Group 4
Clinical Assessment and Emerging Technology for Early Detection

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Current paradigm

STEP 1
- New Patient
- Emergency Patient
- Recall Patient

STEP 2
- Symptom & Risk Factor Assessment
  - Risk Factor -
    - Risk Factor Education
  - Risk Factor +
    - Risk Factor Modification

STEP 3
- Extra-Oral Examination
  - Normal Examination
  - Abnormal Examination
    - Referral to Appropriate Specialist

STEP 4
- Intra-Oral Examination +/- screening adjuncts
  - Normal Examination
  - Abnormal Examination
    - Record/Photograph

STEP 5
- No need to refer
  - Referable
    - Non-Suspicious Finding
    - Suspicions for Malignancy or Potentially Malignant Lesion
      - Lower Suspicion
      - Higher Suspicion
      - Highest Suspicion
        - Biopsy by Non-Expert +/- adjunctive techniques
          - SCCA Dysplasia
          - Record

STEP 6
- Referral to Expert Diagnosis &/or Management
  - Record

[Diagram showing flowchart with various decision points and outcomes, including referrals, examinations, and diagnostic steps.]
The challenge isn’t detecting advanced cancer, but the risk assessment of lesions (or patients) with malignant potential.

Secondary Prevention
REVIEW ARTICLE

Nomenclature and classification of potentially malignant disorders of the oral mucosa

S. Warnakulasuriya¹, Newell. W. Johnson², I. van der Waal³

“OPMDs”
OPMDs
Clinical Diagnostic Spectrum:

Leukoplakia
Speckled leukoplakia
Leukoerythroplakia
Erythroleukoplakia
Speckled erythroplakia
Granular erythroplakia
Erythroplakia
+/- Ulceration, +/- Exophytic
How well does the conventional oral examination (COE) perform?

1. Can it determine PMDs based on clinical features only?

2. Can it differentiate between benign vs dysplasia vs carcinoma?

3. If not already a cancer, can it predict the likelihood for malignant transformation (MT)?

4. Who is the examiner? (novice vs expert)
   - Baseline vs surveillance setting?
1. COE by “frontline” clinicians

TP: True Positive
FP: False Positive
FN: False Negative
TN: True Negative

Sensitivity (Se): TP/TP+FN
Specificity (Sp): TN/TN+FP
PPV: TP/TP+FP
NPV:TN/TN+FN
FN rate=1-sensitivity
FP rate=1-specificity

Higher prevalence >10%: high Se, lower Sp
Lower prevalence <10%: lower Se, high Sp

In general population COE good for detecting “absence” of disease

Walsh T et al Cochrane Collaboration 2013
3. COE and Predictors of MT

REVIEW ARTICLE

Natural history of potentially malignant oral lesions and conditions: an overview of the literature

Séamus S. Napier¹, Paul M. Speight²

¹Department of Tissue Pathology, Institute of Pathology, Royal Group of Hospitals, Belfast, Northern Ireland; ²Department of Oral Pathology, School of Clinical Dentistry, University of Sheffield, Claremont Crescent, Sheffield, UK

Lesion site: tongue/fom/retromolar trigone> buccal mucosa/palate/gingivae
Lesion appearance: non-homogeneous> homogeneous
Lesion size: large (>1 i/o subsite)> small
Multifocal lesions

Natural history of PMDs?

Severity? Progression vs Regression?, Indolent vs Aggressive?

Figure 1 Oral lesion heterogeneity. Oral mucosal lesions, whether histologically normal or atypical, do not follow a linear progression pattern. Rather, a small majority of dysplastic lesions will progress to cancer, while the majority will either remain quiescent or even regress. Similarly, lesions lacking histologic atypia may represent reactive and molecularly benign lesions or they may be molecularly premalignant.

Lingen et al 2011 (Oral Diseases)
Standard Diagnostic Algorithm
Histopathologic Diagnoses

- Epithelial hyperkeratosis/hyperplasia
- Mild dysplasia
- Verrucous hyperplasia
- Moderate dysplasia
- Severe dysplasia
- Carcinoma-in-situ
- Carcinoma (squamous cell/verrucous)
Diagnostic Adjunct

A technique applied to an identified lesion which aides in the characterization of the lesion to better identify high-risk lesions and/or select appropriate regions for further evaluation; its application should accelerate the pathway to a definitive diagnosis, improve diagnostic accuracy and reduce false negative rates due to sampling error. Can it also predict the future nature of the lesion?
Settings where adjuncts might be performed

Home

Community

Primary Care:
- DDS/DMD/RDH
- MD/NP/RN/PA

Secondary Care
- OM/OP/OS
- ENT/HNS/RO

Novice

Expert
Mobile Phones
Light-based adjuncts
Vital stains
Cytopathologic adjuncts
Salivary Diagnostics?

