Influence of fluorescence on screening decisions for oral mucosal lesions in community dental practices

Denise M. Laronde, P. M. Williams, T. G. Hislop, Catherine Poh, Samson Ng, Chris Bajdik, Lewei Zhang, Calum MacAulay, Miriam P. Rosin

BACKGROUND: Quality of oral screening examinations is dependent upon the experience of the clinician and can vary widely. Deciding when a patient needs to be referred is a critical and difficult decision for general practice clinicians. A device to aid in this decision would be beneficial.

The objective of this study was to examine the utility of direct fluorescence visualization (FV) by dental practitioners as an aid in decision-making during screening for cancer and other oral lesions.

METHODS: Dentists were trained to use a stepwise protocol for evaluation of the oral mucosa: medical history, head, neck and oral exam, and fluorescent visualization exam. They were asked to use clinical features to categorize lesions as low (LR), intermediate (IR), or high (HR) risk and then to determine FV status of these lesions. Clinicians made the decision of which lesions to reassess in 3 weeks and based on this reassessment, to refer forward.

RESULTS: Of 2404 patients screened over 11 months, 357 initially had lesions with 325 (15%) identified as LR, 16 (4.5%) IR, and 16 (4.5%) HR. Lesions assessed initially as IR and HR had a 2.7-fold increased risk of FV loss persisting to the reassessment appointment versus the LR lesions. The most predictive model for lesion persistence included both FV status and lesion risk assessment.

CONCLUSION: A protocol for screening (assess risk, reassess, and refer) is recommended for the screening of abnormal intraoral lesions. Integrating FV into a process of assessing and reassessing lesions significantly improved this model.

Keywords: early detection of cancer/methods; fluorescence visualization; mouth neoplasms/prevention and control; oral cancer screening; pre-cancerous conditions/diagnosis; referral and consultation

Introduction

The advent of adjunct tools for use as part of the conventional oral examination has been a driving force for change in the screening activity in community practices. Such devices presently include toluidine blue, brush cytology, reflectance visualization and, more recently, autofluorescence imaging. However, validation of these tools has been mainly restricted to high-risk referral clinic settings with use by experienced personnel, with little work carried out in community settings.

In this paper, we examined one such tool, a handheld device used to measure alterations in tissue fluorescence. Loss of tissue autofluorescence has been associated with cancer and pre-malignant lesions at several sites, including the lung, cervix, and oral cavity. Current evidence for utility of this device in identifying dysplastic lesions has found fluorescence visualization (FV) to be sensitive in the detection of high-grade dysplasias in the oral cavity (1), pre-cancerous occult lesions (2) and in enhancing delineation of surgical margins (3, 4); however, these studies have all been carried out in high-risk referral clinic settings with experienced personnel. There are various reports of confounding factors such as inflammation, infection, or highly pigmented areas, which may cause a decrease in the FV signal and hence affect specificity (5–8).

This study evaluates the use of FV by community practitioners as an adjunct to clinical evaluation following a conventional examination. Our goal was to determine whether FV added any value to white light oral cancer screening as introduced in the Guidelines for the Early Detection of Oral Cancer in British Columbia 2008 (9). The intent was to use this information to determine the value of
Narrow band light imaging of oral mucosal in routine dental patients: Assessment of value in detection of mucosal changes

Edmond Truelove DDS, MSD
Professor Department Oral Medicine
University of Washington

The purpose of the study was to evaluate the value of adding narrow band light source (NBLS) imaging (VELscope) of the oral mucosa to the standard oral examination process for detection of any type of mucosal change not visible during routine white light examination. Six hundred and twenty dental subjects presenting for regular dental evaluation or for treatment of acute dental problems were given a standard oral soft tissue examination by dental students supervised by faculty of the clinic. Results of the examination of the oral mucosa were recorded after which the tissues were again examined with NBLS (VELscope) and areas of loss of fluorescence recorded. The nature of the tissue change was classified clinically as a normal variation, inflammatory, traumatic, dysplastic, or other and the patient was classified as normal, needing a follow-up visit, or immediate biopsy. Risk factors related to oral dysplasia were also recorded. Addition of the NBLS assessment added between one and two minutes to the examination process. Of the 620 examinations, an area of loss of fluorescence suggestive of pathology was detected in 69 subjects. After a second immediate evaluation 41 of the 69 were reclassified as not requiring return and 28 were scheduled for follow up or biopsy. Of the 28, 19 were scheduled for return and 5 received immediate biopsy using a 4 mm punch biopsy technique. Of the 19 scheduled for return 15 returned for follow up with 11 areas of loss of fluorescence resolved and 4 undergoing biopsy due to persistence. Of the 9 biopsied lesions 3 were found to represent mild dysplasia and 2 were found to have mild to moderate dysplasia. Two patients received a diagnosis of lichen planus and 2 as inflammatory. Nine of the 28 patients had a history of tobacco use, 5 had very poor oral hygiene, and 4 reported autoimmune disease. None of the lesions had been detected using standard white light examination.

Summary: In this quality improvement study of routine dental patients, adding NBLS imaging to the routine clinical examination resulted in detection of changes not seen with white light examination in a significant number of patients and of these a small but important number were found to have otherwise undetected persistent changes representing inflammatory lesions or potentially dangerous oral dysplasia. Adding this adjunctive diagnostic procedure improved the quality of the examination process and detection of lesions not otherwise visualized.
Efficacy of Optically-guided Surgery in the Management of Early-staged Oral Cancer

This study is currently recruiting participants.
Verified on February 11 by University of British Columbia
First Received on December 22, 2009. Last Updated on February 23, 2011 History of Changes

| Sponsor: | University of British Columbia |
| Collaborators: | Terry Fox Research Institute, British Columbia Cancer Agency |
| Information provided by: | University of British Columbia |
| ClinicalTrials.gov identifier: | NCT01039296 |

Purpose

Oral squamous cell carcinoma (SCC) is a global disease responsible for ∼300,000 new cancer cases each year. Local recurrence (∼30% of cases) and formation of second primary malignancy are common. Cosmetic and/or functional compromise associated with treatment of disease stage is often significant. These statistics underscore the urgent need to develop a better approach in order to control this deadly disease.

