ventro-lateral tongue (60), floor of mouth (53) and buccal mucosa (34); Table 1.

Table 1: Site of Origin of 224 Mucosal Lesions

<table>
<thead>
<tr>
<th>Oral Site</th>
<th>No. of Lesions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventro-lateral Tongue</td>
<td>60 (27)</td>
</tr>
<tr>
<td>Floor of Mouth</td>
<td>53 (24)</td>
</tr>
<tr>
<td>Buccal Mucosa</td>
<td>34 (15)</td>
</tr>
<tr>
<td>Palate/Fauces/Retromolar</td>
<td>31 (14)</td>
</tr>
<tr>
<td>Alveolar Mucosa</td>
<td>26 (11)</td>
</tr>
<tr>
<td>Labial Mucosa / Commisure</td>
<td>11 (5)</td>
</tr>
<tr>
<td>Tongue Dorsum</td>
<td>9 (4)</td>
</tr>
</tbody>
</table>

4.2 Patient acceptability

50 patients (35 male and 15 females) completed VAS satisfaction questionnaires. The mean VAS satisfaction score for Orcellex® brush biopsy was 88.25 ± 17.71 mm, whilst incision biopsy scored 57.35 ± 13.66 mm. This was a significant difference in patient satisfaction, with kappa agreement low at -0.003.

4.3 Orcellex® brush biopsy cytology

The results for cytology diagnoses are listed in Table 2; only 2 ‘low cellularity’ cases (<1%) were identified and 40 were reported as ‘normal’ in appearance (no atypical or malignant cells). Whilst the single commonest diagnosis was hyperkeratosis (62), cellular atypia and varying degrees of dyskaryosis were seen in 102 cases, with a further 3 being diagnosed as SCC.

4.4 Incision biopsy histopathology

The results for incision biopsy histopathology are shown in Table 3; hyperkeratosis and hyperkeratosis with lichenoid inflammation were the most common diagnoses (101), whilst dysplasia was identified in 83 cases. Proliferative verrucous leukoplakia (PVL) was diagnosed in 21 lesions and in 6 cases, SCC was identified.

4.5 Accuracy of Orcellex® brush biopsy cytology

Assuming histopathology analysis as the more definitive test result, Table 4 cross-tabulates cytology with histopathology diagnoses. Exact agreement between categories was seen in only 44 cases (20%): 18 hyperkeratoses, 5 candidal lesions, 7 mild, 5 moderate, 8 severe dyskaryosis/dysplasias and 1 SCC. Diagnostic categories such as hyperkeratosis with lichenoid inflammation and PVL required histopathological recognition of tissue architecture changes which were not, of course, provided by cytology techniques.
Due to differences in terminology between cytology and histopathology, results were re-classified using a binary system in which ‘normal’ or non-premalignant lesions (0) were contrasted with those thought to be ‘abnormal’ or premalignant (1). ‘Normal’ cytology included normal, hyperkeratosis and candida infection categories, whilst histopathology diagnoses of hyperkeratosis, candida and inflammation were also graded 0. Table 5 summarizes the cross-tabulation of binary results and shows that cytology and histopathology diagnoses agreed in only 109 cases (48.7%) whilst in 115 (51.3%) there was disagreement. No significant correlation was seen between brush biopsy cytology and histopathology grading (McNemar chi-square statistic = 0; kappa agreement = -0.006). Sensitivity (proportion of ‘abnormal’ correctly identified), specificity (proportion of ‘normal’ correctly identified), positive predictive value (proportion of ‘abnormal’ test results correctly diagnosed) and negative predictive value (proportion with ‘normal’ result correctly diagnosed) for Orcellex® brush biopsy are summarized in Table 6. Sensitivity, specificity and negative predictive value were all relatively low, although the positive predictive value was higher.

Figure 2: Receiver Operating Characteristic (ROC) space analysis for Orcellex® brush biopsy, illustrating that cytology diagnosis lies very close to the ‘line of no discrimination’ and is thus no closer to the ‘ideal’ diagnostic method compared with histopathology assessment.

| Table 5: Cross-tabulation of binary cytology scores with binary incision biopsy histopathology diagnoses (‘normal’ or ‘non-premalignant’ / ‘abnormal’ or ‘premalignant’) |
|--------------------------------------------------|--------------------|-----------------|------|
| **Binary Cytology Score** | **Binary Histopathology Score** | **Total** |
| ‘Normal’ | Normal 0 | Abnormal 1 | 117 |
| Abnormal 1 | 29 | 78 | 107 |
| Total | 60 | 164 | 224 |

Utilizing histopathology diagnosis as the ‘gold standard’, Receiver Operating Characteristic (ROC) space analysis was performed, suggesting that Orcellex® biopsy cytology does not improve upon conventional diagnostic accuracy; Figure 2.