POC TEST

OncAlert
Rapid Point-of-Care Assessment Test
Questions to consider about adjuncts

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is it available for use?</td>
<td>Resource rich vs poor settings?</td>
</tr>
<tr>
<td>How easy is it to use chairside?</td>
<td>Patient vs Clinician?</td>
</tr>
<tr>
<td>How quickly do you get results?</td>
<td>POC vs Lab?</td>
</tr>
<tr>
<td>What’s the cost and who pays?</td>
<td>Health care system?</td>
</tr>
<tr>
<td>Can it help lesion/patient risk stratification?</td>
<td></td>
</tr>
<tr>
<td>Can it help predict malignant transformation?</td>
<td></td>
</tr>
<tr>
<td>Can it help in patient referral/management?</td>
<td></td>
</tr>
<tr>
<td>What’s the evidence base?</td>
<td>Se/Sp/PPV/NPV/AUC?</td>
</tr>
</tbody>
</table>
How do we rate these adjuncts?
The Ideal Diagnostic Adjunct

- Widely available
- Can be easily and consistently used by non-experts
- High patient acceptance
- Immediate results
- Inexpensive/covered by insurance vs healthcare
- Helps separate high vs low risk lesions/patients
- Helps move patients into higher care settings
- High accuracy (sensitivity/specificity)
What’s the evidence?

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...and from which clinical domains?

**Community:**
- Community healthcare worker

**Primary Care:**
- Dentist
- Dental hygienist
- Primary care physician
- Nurse practitioner
- Physician assistant
- Nurse

**Secondary Care:**
- Oral Medicine Expert
- Oral Pathologist
- Oral Surgeon
- Otolaryngologist

**Tertiary Care:**
- Head and Neck Surgeon
- Radiation Oncologist
- Medical Oncologist
- Oncology NP/PA/RN
Light-based adjuncts
Precancer and cancer alter the optical properties of the epithelium and stroma.

**Epithelium**
- Fluorophores: Keratin, FAD, NADH
- Alterations in metabolic activity, cellular density and morphology, and epithelial thickness

**Stroma**
- Fluorophores & absorbers: Collagen, hemoglobin
- Degradation of collagen matrix; angiogenesis; inflammation

Pavlova et al, Clin Cancer Res 2008;14:2396-2404
Optical imaging technologies / visualization adjuncts

<table>
<thead>
<tr>
<th>Macroscopic: Large Field of View</th>
<th>Point Probes: Limited Field of View</th>
</tr>
</thead>
<tbody>
<tr>
<td>Practiced/Designed/ Implemented in such a way to be Sensitive to tissue at risk of malignant transformation vs some Specificity performance loss</td>
<td>Practiced/Designed/ Implemented to be Specific to localize and improve the user’s confidence that the tissue is likely to be at risk of malignant transformation. Always used with some form of Macroscopic imaging</td>
</tr>
</tbody>
</table>

- Standard visual/tactile
- High Resolution Digital handheld camera
- Vizilite, Microlux, Orascoptic DK
- Toluidine Blue, Lugol's Iodine
- VELscope, Identafi, Oral ID

- Confocal microscopy
- High resolution microendoscopy
- Optical Coherence Tomography
- Spectroscopy
  - Fluorescence
  - Reflectance
  - Raman
  - Time resolved
Direct viewing enhanced by illumination and/or acetic acid

Vizilite, Microlux, Orascoptic DK

- Chemiluminescent or LED illumination
- “Acetowhitening” – premise: sites with epithelial proliferation preferentially reflect blue-white light
- Generally described as having high sensitivity, low specificity
NBI (Narrow Band Imaging)

During endoscopic observation, NBI enhances visualization of the capillary network and mucosal morphology.

NBI is an optical imaging technology that enhances the visibility of vessels and other structures on or near the mucosal surface. The gastrointestinal tract is mainly composed of blood vessels and mucosa; narrow band imaging, which is strongly absorbed by hemoglobin and penetrates only the surface of tissues, is ideal for enhancing the contrast between the two. As a result, under narrow band imaging, capillaries on the mucosal surface are displayed in brown and veins in the submucosa are displayed in cyan on the monitor.

Capillaries in the mucosal tissue
Veins beneath the surface mucosal tissue

* Allows NBI examination when used with a video system that is NBI compatible.
192 lesions from 170 patients

17 visible white light lesion no history of dysplasia at site (non dysplastic1)
17 visible white light lesion history of dysplasia at site - increased risk (non dysplastic2)
66 Low grade dysplasia – moderate risk of transformation into Cancer
52 High Grade (historically in BC 56% of these progress to cancer with in 3 years and 70% in 5 years and in BC all are treated - very high risk)
40 Squamous cell Carcinoma (SCC)
FV Guided Surgery
BC Study 246 Patients

Eligible patients (N=246)

SCC (N=156)
- T1=104
- T2=52

HGL (N=90)
- D3=41
- CIS=49

FV-guided (N=92 59%)
- Control (N=68 41%)