It is becoming increasingly apparent that oral cancers develop within wide fields of diseased tissue characterized by genetically altered cells that are widespread across the oral cavity and present in clinically and histologically normal oral mucosa. Complete removal of these lesions is difficult because high-risk changes frequently go beyond clinically-visible tumor. In recognition of this, current best practice is to remove SCC with a significant width (usually 10 mm) of surrounding normal-looking oral mucosa. However, since occult disease varies in size such approach often results in over-cutting (causing severe cosmetic and functional morbidity) or under removal of disease tissue, as evidenced by frequent positive surgical margins and high local and regional recurrence - a failure of the best practice.

There is a wealth of literature that supports the use of tissue autofluorescence in the screening and diagnosis of precancers in the lung, uterine cervix, skin and oral cavity. This approach is already in clinical use in the lung and the mechanism of action of tissue autofluorescence has been well described in the cervix. Changes in fluorescence reflect a complex interplay of alterations to fluorophores in the tissue and structural changes in tissue morphology, each associated with progression of the disease.

As one of the internationally leading teams in applying tissue fluorescence technology, we have shown that direct fluorescence visualization (DFV) tools can identify clinically-visible or occult premalignant and malignant lesions that are associated with lesions at risk, with high-grade histology and high-risk molecular change. In a recently completed, retrospective study, we have shown that DFV helped surgeons in the operating room to determine the extent of the high-risk DFV field surrounding the cancer and resulted in remarkably lower 2-year recurrence rates (0% for DFV-guided vs. 26% for those without DFV-guided approach). There is need to design a larger scale prospective, randomized controlled (Phase III) trial to gather strong evidence in proving the efficacy of the surgery approach using this adjunct tool.

To establish the evidence supporting the change in clinical practice using DFV-guided surgery. There are 3 objectives.

2.1. Objective 1 (Clinical evidence). To assess the effect of DFV-guided surgery on the recurrence-free survival of histologically confirmed disease within the context of a randomized controlled trial (efficacy). Hypothesis: DFV-guided surgery will increase the recurrence-free survival.

2.2. Objective 2 (Quality of Life evidence). To establish the cost per recurrence prevented for this approach and assess quality of life issues. Hypothesis: DFV-guided surgery can be delivered in a cost effective manner and improve the quality of life of patients.

2.3. Objective 3 (Scientific/Molecular evidence). To assess the presence of previously validated molecular markers (microsatellite analysis, LOH) and histological change (quantitative pathology) in surgical margins in a nested case-control study involving a tumor bank created within this project. Hypothesis: DFV-guided surgery will spare normal tissue at the same time improving capture of high-risk tissue.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Intervention</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Cancer</td>
<td>Procedure: Fluorescence visualization device</td>
<td>Phase III</td>
</tr>
<tr>
<td>High-grade Precancer</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Study Type:** Interventional  
**Study Design:**  
- Allocation: Randomized  
- Endpoint Classification: Safety/Efficacy Study  
- Intervention Model: Parallel Assignment  
- Masking: Double Blind (Subject, Investigator)  
- Primary Purpose: Treatment

**Official Title:** Efficacy of Optically-guided Surgery in the Management of Early-staged Oral Cancer

**MedlinePlus related topics:** Cancer Oral Cancer

**U.S. FDA Resources**

**Further study details as provided by University of British Columbia:**

**Primary Outcome Measures:**
- Recurrence-free survival [Time Frame: 5 years] [Designated as safety issue: Yes]

**Secondary Outcome Measures:**
- Histological and molecular evidence of positive margins and quality of life [Time Frame: 5 years] [Designated as safety issue: No]

**Estimated Enrollment:** 200  
**Study Start Date:** February 2011  
**Estimated Study Completion Date:** June 2015  
**Estimated Primary Completion Date:** December 2014 (Final data collection date for primary outcome measure)

<table>
<thead>
<tr>
<th>Arms</th>
<th>Assigned Interventions</th>
</tr>
</thead>
</table>
| A Active Comparator  
  All subjects in this study will receive surgery to treat their oral lesions. The margins (or boundaries) of the tissue to be removed during surgery will be defined by 2 different procedures (or study arms) in the operating room.  
  The Control arm: Surgical boundaries for oral lesions will be defined under regular white light.  
  Intervention: Procedure: Fluorescence visualization device | Procedure: Fluorescence visualization device  
  The trial will randomize 200 patients - 100 in the FV arm (using FV guided the surgery margin) |
| B Experimental  
  All subjects in this study will receive surgery to treat their oral lesions. The margins (or boundaries) of the tissue to be removed during surgery will be defined by 2 different procedures (or study arms) in the operating room.  
  The FV arm (experimental arm): Surgical boundaries for oral lesions will be defined by FV.  
  Intervention: Procedure: Fluorescence visualization device | Procedure: Fluorescence visualization device  
  The trial will randomize 200 patients - 100 in the control arm (using conventional white light approach) |
Detailed Description:
1.1. Objective 1 (Clinical evidence): To assess the effect of FV-guided surgery on the recurrence-free survival of histologically confirmed disease within the context of a randomized controlled trial (RCT).