5. Discussion

5.1 Patients and oral lesions

This paper reports upon one of the largest series of PMD patients and lesions (224) examined by brush biopsy and compared, within 2 weeks to avoid lesion progression, with incision biopsy results; other recent studies have involved much smaller patient numbers of 35 or 79 (Guner et al., 2011; Mehrotra et al., 2011). The majority of lesions in this study presented clinically as leukoplakia on ventro-lateral tongue, floor of mouth and buccal mucosa sites, which is consistent with previous PMD studies (Thomson and Wylie, 2002; Hamadah and Thomson, 2009; Diajil et al., 2013).

5.2 Orcellex® brush biopsy

The Orcellex® brush biopsy technique meets all the important criteria of a pragmatic diagnostic technique: readily available, easy to use, minimally invasive and efficient in collecting sufficient numbers of cells for examination (Mehrotra, 2012). From the clinician viewpoint, the brush provides a simple and reliable technique which is easy to learn, with an optimal head design which facilitates application to all accessible oral mucosal surfaces. The Orcellex® brush has the additional advantage of ensuring that atypical basal and parabasal cells are harvested, along with more superficial cell layers, allowing representative trans-epithelial sampling which is important for accurate oral...
cytology diagnosis. This is highly pertinent in clinical practice because the majority of presenting lesions manifest as leukoplakia, due to thickened layers of keratin which prevent abnormal cells from readily reaching the surface layers.

5.3 Patient Acceptability
There is no doubt that Orcellex® brush biopsy was well tolerated in this study, proved popular with patients (who volunteered no adverse comments) and, overall, showed high patient acceptance. VAS satisfaction scores recorded for the brush were much higher than subsequent incision biopsies (88.25 and 57.35, respectively), which is probably unsurprising due to the need for local anaesthetic administration, invasive scalpel or punch techniques and mucosal suturing. Indeed, it is not an uncommon scenario in specialist clinical practice for patients to decline invasive biopsy procedures, preferring observation only or brush biopsy when available, especially during prolonged follow-up periods when multiple biopsies have already been required or when large or multi-focal mucosal lesions necessitate field mapping procedures (Thomson & Hamadah, 2007; Hamadah et al., 2010).

5.4 Liquid-Based Cytology
Specimen filtration during the automated BD Prep Stain TMLBC preparation process effectively removes mucous, blood and inflammatory cells from brush samples and significantly improves cell distribution and smear thickness, leading to better diagnostic accuracy. This is clearly demonstrated in this study, in which only 2 out of 224 samples were deemed ‘low cellularity’. Experienced cytologists assessed all study samples, with consensus grading when required, which is a significant advantage in clinical practice. A potential disadvantage of LBC, however, is the inevitable destruction of epithelial fragments and the lack of tissue architecture and structural hierarchy which is so valuable in histopathology assessment. The cytologists also noted cell morphological appearances to be much more subtle in oral compared to cervical cytology.

5.5 Accuracy of Technique
This is the first study to our knowledge to assess the accuracy of Orcellex® brush biopsy using incision biopsy diagnosis as a ‘gold standard’ comparator. Sensitivity and specificity appear disappointingly low for the technique (47.6 and 51.7%, respectively) and although positive predictive value was quite high (72.9%), the low negative predictive value (26.5%) raises concern that brush cytology may not be reliable in identifying abnormal mucosa. A number of previous studies investigating the use of the OralCDx® brush (CDx Laboratories Inc, Suffern, NY, USA) in conjunction with microscopic computer analysis have certainly supported brush cytology as a diagnostic adjunct and shown high sensitivity and specificity (Mehrotra et al., 2009), but critics have noted that the technique is of limited value in detecting mucosal change not readily visible on conventional examination and also observed high false positive results when applied as a screening tool (Balevi, 2011; Messadi, 2013).

Unfortunately, direct comparison of cytology and histopathology is not always feasible, due to variations in diagnostic terminology and the lack of tissue structure following brush cytology which is often necessary to establish a histopathology diagnosis. Interestingly, review of Table 4 shows that whilst no histopathology specimens were deemed ‘normal’ 40 cytology samples were reported to be, although review of incision biopsy data found 13 to be dysplasia, 4 PVIL and 1 SCC, confirming the poor negative predictive value for Orcellex®. Whilst 6 SCC lesions were recognised on incision biopsy, only 1 was correctly identified on cytology and 3 others reported as hyperkeratosis or normal (as above). In addition, 2 suspected cytology SCC lesions were re-classified as dysplasia on histopathology assessment. Although too small to be of statistical significance in this study, and recognizing that cytology is limited in its ability to confirm invasive carcinoma, potential misdiagnoses such as these can be of considerable clinical significance.