FV-guided (N=62 69%)
- Control (N=28 31%)

the global oral cancer forum

Henry Schein Cares
Helping Health Happen.
FV Appears to Reduce Recurrence, etc

Oral Cancer Extent:
At Risk Tissue Evaluation

Standard Visual/Tactile

Toluidine Blue

Fluorescence Visualization
Surveillance using autofluorescence

Day 1
Right Lateral Tongue: Abnormal Low Risk

Day 121 (Prior to Biopsy)
Right Lateral Tongue: Leukoplakia, Abnormal Low Risk

Day 196
Right Lateral Tongue: Irritation, Abnormal Low Risk

Day 294
Right Lateral Tongue: Leukoplakia, Abnormal Low Risk

Day 379
Right Lateral Tongue: Leukoplakia, Abnormal Low Risk

Day 477
Right Lateral Tongue: Inflammatory, Abnormal Low Risk

Day 611 (Prior to Biopsy)
Right Lateral Tongue: Abnormal High Risk

Day 121
Histopathology: Mild dysplasia

Day 387
Histopathology: Mild dysplasia

Day 611
Histopathology: Moderate dysplasia
Some “confounders” for autofluorescence

- **Hematoma**
  - FVL false +

- **Candidiasis**
  - FVL false +
**In vivo** Optical Coherence Tomography (OCT) Imaging

0.9mm OD probe

inserted into

1.5mm OD closed-ended sheath with optional holder

pullbacks < 50 mm

pullbacks < 35 mm
OCT: Rotary pullback image presentation

Rotation (<100Hz)
Pullback (<15mm/s)

OCT is like ultrasound but using light, not sound
High Resolution 10-20 um
Largish imaging swaths
2-3mm by 5cm

1mm
33 mm
OCT Normal vs. lesion: lateral tongue

Contralateral normal

Lesion

33 mm
OCT Pre-surgery: lateral tongue

modified dental mirror probe holder
OCT-AFI
Combining Co-registered OCT with Auto-Fluorescence Imaging (AFI) within same probe

AFI uses similar illumination wavelengths as Autofluorescence Visualization

Apparent loss of Intact Basement membrane

Drop in Autofluorescence
Point probe - spectroscopy

UV-visible fluorescence spectroscopy

In vivo clinical study, 115 subjects

Normal & Mild Dysp (avg)

Mod/Severe Dysp & Cancer (avg)

Schwarz et al, Cancer 2009;115:1669-1679

Raman spectroscopy

Schwarz et al, Cancer 2009;115:1669-1679

Cals et al, Lab Invest 2015;95:1186-1196
## Point probe – confocal in vivo microscopy

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</tr>
</thead>
<tbody>
<tr>
<td><strong>Mode</strong></td>
<td>Reflectance</td>
<td>Reflectance</td>
<td>Fluorescence</td>
<td>Fluorescence</td>
<td>Fluorescence</td>
<td>Fluorescence</td>
</tr>
<tr>
<td><strong>Type</strong></td>
<td>Fibered rigid probe</td>
<td>Not fibered</td>
<td>Rigid probe</td>
<td>Rigid probe</td>
<td>Fibered flexible probe</td>
<td>Fibered probe integrated into a colonoscope</td>
</tr>
<tr>
<td><strong>Excitation wavelength</strong></td>
<td>1064 nm</td>
<td>830 nm</td>
<td>488 nm</td>
<td>488 nm</td>
<td>660 nm</td>
<td>488 nm</td>
</tr>
<tr>
<td><strong>Penetration depth</strong></td>
<td>NS[a]</td>
<td>0–450 μm</td>
<td>0–300 μm</td>
<td>0–250 μm</td>
<td>60 μm</td>
<td>0–250 μm</td>
</tr>
<tr>
<td><strong>Frame rate</strong></td>
<td>15 frames/s NS</td>
<td>6 frames/s for 1000 × 1000 pixels</td>
<td>1–2 frames/s for 512 × 512 pixels</td>
<td>0.8 images/s for 1024 × 1024 pixels</td>
<td>8–12 images/s for 500 × 400 pixels</td>
<td>0.8 frame/s for 1204 × 1204 pixels 1.6 frame/s</td>
</tr>
<tr>
<td><strong>Field of view</strong></td>
<td>230 × 230 μm</td>
<td>500 × 500 μm</td>
<td>390 × 390 μm</td>
<td>475 × 475 μm</td>
<td>240 × 200 μm and mosaicing</td>
<td>475 × 475 μm</td>
</tr>
<tr>
<td><strong>Lateral resolution</strong></td>
<td>2 μm</td>
<td>0.5–1 μm</td>
<td>2 μm</td>
<td>0.7 μm</td>
<td>1.4 μm</td>
<td>0.7 μm</td>
</tr>
<tr>
<td><strong>Axial resolution</strong></td>
<td>&lt;10 μm</td>
<td>5 μm</td>
<td>6 μm</td>
<td>7 μm</td>
<td>10 μm</td>
<td>7 μm</td>
</tr>
<tr>
<td><strong>Distal tip diameter</strong></td>
<td>10 mm</td>
<td>NS</td>
<td>8 mm</td>
<td>6.3 mm</td>
<td>2.6 mm</td>
<td>12.8 mm</td>
</tr>
</tbody>
</table>

[a] Not specified.