Hypothesis: FV-guided surgery will increase the recurrence-free survival. Approaches: This Aim requires the establishment of a randomized controlled trial of 200 patients which will compare outcome for patients in 2 arms: one with conventional surgery with margin delineated under white light, and the other using FV guidance for margin delineation. Please see attached Appendix 1 for a step-by-step protocol. This comprises a multidisciplinary team of surgeons, pathologists, project coordinators, and FV specialists. In addition to the presurgery assessment, all participating patients will have 3-month follow-ups for the first 2 years and 6-month for the rest of the study. Biopsy will occur when clinically warranted or at 2-year post-surgery.

1.2. Objective 2 (Quality of Life evidence): To establish the cost per recurrence prevented for this approach and assess quality of life issues.

Hypothesis: FV-guided surgery can be delivered in a cost-effective manner and improve the quality of life of patients.

Approaches: This Aim requires the collection of economic and quality of life (QoL) data to establish the cost per recurrence prevented for FV-guided surgery and to assess quality of life impacts. To assess potential psychosocial consequences of FV-guided surgery we will measure global QoL. We will use the validated EQ-SD and Functional Assessment of Cancer Therapy Head and Neck Module (FACT-HN) to determine the participant’s QoL at each assessment. The questionnaires will be applied at pre-surgery baseline, and at 6-week, 3-month, and 24-month post-surgery follow-ups.

1.3. Objective 3 (Scientific/Molecular evidence): To assess the presence of previously validated molecular markers (microsatellite analysis, LOH) and histological change (quantitative pathology) in surgical margins in a nested case-control study involving a tumor bank created within this project.

Hypothesis: FV-guided surgery will spare normal tissue at the same time improving the capture of high-risk tissue.

Approaches: This Aim requires the retrieval and cutting of the archive material for a nested control study. The estimate number of cases reaches outcome is 30% of FV group (100) + 25% of control group (100). Additionally, 60 matched controls will be selected (matched by gender, age, smoking habit, and anatomical site). This Aim is critical to demonstrate a shift in field, sparing normal tissue while catching high-risk occult tissue. Samples for the nested molecular analysis will be performed in Roslin’s Lab (for microsatellite analysis) and Cancer Imaging at BC Cancer Agency (Dr. MacAskill for qualitative Pathology). The protocols used to analyze these samples have been published.

2.0. STUDY TOOL - VELSCOPE®: We have recently developed a simple hand-held field-of-view device for direct visualization of tissue fluorescence in the oral cavity. This tool is currently commercially available as VELSCOPE® (LED Med Inc., White Rock, BC). We have begun a longitudinal study to explore the effect of FV in defining the surgical margin on outcome of oral cancer surgery. Between 2004 and 2008, 60 patients with a single oral cancer entered the study. Each case was treated with surgical excision alone and followed for a minimum of 12 months. Thirty-eight patients had FV-guided surgery, with the surgical margin placed at 10 mm beyond the perimeter of autofluorescence loss. The remaining patients (control group) had the surgical margin placed at 10 mm beyond the tumor edge defined by standard white-light examination. Among those, 7 of the 60 cases (12%) have developed a recurrence of severe dysplasia, carcinoma in situ or squamous cell carcinoma at the treated site, all in the control group (25% versus 0%, P = 0.002). These data suggest the potential utility of autofluorescence changes within this clinical setting. There is a need to design a larger scale randomized controlled clinical trial to confirm the efficacy of FV-guided surgery.

We are also using FV to monitor the potential re-emergence of regions of autofluorescence loss at treated sites in the cases accrued to the longitudinal study and are currently completing an interim assessment of these monitoring results. Autofluorescence loss persists in some cases, increasing in size and intensity over time and giving rise to a clinical lesion containing dysplasia or cancer.

3.0. Core members of the clinical trial and project management: We have a well-built core group with long-term and strong working relationships, including surgeons (Drs. Anderson (Co-PI) and Durham). Pathologists (Drs. Berean (Co-PI) and Zhang), and Oral Medicine (Drs. Poh (PI) and Williams), and are in a world-leading position in using fluorescence visualization in operating room and in follow-up. Dr. J. Lee, collaborator from M.D. Anderson Cancer Centre and has extensive experience in clinical trials with special expertise in randomized controlled trials. He will be the trialist in this project, designing and implementing the trial protocol, overseeing the trial’s conduct, and serving as the principal investigator (PI) for the study. Professor Juahua Chen, Department of Statistics, the University of British Columbia will serve as the biostatistician to the trial and will be responsible for the data analysis and submission of interim analyses to the Data Safety Monitoring Board.

4.0. Design: The study will be a double-blinded, randomized controlled, Phase III study to evaluate the effect of FV guided surgery in patients diagnosed with severe dysplasia, carcinoma in situ and invasive squamous cell carcinoma undergoing surgery treatment with an intent-to-cure. The trial will randomize 200 patients - 100 in the FV arm (using FV guided surgery) and 100 in the control arm (using conventional white light approach). The trial period is 5 years - 2 years to complete accrual and 3 more years of follow-up.

- Eligibility

| Ages Eligible for Study | 19 years and older |
| Accepts Healthy Volunteers | No |

Criteria

Inclusion Criteria:
- Patients diagnosed with severe dysplasia, carcinoma in situ, invasive squamous cell carcinoma (T1 or T2) of the oral cavity (C0-D site codes: C02.0-C06.9) who will be undergoing curative resection (primary disease).