Hyperkeratosis with lichenoid inflammation is a common histopathology diagnosis in clinical practice and was seen in 51 lesions (22%) in this study, whereas their equivalent cytology reports listed: low cellularity / normal (13), hyperkeratosis (17), candida (2) or varying degrees of cellular atypia and dyskaryosis (19). Similarly, the 21 histology confirmed PVILs (high-risk progressive lesions characterized by verrucous hyperplasia and sub-epithelial lymphocytic infiltration; Hansen et al., 1985), showed a range of normal (4), hyperkeratosis (2) and atypia/dyskaryosis (8) features on cytology examination. It seems clear, therefore, that cytology findings may need to be regarded as potentially ‘non-specific’ in nature.

In terms of dyskaryosis versus dysplasia, mild dysplasia was correctly identified by cytology in only 7 out of 41 histology confirmed lesions (17%), although this rose to 5 out of 24 (21%) for moderate and 8 out of 18 (44%) for severe dysplasia, probably reflecting more efficient sampling of superficial dyskariotic cells in the higher grade dysplastic lesions.
Whilst histopathological examination of scalpel biopsy specimens clearly remains the ‘gold standard’ for provisional incision biopsy diagnosis in PMD management, we have previously shown that disease severity frequently requires up-grading following formal excision biopsy examination (Thomson and Wylie, 2002; Hamadah and Thomson, 2009; Goodson et al., 2011). It may not be necessary, or indeed feasible, to excise every suspicious mucosal lesion but it is then important to remember that incision biopsy results may not be representative of the true nature and extent of disease. Orcellex® brush biopsy, whilst not a substitute for histopathology examination, may offer additional opportunities for abnormal cell identification particularly during sampling of large, widespread or multi-focal lesions and may have a role in long-term monitoring and active surveillance of PMD patients during clinic follow-up.

5.6 Additional analytical techniques.
A particular advantage of the LBC technique is that additional analytical techniques such as immunocytochemistry, HPV testing, DNA ploidy, molecular analysis and cytomorphometry may be applied to cells remaining in the collection fluid following diagnostic smear preparation (Kujan et al., 2006; Mehrotra, 2012). In relation to immunocytochemistry, for example, high Ki-67 labelling may be seen in cytology specimens exhibiting severe dyskaryosis and, as we have previously found high Ki-67 proliferative indices to be associated with increased dysplasia and disease progression in PMD lesions (Thomson et al., 2008), there may well be a prognostic role for such cytology labelling in the future.

Research Highlights
• This paper analyses the results of Orcellex® brush biopsy and LBC diagnoses from a large cohort of patients attending a specialist PMD service.
• Brush biopsy and LBC are useful adjunctive techniques in specialist PMD clinics, but are not in themselves reliable substitutes for ‘gold standard’ histopathology assessment.
• Further research is warranted to determine the precise role of Orcellex® brush cytology in PMD diagnosis and management, especially in relation to multiple lesion disease and long-term patient monitoring.

Limitations of Research
This study took place within a specialist PMD clinical service and did not, therefore, assess the potential role of Orcellex® brush biopsy as a primary screening tool. As a one-centre, UK-based, non-randomized patient cohort study, there may be limitations to the global significance and applicability of the study results.

Recommendations
Orcellex® brush biopsy and LBC techniques are recommended as diagnostic adjuncts for diagnosis, assessment and clinical follow-up of mucosal lesions in PMD patients in specialist practice. Further assessment of these techniques, particularly in comparison with excision biopsy histopathology, is warranted in larger, multi-centre clinical trials and in the longer term monitoring of post-treatment cases.

Conclusions
Orcellex® brush biopsy and LBC are practical, acceptable and effective diagnostic adjunctive techniques, but the results of this study suggest they are not as accurate as incision biopsy histopathology and cannot be regarded as a substitute investigation. Further studies are necessary to refine the sensitivity and specificity of brush biopsy in relation to definitive excision biopsy diagnosis. The Orcellex® brush may have an important role as a diagnostic adjunct during the diagnosis, management and post-treatment surveillance of PMD patients in specialist practice.

Authors’ Contributions, Competing Interests and Funding Aspects
ML Goodson led the data collection and analysis, assisted by G Aubourg, whilst PJ Thomson and ML Goodson coordinated the clinical assessment and management of all patients in this study. V Wadehra and SJ Johnson provided specialist cytology diagnostic services and P Sloan and CM Robinson oral pathology expertise. All authors contributed to the intellectual content and writing of the manuscript. No competing or conflicts of interest are declared, nor was any funding received for this study.

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