Table 1 from: Abbaci et al, Oral Oncol 2014
Point probe – low cost in vivo microscopy

High resolution microendoscope (HRME)

Research device – cost of goods $2500

Automated real-time processing (6 sec)

Normal

Abnormal
Benign lesions – improving specificity

- Pemphigoid
- Lichen Planus
Evidence-based Approaches

Large Field of View
• Standard visual/tactile
• Vizilite, Microlux, Orascoptic DK
• VELscope, Identafi, Oral ID
• Demographics
• Risk factors
• Clinical Knowledge-Patient History

Limited Field of View
Localization of most at risk tissue
Biopsy Site Selection
When to Biopsy

• High resolution microendoscopy/ Confocal
• Optical Coherence Tomography
• Spectroscopy
  - Fluorescence/Reflectance/Raman

Research Community
Oral Cancer/Intra Epithelial Neoplasia
Risk Assessment/ Risk Indicators

DENTIST

OM/ENT

PATHOLOGY

COMMUNITY

REGIONAL HOSPITAL CLINICS

INT RISK

HIGH RISK

CANCER CENTRES

Molecular Based
(LOH, DNA based, Expression, IHC, etc)
Quantitative Pathology

Next Gen Sequencing
DNA, coding RNA
Non coding RNA
Proteomics
Roles of Light Based Adjuncts

1. Discovery (Screening)
   • General Practitioners

2. High Risk Tissue Localization
   • General Practitioners / Oral Specialists

3. Biopsy Guidance
   • General Practitioners/Oral Specialists/Oral Surgeons

4. Surgical Margin Delineation
   • ENT, Head & Neck Surgeons

5. Surveillance and Monitoring
   • GP’s & Specialists
In a low-resource setting:

- **Challenges due to limited health infrastructure**
  - Lab/diagnostic facilities limited
  - Treatment options limited
  - Transportation may be difficult
  - Patient may be seen only once

- **Roles of light based adjuncts:**
  - Identify high risk lesions
  - Enable best use of diagnostic resources available
  - Enable “see & treat” strategies
High-resolution microendoscopy system incorporated into a van for mobile cervical cancer detection in rural Brazil (similar setup for oral cancer detection in progress)

Barretos Cancer Hospital
M.D. Anderson Cancer Center
Rice University
Inaugural trip
November 2015:
4370 km (2700 miles)

2\textsuperscript{nd} trip January 2016

3\textsuperscript{rd} trip February 2016
Some Important things to remember...

• Combine the information from what you see with optical adjuncts with your white light visual & tactile exam.

• A comprehensive patient history can be the key to helping you understand what you see with any adjunct device/methodology other tool for that matter.

• Review some of the many resources available on the basics of oral cancer – knowing where it is most likely to occur, for example, can be very helpful in deciding how to follow up on a patient
Vital Staining: Historical Perspective

In Vivo Staining Test for Delineation of Oral Intraepithelial Neoplastic Change: Preliminary Report

Harold H. Niebel,* D.D.S., and Bernhard Chomet,† M.D., Chicago

JADA 1964

A report on the diagnostic and therapeutic potential of a simple in vivo procedure—the toluidine blue staining test—to delineate intraepithelial neoplasms of the oral mucosa. This test may become another valuable tool in the diagnosis and treatment of oral cancer.

Blue Man Group 1991
- Metachromatic vital stain
- Binds to free anionic groups (sulphate, phosphate, carboxylate radicals of large molecules eg nucleic acids)
- Tolonium chloride is one dye in TB
Vital staining accuracy: 14 studies

Meta-analysis:
Sensitivity of 0.84 (95% CI 0.74 to 0.90)
Specificity of 0.70 (95% CI 0.59 to 0.79)
Toluidine Blue

- Widely available/inexpensive
- Painless, albeit a little messy
- Immediate results
- Generally not covered by insurance
- Principally used by experts (eg surveillance setting)
- Helps separate high vs low risk lesions/patients
- High accuracy in SCCA/high grade dysplasia
- PPV increases with prevalence of OPMDs
- Helps with biopsy site selection
- May be predictive for malignant transformation
Ulcerative lichenoid mucositis

TB+ (ulcer stains) false +

Filiform papillae (arrow) TB+
Case No.2115419 (38yrs, male)

Squamous cell ca

2% lugol app

PGA sheet + fibrin glue app

Slide Courtesy Dr. Toru Nagao, Japan
Camile
Cytopathology

• Commercially available in resource-rich countries
• Painless
• Mostly lab assays
• Range of cost, mostly covered by health insurance
• Malignant transformation data lacking
• More potential with other adjuncts?
Brush Biopsy Cytology

- To overcome the innate limitations of conventional exfoliative cytology, pathologists have striven to improve cytology associated techniques and instrumentation.
- Oral cytobrush was modified from the cytobrush used in obstetrics and in most comparisons has proven advantageous over the wooden/metallic spatula and even comparable with incisional biopsy.
- Although brush biopsy has the advantage of collecting cells from a wide area and all three layers of epithelium by accessing the basement membrane (Divani, 2009), potential disadvantages of this technique include difficulty detaching cells in ulcerated and necrotic surfaces and visualising cells out of context.
- Similar to other techniques, the efficacy of brush biopsy is highly dependent upon adequate training of the operator.
Brush Biopsy Cytology

Different types of sampling devices:

- Cervex brush
- Cytobrush
- Plastic spatula
- Wooden spatulae
• OralCDx consists of a highly specialized, computer assisted analysis of an oral brush biopsy.