Exclusion Criteria:
- Patients with a non-oral malignancy diagnosed (not including non-melanoma skin cancer and lymphoma outside of head and neck region) within the past 3 years.
- Patients with evidence of distant metastasis (as determined by CAT and X-ray) at the time of recruitment.
Contacts and Locations

Please refer to this study by its ClinicalTrials.gov identifier: NCT01038289

Contacts

Contact: Helen Chiu  604-675-6057  hchiu@bccancer.bc.ca
Contact: SiuMa Lam  604-675-6057  saml@bccancer.bc.ca

Locations

Canada, Alberta

University of Calgary
Calgary, Alberta, Canada
Contact: Joseph Dorf, Dr.  jodorf@gmail.com

Canada, British Columbia

BC Cancer Agency (Vancouver & Fraser Valley Centres) & Vancouver General Hospital
Vancouver, British Columbia, Canada
Contact: Helen Chiu  604-675-6057  hchiu@bccancer.bc.ca
Contact: SiuMa Lam  604-675-6057  saml@bccancer.bc.ca

Canada, Manitoba

CancerCare Manitoba, University of Manitoba
Winnipeg, Manitoba, Canada
Contact: Paul Kerr, Dr.  Pkerr@exchange.hsc.mb.ca

Canada, Nova Scotia

Victoria General Hospital, Dalhousie University
Halifax, Nova Scotia, Canada
Contact: Rob Hall, Dr.  drahall@hotmail.com

Canada, Ontario

London Health Science Centre, University of Western Ontario
London, Ontario, Canada
Contact: John Yoo, Dr.  john.yoo@lhsc.on.ca

Ottawa General Hospital, University of Ottawa
Ottawa, Ontario, Canada
Contact: Mike O'Neil, Dr.  lesandmike@hotmail.com

Sunnybrook Hospital
Toronto, Ontario, Canada
Contact: Kevin Higgins, Dr.  kevin.higgins@sunnybrook.ca

Canada, Quebec

McGill University Health Centre
Montreal, Quebec, Canada
Contact: Karen Kost, Dr.  kmkost@yahoo.com
Sponsors and Collaborators

University of British Columbia
Terry Fox Research Institute
British Columbia Cancer Agency

Investigators

Principal Investigator: Catherine Poh, DDS, PhD  University of British Columbia
Principal Investigator: Scott Durham, Dr.  University of British Columbia
Principal Investigator: Miriam Rosen, Ph.D  Simon Fraser University
Study Director: Calum MacAulay, Ph.D  BC Cancer Agency Research Centre
Study Director: Penelope Brashear, Ph.D  University of British Columbia
Study Director: Stuart Peacock, Ph.D  BC Cancer Agency Research Centre
Study Director: Kelly Corbett, Ph.D  Simon Fraser University
Study Director: Kenneth Berean, Dr.  University of British Columbia
Study Chair: Donald Anderson, Dr.  University of British Columbia
Study Chair: Michele Williams, DDS  British Columbia Cancer Agency
Study Chair: Joseph Dott, Dr.  University of Calgary
Study Chair: Robert Hart, Dr.  Dalhousie University
Study Chair: Mike Odeh, Dr.  University of Ontario
Study Chair: Paul Kerr, Dr.  University of Manitoba
Study Chair: John You, Dr.  University of Western Ontario, Canada
Study Chair: Kevin Higgins, Dr.  Sunnybrook Hospital
Study Chair: Karen Kost, Dr.  McGill University Health Center
A Cross-Sectional Study Evaluating Chemiluminescence and Autofluorescence in the Detection of Clinically Innocuous Precancerous and Cancerous Oral Lesions
Ravi Mehrotra, Mamta Singh, Shaji Thomas, Preeti Nair, Shruti Pandya, Niraj Shakti Nigam and Pankaj Shukla
J Am Dent Assoc 2010;141;151-156

The following resources related to this article are available online at jada.ada.org (this information is current as of February 7, 2010):

Updated information and services including high-resolution figures, can be found in the online version of this article at:
http://jada.ada.org/cgi/content/full/141/2/151

Information about obtaining reprints of this article or about permission to reproduce this article in whole or in part can be found at:
http://www.ada.org/prof/resources/pubs/jada/permissions.asp
A cross-sectional study evaluating chemiluminescence and autofluorescence in the detection of clinically innocuous precancerous and cancerous oral lesions

Ravi Mehrotra, MD; Mamta Singh, MD; Shaji Thomas, MDS; Preeti Nair, MDS; Shruti Pandya, MSc; Niraj Shakti Nigam, BDS; Pankaj Shukla, MD

Cancer of the oral cavity is the sixth most common malignancy reported worldwide, and it has one of the highest mortality rates among all cancers. In 2008, an estimated 35,000 people developed cancer of the oral cavity and oropharynx in the United States, and approximately 7,500 people died of the disease. In India, oral cancer is the most prevalent cancer in men and the third most prevalent cancer in women, and it makes up 40 percent of all cancers in the country. Early diagnosis of oral cancer greatly increases the probability of achieving a cure with minimum impairment and deformity.

Light-based oral cancer screening aids have been developed with the stated goal of assisting dentists in

Dr. Mehrotra is a professor, Department of Pathology, Moti Lal Nehru Medical College, Lowther Road, Allahabad 211 001, India, e-mail "rm8509@gmail.com". Address reprint requests to Dr. Mehrotra.
Dr. Singh is a professor, Department of Pathology, Moti Lal Nehru Medical College, Allahabad, India.
Dr. Thomas is a professor, Department of Oral and Maxillofacial Surgery, People’s College of Dental Sciences & Research Centre, Bhopal, India.
Dr. Nair is a professor, Department of Oral Medicine and Radiology, People’s College of Dental Sciences & Research Centre, Bhopal, India.
Ms. Pandya is a research scholar, Department of Pathology, Moti Lal Nehru Medical College, Allahabad, India.
Dr. Nigam is a consultant dental surgeon, Vidiash, India.
Dr. Shukla is a consultant pediatrician and civil surgeon, District Hospital, Vidiash, India.

A B S T R A C T

Background. ViziLite Plus with TBlue system (Zila Pharmaceuticals; now Zila, a division of Tolmar, Fort Collins, Colo.) and VELscope (LED Dental, White Rock, British Columbia, Canada) are oral cancer screening aids that have been developed to assist dentists in identifying precancerous and cancerous oral lesions.