• It is used to confirm the nature of mucosal lesions while identifying pre-cancerous and cancerous lesions that are not clinically suspected.
What is the accuracy of OralCDx?

- A multi-centre US study using 1000 patients detected approximately 100% of histologically confirmed oral pre-cancers and cancers.

- In 945 patients, OralCDx® independently detected every case of histologically confirmed oral dysplasia and carcinoma with a sensitivity of 100%.

- The specificity for the OralCDx® "positive" result was 100%, while the specificity for the OralCDx® "atypical" results was 92.9%.

- OralCDx® uncovered pre-cancer and cancer among 4.5% of clinically benign-looking lesions that would not have received additional testing, other than clinical follow-up.
What is the accuracy of OralCDx?

- The Berlin Group presented data on 103 brush biopsy cases. The sensitivity and specificity of OralCDx® to detect dysplasia and OSCC was 92.3% and 94.3% respectively.
- The positive and negative likelihood ratios were 16.2 (95% CI: 6.2-42.1) and 0.08 (95% CI: 0.02-0.31), supporting the findings of the Sciubba study and the use of OralCDx® as a screening tool for oral mucosal lesions (Scheifele et al 2004).
- Mehrotra et al. assessed 85 consecutive patients presenting with an oral lesion deemed to be minimally suspicious by clinical examination, and undertook OralCDx® brush biopsy followed immediately by a matched scalpel biopsy at the same location.
- The sensitivity was 96.3%, while the specificity of a “positive” brush result was 100% and that of an “atypical” result 90.4%. The PPV and NPV were 84 and 98% respectively (Mehrotra, 2011).
What is the accuracy of OralCDx?

• The Eastman Group (London), presented data on 112 brush biopsy cases (Poate et al 2004). Poate et al. found that the sensitivity and specificity of OralCDx® for the detection of OED or OSCC was 71.4% and 32% respectively, while the PPV of an abnormal brush biopsy result (positive or atypical) was 44.1%, and the NPV was 60%.

• Seijas-Naya and colleagues reported a sensitivity, specificity, PPV and NPV of 72.7%, 92.3%, 88.8%, 80% respectively for OralCDx®.

• The sensitivity and specificity of all subsequent studies did not reach what was originally stated by the US multi-centre study (Sciubba et al., 1999).
Liquid based cytology

To improve the characteristics of conventional exfoliative cytology through reducing cell necrosis, blood contamination and inflammation, liquid based cytology (LBC) was developed. In this technique, after collection, cells are transferred into a vial containing conservative liquids to ensure immediate fixation, even distribution and a significantly higher number of collected cells. Despite its extensive use in detecting cervical cancer, LBC is not commonly used in the oral health setting.
Liquid based cytology
Adequate samples were more likely to be obtained with a curette (90.6%) or OralCDx® (80.0%) than a Cytobrush® (48.6%); P < 0.001. Similarly, the RNA quantification was higher with a curette, or OralCDx® compared with the Cytobrush® (Reboiras-Lopez, 2012).

There were statistically significant differences between the Cytobrush® and curette (P = 0.008) and between the Cytobrush® and OralCDx® (P = 0.034).

Although material was obtained with all three instruments, adequate samples were more likely to be obtained with the curette or OralCDx® than with the Cytobrush®.

Furthermore, they reported that the OralCDx® brush was a less aggressive instrument than the curette, so could be a useful tool in a clinical setting particularly as molecular biomarkers found their way into clinical practice (Reboiras-Lopez, 2012).
Liquid based cytology

- Navone et al. (Navone, 2007) used LBC and conventional cytology to study 473 patients referred for presence of OSCC or OPML.
- All patients, after sampling for cytology, received surgical biopsy and histological examination.
- Eighty-nine of the 473 samples were processed using conventional cytology and 384 by LBC (ThinPrep®).
- 12.4% of cases were inadequate in conventional oral cytology compared with 8.8% in LBC.
- The sensitivity, specificity, positive and negative predictive values were 85.7, 95.9, 95.4 and 87.0%, respectively, for conventional samples, versus 95.1, 99.0, 96.3 and 98.7% for LBC.
- This has been supported by Delavarian et al. (Delavarian, 2010) who assessed 25 patients and obtained values of 88.8, 100, 100 and 80%, respectively.
DNA Cytometry

- DNA cellular content (ploidy) has been reported to be a reliable marker for both malignant and premalignant lesions.
- DNA ploidy has been studied by both flow and image cytometry.
- Marsico et al. (Marsico, 2008) combined LBC and flow cytometry to examine 211 OPMLs, compared to a conventional histopathological diagnosis on scalpel biopsy.
- Flow cytometry demonstrated aneuploidy in 54.8% of OSCCs, 50% of OPMLs with dysplasia, and in 15.1% of OPMLs without dysplasia.
DNA Cytometry

- McCullough and Farah took advantage of the positive characteristics assessed DNA content using a Cytobrush®, LBC (ThinPrep) and virtual microscopy with Feulgen stain for the early detection of OED and neoplasia in oral mucosal lesions compared to incisional biopsy and histopathological assessment. (McCullough, 2009)
- In 17 OPMD/OSCC they were unable to observe variation between abnormal samples compared to a database of 100 normal controls.
- They concluded that DNA content assessment of oral cytology utilising Cytobrush®, LBC and virtual microscopy was not useful as an adjunctive prognostic tool in the analysis of the malignant potential of oral mucosal lesions.
Kämmerer and colleagues assessed the ability of DNA image cytometry (DNA-ICM) to enhance the morphological interpretation of pure oral brush biopsies (Kammerer, 2013).

They found that DNA-ICM has the potential to substantially improve the sensitivity of a pure morphological interpretation of oral brush biopsies (from 55% to 76%), while the combination of DNA-ICM and brush biopsy had a specificity of 100% similar to that of brush biopsy or DNA-ICM alone for the detection of dysplasia.
DNA Cytometry

• In an effort to increase cell number, Navone et al. (Navone, 2008) used a dermatological curette approach (scraping/micro-biopsy) and LBC in a prospective study of 164 patients with OPMLs, to detect the presence of dysplasia/carcinoma.

• Micro-biopsy diagnosis was in agreement with scalpel biopsy in 91.14% cases and showed a better sensitivity than scalpel biopsy (97.65% vs. 85.88%), corresponding to two of 158 false-negative cases by micro-biopsy vs. 12 of 158 by scalpel biopsy.
DNA Cytometry

- Given the disparate results surrounding the use of OralCDx® oral brush biopsy system with computer assisted neural network analysis and DNA-ICM in diagnosing oral cancer and precancerous conditions, a recent meta-analysis was conducted to compare the accuracy of the two systems in diagnosing both conditions.
- Thirteen studies (eight of OralCDx® brush biopsy and five of DNA-ICM) were identified as having reported on 1981 oral mucosal lesions.
- The meta-analysis found that the area under the summary receiver operating characteristic curves of the OralCDx® brush biopsy and DNA-ICM were 0.8879 and 0.9885, respectively.
- The pooled sensitivity, specificity, and diagnostic odds ratio of the OralCDx® brush biopsy were 86%, 81%, and 20.36, respectively, while these were 89%, 99%, and 446.08 for DNA-image cytometry, respectively.
- Results of a pairwise comparison between each modality demonstrated that specificity, area under the curve, and $Q^*$ index of DNA-ICM was significantly higher than that of the OralCDx® brush biopsy, but no significant difference in sensitivity was found.
- The authors concluded that based on available published studies, DNA-ICM was more accurate than OralCDx® brush biopsy in diagnosing oral cancer and precancerous mucosal lesions (Ye, 2015).
Commercially Available DNA-ICM

- PMI Labs - OralAdvance™ (Perceptronix)
- Cost $150 CAD
- Report in 2-3 working days
- Study published in 2012 (The use of quantitative cytology in identifying high-risk oral lesions in community practice. Samson P. Ng, Indervir S. Mann, Christopher Zed, Alexei Doudkine, Jasenka Matisic)
- Sensitivity and specificity of OralAdvance™ in the detection of high-risk potentially malignant disorders and squamous cell carcinoma were found to be 87% and 97% respectively.
- Offered through Second Step Laboratories with VELscope (LED Dental) users in Canada. (Cytology kit $15 CAD)
- As of January 1st 2015, PMI no longer provides laboratory services
### Questions to consider about Cytology

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Is it available for use?</td>
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<td>How easy is it to use chairside?</td>
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<td>How quickly do you get results?</td>
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<td>What’s the cost and who pays?</td>
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<tr>
<td>Can it help lesion/patient risk stratification?</td>
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<td>Can it help predict malignant transformation?</td>
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<tr>
<td>Can it help in patient referral/management?</td>
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<tr>
<td>What’s the evidence base?</td>
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<tr>
<td></td>
<td>Spatula</td>
<td>Cytobrush</td>
<td>Curette</td>
<td>OralCDx LBC</td>
<td>Cytobrush + LBC</td>
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<tr>
<td>--------------------------------</td>
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<tr>
<td>Is it available for use? Resource rich vs poor settings?</td>
<td>Both</td>
<td>Both</td>
<td>Both</td>
<td>RR</td>
<td>RR</td>
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<td>How easy is it to use chairside? Patient vs Clinician?</td>
<td>Easy</td>
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<td>How quickly do you get results? POC vs Lab?</td>
<td>Quick</td>
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<td>Quick</td>
<td>Slow</td>
<td>Quick</td>
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<td>What’s the cost and who pays? Health care system?</td>
<td>Low</td>
<td>Low</td>
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<td>High</td>
<td>Low</td>
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<td>Can it help lesion/patient risk stratification?</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Can it help predict malignant transformation?</td>
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<td>Yes</td>
<td>Yes</td>
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<tr>
<td>What’s the evidence base? Se/Sp/PPV/NPV/AUC?</td>
<td>Poor</td>
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<td>Good</td>
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<td>How accurate compared to gold standard?</td>
<td>Poor</td>
<td>Decent</td>
<td>Good</td>
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Limitations - Cytopathology