Methods. The authors screened patients with an overhead examination light and then with VELscope or ViziLite. Patients with a clinically innocuous lesion underwent a biopsy, and the authors compared the results of tissue pathological analysis with findings from the screening aid tests to determine the sensitivity and specificity of each device. The authors tested these devices to determine their ability to aid in the decision-making process regarding whether further evaluation of a clinically innocuous lesion was required.

Results. The authors biopsied 102 lesions and examined them with the ViziLite. They found three dysplasias and one malignancy, none of which were detected with the ViziLite (sensitivity = 0 percent, confidence interval [CI] = 0-60.2 percent; specificity = 75.5 percent, CI = 66.7-82.8 percent). The authors biopsied another 156 lesions and examined them with VELscope. They found 11 dysplasias and one malignancy, six of which were detected with VELscope (sensitivity = 50 percent, CI = 21.1-78.9 percent; specificity = 38.9 percent, CI = 30.8-46.9 percent).

Conclusions. The study results indicate that use of ViziLite or VELscope along with a conventional screening examination for lesions deemed clinically innocuous was not beneficial in identifying dysplasia or cancer. Additional clinical studies are needed before these devices can be recommended.

Clinical Implications. Clinicians and patients could have a false sense of security after obtaining a negative ViziLite or VELscope examination result because potentially large numbers of precancerous and cancerous lesions will be missed by both devices.

Key Words. Oral cancer; dysplasia; oral cancer screening aids.

JADA 2010;141(2):151-156.
identifying precancerous and cancerous oral lesions at their earliest stage. Specifically, these devices are intended to be used as adjuncts to the conventional oral cavity examination to help visualize potentially dysplastic and cancerous oral lesions. We evaluated two of these products in this study: the ViziLite Plus with TBlue system (Zila Pharmaceuticals; now Zila, a division of Tolmar, Fort Collins, Colo.) and VELscope (LED Dental, White Rock, British Columbia, Canada).

Investigators assess the accuracy of an oral cancer screening aid by comparing the screening aid findings with those of pathological testing in a masked fashion (that is, the clinician using the screening aid is unaware of the patient’s pathological diagnosis and the pathologist is unaware of the findings from use of the screening aid) and in a general population setting. To date, no published prospective clinical trials, to our knowledge, have evaluated the ability of ViziLite or VELscope to detect oral precancerous and cancerous lesions when used as a screening tool. Consequently, recent reviews in the literature of these devices have questioned the benefits of these light-based systems because their accuracy remains unknown.

Unlike new pharmaceuticals and medical devices that require approval by the U.S. Food and Drug Administration (FDA) before they can be marketed in the United States, certain grandfathered medical devices such as ViziLite and VELscope may be marketed without FDA approval. If a manufacturer claims that a medical device is “substantially equivalent” to another medical device that was sold before 1976 (when the FDA first began regulating medical devices), the FDA may grant a 510(k) clearance that allows the manufacturer to market that device without substantive review of its safety and efficacy.

The 510(k) clearance of the ViziLite Plus with TBlue system was based on the manufacturer’s claim that the device was “substantially equivalent” to colposcopy examination lights sold to illuminate the uterine cervix during a gynecologic examination. The 510(k) clearance of VELscope was based on the manufacturer’s claim that it, in turn, was “substantially equivalent” to the ViziLite system.

The purpose of our study was to evaluate the use of these two systems as adjunct aids in diagnosing lesions deemed clinically innocuous according to conventional light examination. We also assessed the sensitivity and specificity of ViziLite and VELscope in the identification of oral dysplasia and carcinoma by independently comparing pathological examination results with those obtained with these visual screening aids.

**PARTICIPANTS, MATERIALS AND METHODS**

In June 2008, 258 patients seeking dental care and found to have clinically innocuous lesions were investigated across a 10-day period by a team of dental and medical specialists (R.M., S.T., P.N.) in the outpatient department of the government-run District Hospital in the Vidisha district in the state of Madhya Pradesh in central India. The team included specialists in oral medicine (P.N.), oral and maxillofacial surgery (S.T.) and oral pathology (R.M.). All three specialists had received significant clinical training and had considerable experience with both the ViziLite and VELscope devices to ensure reproducible and accurate clinical findings and screening aid results. However, we did not calibrate the examiners. The institutional ethical committee of the District Hospital at Vidisha approved the study.

We enrolled in the study patients who were 18 years and older after they provided written consent. One of the three specialists examined each patient with a conventional overhead light; we then assigned patients randomly to either the VELscope or ViziLite devices depending on which examiner screened them. The specialists rotated between the two devices to prevent fatigue as well as to ensure unbiased selection of patients. Before the examination, patients rinsed their mouths thoroughly with water.

We defined all identified oral lesions according to Sciubba’s definitions:
- **Class I:** lesion “causing suspicion of intraepithelial neoplasia” or frank malignancy necessitating immediate biopsy;
- **Class II:** clinically innocuous lesion “that in the investigators’ opinion required no further attention other than clinical follow-up.”

**Exclusion criteria.** We excluded patients with Class I lesions detected with a conventional overhead examination light (and referred them for treatment) and those without any oral lesions. We included patients with Class II lesions for subsequent evaluation with the light-based adjunct screening tools. Furthermore, we excluded oral lesions that were submucosal (for

**ABBREVIATION KEY.** FDA: Food and Drug Administration.
example, cyst, salivary gland tumor) or covered with a clinically intact normal epithelium (for example, hemangioma, fibroma). In addition, we excluded from the study patients with pigmented lesions such as nevi and amalgam tattoos and lip lesions, specifically those on the vermilion border or cutaneous surfaces, as well as patients who refused to undergo a scalpel biopsy. Finally, we excluded patients with medical problems and those who wore dental appliances, such as orthodontic or other fixed prostheses, that might interfere with the examination.

**Intraoral examinations.** The clinicians performed the examinations with the VELscope and ViziLite devices according to the manufacturers’ instructions. In addition to evaluating the Class II lesion, the clinician examined the entire oral cavity of every patient with the light-based screening aid in an attempt to identify new lesions not apparent during the oral examination with the conventional overhead light. It would have been best for all patients to be examined by multiple examiners with both VELscope and ViziLite. Unfortunately, we conducted this screening at a rural facility with time constraints and limited resources. Only one VELscope device and a limited supply of ViziLite kits were available.

Patients underwent an examination with the conventional overhead light and then, depending on which screening aid was available, underwent an examination with VELscope or ViziLite. The assignments were completely random. As explained earlier, we excluded patients with Class I lesions (that is, suspicious enough to warrant a biopsy). Consequently, they did not undergo examinations with the light-based devices because the examiners already had determined that they needed to undergo a biopsy. Findings with the light-based devices for these patients—whether positive or negative—would be meaningless.

After a participant rinsed with a dilute 1 percent acetic acid solution and the clinician examined the mouth with a chemiluminescent light, normal mucosa—a negative ViziLite finding—appeared blue or dark, while abnormal mucosa—a positive ViziLite finding—appeared acetowhite. The ViziLite Plus with TBlue system also contains a toluidine blue dye, which is intended to be used only to mark lesions for follow-up examination that are positive according to the ViziLite screening. The manufacturer claims no diagnostic capability for the dye.

The VELscope is a portable device that is used to examine the oral cavity. Normal mucosa—a negative VELscope finding—appears as a bright green glow, while abnormal mucosa—a positive VELscope finding—is identified by a loss of fluorescence and appears dark.

Three of us (R.M., N.S.N., P.S.) obtained demographic information about each patient, including age, sex and tobacco use. The examiners performed detailed clinical examinations in each patient to assess the site and size of all oral mucosal lesions, and they recorded this information on a standard form.

Using the standard scalpel technique, two of us (S.T., P.N.) obtained biopsy samples from patients, who were under local anesthesia. The samples were processed at laboratories in Mumbai, India. Hospital pathologists first analyzed the specimens and then an independent pathologist with expertise in oral dysplasia and cancer analyzed the specimens; we used the independent pathologist’s findings in the final data analysis. The pathologists were masked with regard to the clinical data and screening aid test results.

We used specimens from patients who underwent scalpel biopsies and ViziLite screening to determine the sensitivity and specificity of the ViziLite Plus with TBlue system; likewise, we used specimens from patients who underwent scalpel biopsies and VELscope screening to determine the sensitivity and specificity of that device.

We calculated statistical confidence intervals (CIs) on the basis of a $t$ distribution and the exact binomial Clopper-Pearson interval. We analyzed the data with statistical software (Mathematica 6.0.3, Wolfram Research, Champaign, Ill.).

**RESULTS**

One hundred two patients who were examined with ViziLite also underwent a biopsy, and 156 patients who were examined with VELscope also underwent a biopsy. The table shows patients’ demographic data and the locations of the lesions identified in both groups.

**ViziLite group.** Of the 102 participants in the ViziLite group who underwent a biopsy, three had dysplasia (one mild, two moderate) and one had cancer; none of these was detected with the adjunct screening device. Consequently, the sensitivity rate of ViziLite—defined as a measure of the likelihood that a patient with dysplasia or carcinoma found on biopsy will have a positive ViziLite result—was 0 percent (0 of four positive findings) (CI, 0-60.2 percent). The ViziLite findings were
negative in 74 patients with benign lesions and positive in 24 patients with benign lesions. The specificity rate—defined as a measure of the likelihood that a patient with a benign lesion will have a negative ViziLite result—was 75.5 percent (74 of 98 negative findings) (CI, 66.7-82.8 percent). The positive predictive value—defined as the probability that a positive ViziLite test result would be confirmed by scalpel biopsy—was 0 percent (CI, 0-14.3 percent). The negative predictive value—defined as the probability that a negative ViziLite test result would be confirmed by scalpel biopsy—was 94.8 percent (CI, 89.9-99.9 percent).

**VELscope group.** Of the 156 participants in the VELscope group who underwent a biopsy, 11 had dysplasia and one had cancer, six of which also were detected with VELscope (five dysplasias [two mild, three moderate] and one cancer). The sensitivity rate of VELscope—defined as a measure of the likelihood that a patient with dysplasia or carcinoma will have a positive VELscope result—was 50 percent (six of 12 positive findings) (CI, 21.1-78.9 percent). VELscope findings were negative in 56 patients with benign lesions and positive in 88 patients with benign lesions. The specificity rate—defined as a measure of the likelihood that a patient with a benign lesion will have a negative VELscope result—was 38.9 percent (56 of 144 negative findings) (CI, 30.8-46.9 percent). The positive predictive value of VELscope was 6.4 percent (CI, 2.4-13.4 percent), and the negative predictive value was 90.3 percent (CI, 82.8-97.9 percent).

Neither ViziLite nor VELscope identified any lesions that were not already apparent during the clinical examination with a conventional overhead light alone.

The pathological test results from the independent pathologist were in agreement with those from the hospital pathologists who initially analyzed all of the biopsy specimens.

**DISCUSSION**

This is the first study, to our knowledge, to compare ViziLite and VELscope screening results with histopathologic findings in lesions deemed to be clinically innocuous according to conventional light examination.