• Lack of accurate universally agreed oral cytology classification system to facilitate research and clinical reporting.
• Site selection limitations could potentially benefit from coupling with other diagnostics such as TBlue or visual optical imaging adjuncts.
• Lack of evidence to predict malignant transformation of OPML to oral cancer.
• Lack of uptake of cytology may be circumvented by development of better oral biomarkers (diagnostic/prognostic).
Next steps - Cytopathology

- LBC not fully explored in oral context compared to cervix. Potential not explored fully.
- Curette with LBC, has potential in resource poor settings.
- LBC with biomarkers should be explored further.
- Need multicentre, longitudinal studies to assess utility in determining malignant transformation of OPML.
Point of Care Platform: “Cytology on a Chip”

Morphometericcs (cytoplasmic & nuclear) + Biomarkers (intracellular and cell surface)

McDevitt J et al (submitted paper)
Salivary Diagnostics

- Commercially available in resource-rich countries
- Painless
- Point of care/lab assay
- Expensive/covered by medical insurance?
- Data from SCCA populations only
Screening or diagnostic adjunct?

• Can saliva be used to **screen** a population and identify “at risk” patients?

• Can saliva be used as a **diagnostic adjunct** to determine the significance of an oral lesion (or lesions) that has (or have) been detected by a frontline examiner?
# Original Article

## Salivary Protein and solCD44 Levels as a Potential Screening Tool for Early Detection of Head and Neck Squamous Cell Carcinoma

Elizabeth J. Franzmann, MD,¹,² Erika P. Reategui, MS,³ Lutecia H. Mateus Pereira, PhD,¹ Felipe Pedrosa, MD,⁴ Debbie Joseph, MD,⁵ Glenn O. Allen, MPH,¹ Kara Hamilton, MPH,⁶ Isildinha Reis, DrPH,⁷,⁸ Robert Duncan, PhD,⁷ W. Jarrard Goodwin, MD,¹,² Jennifer J. Hu, PhD,¹,⁷ Vinata B. Lokeshwar, PhD¹,⁹

<table>
<thead>
<tr>
<th>Predicted probability cut point</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Accuracy %</th>
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<tbody>
<tr>
<td>Best accuracy</td>
<td>0.47</td>
<td></td>
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<tr>
<td>Better sensitivity</td>
<td>0.43</td>
<td>60.3</td>
<td>72.6</td>
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<tr>
<td>Better specificity</td>
<td>0.58</td>
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</table>

AUC = 79.6

**Opportunistic Screening**

Marketed by Vigilant Biosciences

Not available in US

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*Head and Neck 2012*
Evaluation of Salivary Transcriptome Markers for the Early Detection of Oral Squamous Cell Cancer in a Prospective Blinded Trial

Jack L. Martin, MD; Neil Gottehrer, DDS; Harvey Zalesin, DDS; Paul T. Hoff, MD; Michael Shaw, PhD; James H.W. Clarkson, MD; Pam Haan, BSN; Mark Vartanian; Terry McLeod, BSN; and Stephen M. Swanick

Predictive Model Generated from Intended-Use Population Data: Intercepts, Marker Coefficients, AIC, and ROC AUC

<table>
<thead>
<tr>
<th>POPULATION</th>
<th>CANCERS/ NON-CANCERS</th>
<th>INTERCEPT (P VALUE)</th>
<th>COEFFICIENT DUSP1 (P VALUE)</th>
<th>COEFFICIENT OAZ1 (P VALUE)</th>
<th>COEFFICIENT SAT (P VALUE)</th>
<th>AIC</th>
<th>ROC AUC (SE)</th>
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<tr>
<td>Invasive cancer only</td>
<td>24/144</td>
<td>-2.7706 (0.001)</td>
<td>-1.0384 (0.043)</td>
<td>+0.6828 (0.149)</td>
<td>-0.9319 (0.020)</td>
<td>106.415</td>
<td>0.856 (0.0438)</td>
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AIC - Akaike's Information Criteria; ROC AUC - area under receiver operating characteristic curve; SE = standard error

Diagnostic adjunct

Se >90%, Sp 60%

Marketed by PeriRx

Compendium 2015
Telemedicine in oral cancer surveillance

M. Abraham Kuriakose MD, FRCS
Mazumdar-Shaw Cancer Center, Bangalore, India
Gaps in PoC diagnostics

1. Primary care: Screening
   - Real-time results: More effective therapy at PoP
   - Networked results: Improved surveillance

2. Referral center: Diagnosis
   - Real-time results combined with treatment at PoC
   - Networked results: Epidemiological monitoring