The poor sensitivity and poor positive predictive values of these devices (ViziLite, 0 percent; VELscope, 50 percent) have significant implications for dentists and physicians who attempt to rely on these aids to determine whether a lesion is benign or precancerous or cancerous. Our study results show that because of their high false-negative rates, potentially large numbers of precancerous and cancerous lesions will be missed with the ViziLite or VELscope. Consequently, both the clinician and patient will have a false sense of security after a negative ViziLite or VELscope finding is reached because many dysplasias probably will have remained undetected and undiagnosed. These high false-negative rates invariably will lead to a delay in diagnosis, and a potentially greater number of oral cancers will be diagnosed at more advanced stages.

Although some published reports have shown that ViziLite improves the sharpness and brightness of oral lesions,10 Oh and Laskin11 concluded that ViziLite produced reflections that made visualization more difficult than that with typical operatory lighting. In another study, Farah and McCullough12 reported that ViziLite did not discriminate between 55 keratotic, inflammatory, potentially malignant and malignant oral mucosal white lesions with positive ViziLite findings that underwent a scalpel biopsy; these results are in agreement with ours. Furthermore, Kerr and colleagues13 reported that a significant number of suspicious red lesions, which typically are revealed to be dysplasia or frank carcinoma on biopsy, were not detected with ViziLite.
Toluidine blue stain. Epstein and colleagues\textsuperscript{14} assessed clinically suspicious lesions with ViziLite and then with toluidine blue stain before biopsy. They found that ViziLite improved the brightness and/or sharpness of the majority of lesions, but the false-positive rate was high; this was reduced with toluidine blue. Toluidine blue is not available commercially or approved by the FDA for evaluating oral lesions. Furthermore, the toluidine blue swab enclosed with ViziLite Plus with TBlue test kits is not approved by the FDA as a diagnostic tool but is intended to be used only as a marker to highlight a lesion. This is stated clearly in the manufacturer’s instructions: “The marking system is not intended to be used as an indicator of lesions warranting further study, including biopsy.”\textsuperscript{7,25}

We found that ViziLite did not detect any of the dysplasias or cancerous lesions in study participants, regardless of whether they were red or white. Furthermore, in our study, as with other studies, ViziLite did not detect any lesions that the clinician did not detect with an overhead examination light alone.

VELscope is based on a principle that excitation by blue or ultraviolet light will generate tissue autofluorescence that is produced by submucosal collagen, elastin and other naturally occurring fluorophores.\textsuperscript{16} Autofluorescence distinguishes fluorescence of naturally occurring tissue components from artificially introduced fluorescent molecules, such as molecular biomarkers.

The mechanism by which clinicians use VELscope’s tissue autofluorescence to detect epithelial carcinomas may be explained by the fact that hemoglobin strongly absorbs the autofluorescent light produced by collagen and elastin.\textsuperscript{17-19} More specifically, the increased presence of submucosal blood resulting from cancer-induced angiogenesis may result in absorption of collagen- and elastin-produced autofluorescent light and, therefore, the tissue area may appear dark during the VELscope examination.

As our study results show, the VELscope examination failed to detect six of 11 dysplasias. Because angiogenesis generally is associated with severe dysplasia or carcinoma in situ, as well as invasive disease, and is absent in healthy oral epithelium, mild dysplasias and moderate dysplasias,\textsuperscript{20} clinicians should not rely on VELscope to detect precancerous lesions.

Huff and colleagues\textsuperscript{21} reported that the addition of VELscope screening to an oral examination with standard overhead lighting resulted in the discovery of a greater number of oral dysplasias in a general dental practice. However, this study has significant limitations because the authors did not report if VELscope actually detected any new dysplastic lesions or even identified all of the lesions that were detected with standard overhead lighting.

In a proof-of-concept study, Lane and colleagues\textsuperscript{22} reported that VELscope had a 98 percent sensitivity and a 100 percent specificity when discriminating between carcinoma in situ or invasive cancer and healthy oral mucosa. However, in their study, unlike ours, the investigators used VELscope only in patients who were already known to have carcinoma in situ or invasive cancer on biopsy. Furthermore, all abnormalities found with VELscope also were observed with the standard examination light alone. Like Lane and colleagues, we did not find any lesions with VELscope that were not apparent during an oral examination involving the use of a standard overhead light.

The increased submucosal hemoglobin that apparently has been detected with VELscope can result from a variety of traumatic and inflammatory conditions, which may account for VELscope’s poor specificity. The high false-positive rate associated with VELscope has raised concerns about its potential harm, causing unnecessary stress and fear among patients, as well as increasing morbidity through unnecessary surgical biopsy procedures.\textsuperscript{23}

Manufacturer’s advice. The manufacturer of VELscope offers advice to reduce the number of false-positive results caused by inflammation and other noncancerous lesions that may result from the presence of submucosal blood.\textsuperscript{24} The company recommends applying pressure to a lesion that appears dark and, therefore, is suspicious to see if it blanches (that is, if the green color returns with pressure). This advice appears problematic because absorption of autofluorescent light resulting from true angiogenesis also may be hidden by this temporary tourniquet action, while absorption of autofluorescent light from the presence of submucosal blood caused by minor trauma may not. Indeed, there is no clinical basis to support this procedure based on our review of the VELscope literature.

Study limitations. Experienced clinicians performed the clinical examinations and examinations with the adjunct screening aids. However, they were not calibrated in using the VELscope and ViziLite devices; they also were not cali-
brated regarding classification of lesions identified during the oral examinations. The two adjunct screening aids are fairly straightforward in their application, so the lack of calibration was not as important as the lack of calibration in classifying lesions. Clinicians should keep this fact in mind when interpreting our results. We made no attempt to assess variability between observers.

Regarding other study limitations, it is worth noting that because this was not an opportunistic screening, in which dentists might use these devices for all adult patients regardless of whether or not they have a visible lesion, our study design does not reflect exactly the way in which these devices are currently used. Again, clinicians should keep this fact in mind when interpreting these results. Because specialists from different fields conducted the examinations in this study, day-to-day practice and use of these adjunct screening aids may differ.