3. Referral center: Treatment
   - Delay in diagnosis

4. Population-wide issue
   - Delay in seeking treatment
   - Delay/ inadequate treatment and follow up

Boppart SA. Sc. Transl Med 2014
Oral cancer prevalence

Countries with high incidence and mortality of oral cancer

Warnakulasurya Oral Oncology 2009
for oral cancer point-of-care diagnostics to have a global impact it must reach “at risk” population
Telemedicine in oral cancer diagnosis

At risk population
Primary health providers
Diagnostic methods
1. Visual examination
2. Light-based adjuncts
3. Vital stains
4. Cytopathology

PoC diagnostics with Telemedicine
Image analysis
Images
Digitized
Specialists
Remote interpretation
SOMNET (www.somweb.se)
Mobile phones per capita

\[ y = 135.68 \exp(0.0019x) \]
\[ R^2 = 0.4243 \]

Mobile phones/1000 population
Mobile phone for oral cancer screening

**METHODS**

The project was initiated by mobile health (mHealth) by the institutional health difference Centre, tertiary-based surveillance.

**KEY**

This paper is an account of the implementation of a mobile phone-based screening program for oral cancer.

**CONTRIBUTIONS**

The study was designed, coordinated, and executed by the authors.

**ABBREVIATION**

JADA: Journal of the American Dental Association

**ORIGIN**

The findings presented in this paper are original and have not been published elsewhere.

**ABSTRACT**

This study aimed to evaluate the feasibility and acceptability of a mobile phone-based screening program for oral cancer. The study was conducted in two phases: a targeted cohort (Frontline Health Care Workers) and an opportunistic cohort (Dental Surgeons). The targeted cohort was made up of trained frontline health care workers who conducted oral cancer screening using mobile phones. The opportunistic cohort consisted of dental surgeons who screened patients in their clinical settings.

**RESULTS**

The mobile phone-based screening program was well-received by both the targeted and opportunistic cohorts. The targeted cohort reported a high level of satisfaction with the mobile phone screening tool, while the opportunistic cohort found it to be a useful addition to their routine clinical practice.

**DISCUSSION**

The mobile phone-based screening program for oral cancer has the potential to improve access to early detection and treatment of oral cancer.

**CONCLUSION**

The study highlights the feasibility and acceptability of using mobile phones for oral cancer screening. Further research is needed to evaluate the effectiveness of this approach in reducing oral cancer mortality.
m-Health in oral cancer - Oncogrid

Server

Sana
Open-Source Mobile Health Platform

OpenMRS
Open-Source Medical Record Program

Oral Cancer Specialist at the Cancer center

Community Health Workers

Birur P., JADA 2015
<table>
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<th>Closed</th>
<th>ID</th>
<th>Name</th>
<th>Age</th>
<th>Gender</th>
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</tbody>
</table>
Study population

Opportunistic screening by dental professionals
N = 1440

Subjects - high risk group with lesion
N = 106

Subjects not involved in mobile phone screening
N = 0

Subjects involved in mobile phone screening
N = 106

Non interpretable images
N = 0

Interpretable images
N = 106

Lesions positive on remote diagnosis
N = 106

Lesions negative on remote diagnosis
N = 0

Targeted screening by frontline health workers
N = 2000

Subjects – high risk group with lesion
N = 130

Subjects not involved in mobile phone screening
N = 47

Subjects involved in mobile phone screening
N = 83

Non interpretable images
N = 32

Interpretable images
N = 51

Lesions positive on remote diagnosis
N = 23

Lesions negative on remote diagnosis
N = 28

Birur P., JADA, in press
Cellscope

- Feasibility of tele-microscopy for oral cancer screening
Work Flow

Brush biopsy

Manual Liquid cytology

Tele-microscopy

sensitivity & specificity
Health Worker- Cellscope
Pathologist - Remote diagnosis
Cytology images

- Increased N/C ratio
- Abnormal cell shapes
- Prominent Nucleoli
- Irregular chromatin
- Cluster of malignant cells
- Irregular nuclear membrane
Results

55 subjects

36 Malignancy
  35 SCC
  1 lymphoma

Stage 1,2 =11
Stage 3,4 =24

19 OPML
18 leukoplakia
1 lichen planus

High grade dysplasia =15
Low grade dysplasia =3
Non dysplastic =1

Direct microscopy
Atypia present = 34

Tele-microscopy detected =33

Direct microscopy
Atypia present = 4

Tele-microscopy detected = 3
Results

- **Tele-microscopy**
  - Sensitivity = 91% in malignancy (64% overall)
  - Specificity = 100%
  - Kappa Value=76%

- **Cytology (direct microscopy)**
  - Sensitivity= 97% in malignancy (71% overall)
  - Specificity=100%
  - Kappa Value=78%
Future of telemedicine
Summary

• Telemedicine has the potential to connect at risk population to the specialists
• Improve efficiency of PoC
• WHO ASSURED criteria for PoC (Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment free, Deliverable to end user)
• Long-term goal of changing standard of care