CONCLUSION

Although ViziLite and VELscope have been promoted as valuable adjuncts in the early detection of oral precancerous and cancerous lesions, the results of our study indicate that they do not add any benefits to a conventional screening examination involving the use of a standard overhead light. Additional clinical studies are needed to evaluate the effectiveness and costs of light-based oral cancer screening aids before they can be recommended.

Disclosure. None of the authors reported any disclosures.

The authors are grateful to the Shusilaben Ramniklal Jhaveri Trust, Mumbai, India, for logistical support for this study.

this new technology in community dental practices and if suitable to develop a framework for knowledge translation and to test the hypothesis that FV is useful in facilitating the clinical decision to refer forward suspect lesions.

Materials and methods

Study participants

This study was approved by the British Columbia Cancer Agency and the Simon Fraser University Research Ethics Board. Dental practitioners participating in this study were recruited using a notice in a Greater Vancouver dental association publication that described the study and requested volunteers. Each of these practices was contacted by telephone (DML) and was given a more in-depth description of the project and its timelines. A total of 18 dentists participated from 15 offices (two offices had two dentists participating), with each dentist signing informed consent.

Data collection

The study included a 1-day workshop to orient dental participants to the study protocols and subsequent follow-up of screening activities in each dental office, with facilitation and referral to dysplasia clinics for patients requiring further assessment.

Description of workshop

The workshop was comprised of three parts. Firstly, before the start of the workshop, two short self-administered questionnaires, adapted from Yellowitz et al. (10) and Horowitz et al. (11), were completed to assess knowledge of oral cancer risk factors and to collect personal demographics on the participating dentists and information on their current screening activities. These findings will be reported elsewhere.

Secondly, a presentation was given, including a short review of oral cancer statistics, etiological factors, clinical risk factors, and oral histopathology. An introduction to fluorescence visualization (FV), as an adjunctive device for lesion examination (1, 2), was also given followed by a presentation of the step-by-step protocol for clinical assessment of patients, including extraoral and intraoral examination as described in Williams and co-workers (12). Finally, the referral pathway for suspicious lesions and follow-up procedures was described.

Thirdly, the workshop concluded with a hands-on clinical session where each participant observed and performed an oral cancer screening examination of patients with active disease under both white light and FV conditions.

Assessment of oral cancer screening activities during follow-up of dental practices

After completion of the workshop, participants were asked to screen all patients over age 21 for the period from November 2007 to September 2008. Each practice was loaned a VELSscope for the duration of the study (LED Dental, Inc., Burnaby, BC, Canada). The study community facilitator (DML) then contacted each dental practice monthly for data acquisition and to address any questions on the study protocol. Participating offices were contacted regularly via email for the duration of the study. Each patient screened was given a unique identifier which was also used at the follow-up clinic and on further diagnostic reports, if required.

The dental offices were asked to complete the screening at each new patient or recall examination. The protocol included the following:

Step 1. Patient History: This step involved recording the patient’s age, gender, personal and family history of oral cancer, tobacco use, and alcohol consumption. The study questionnaire was developed as an adjunct to the dental offices’ own medical history with the intent to minimize overlap and time spent by the clinician.

Step 2. Visual Screening Examination: This step involved both an extraoral and intraoral examination. The extraoral examination included inspection and palpation of the head and neck region, focusing on asymmetry, and swelling or tenderness. Participants were asked to refer to a medical doctor any patient with fixed, firm, or unexplained lymph nodes or asymmetries. An intraoral exam under incandescent (white light) conditions was then undertaken. If an anomaly was present, the site, color, texture, and appearance of the lesion were documented by checking off the appropriate boxes on a screening form and drawing the anomaly’s location on an oral cavity diagram (Fig. 1). Benign common mucosal changes not to be recorded included amalgam tattoos, Fordyce’s granules, vascularities, and pigmentation due to skin color.

Step 3. Lesion Assessment: This step involved assessing the risk of an anomaly. Low-risk lesions (LR) included obvious trauma, aphthous lesions, melanotic macules, candidiasis (including median rhomboid glossitis), and geographic tongue. Anomalies without apparent cause, non-healing ulcers, red or white patches, and lichenoid lesions were considered high-risk lesions (HR). Lichenoid lesions were later reclassified as intermediate-risk lesions (IR) because lichenoid lesions have a variation in clinical presentation from faint white striae to red and erosive and some may have increased cancer risk. Lesions in this latter group require further follow-up for clinical management.

Step 4. Direct FV: The FV component was the final step in the oral screening protocol. The FV examination followed the same methodical examination of all oral mucosa tissue as the conventional exam; however, it was performed under reduced room lighting whenever possible and with a handheld autofluorescence imaging device, marketed as the VelScope™ (LED Dental, Inc.). This device uses a blue/violet light (400–460 nm wavelength) to illuminate oral tissue, with long-pass and notch filters to allow clinicians to directly view fluorescence (1, 13). Lesions that retained the normal green autofluorescence under FV were classified as FV negative (FV−); those that showed a reduction in the normal pale green, appearing as dark patches, were classified as FV positive (FV+). Where the clinician was unsure of FV loss, these lesions were classified as FV equivocal (FVE).

Participating clinicians were further asked to document sites which appeared clinically normal but had a loss of FV (FV−).
Lesion follow-up
Patients with low-risk (LR) lesions without an obvious cause or with intermediate-risk (IR) and high-risk (HR) lesions were asked to return for reassessment in 3 weeks. If the lesion was still present after 3 weeks, the dental practice was requested to notify the study’s community facilitator (DML) who reassessed the patient’s lesion, both clinically and with FV, at the dental office. The community facilitator then referred any suspicious lesions to an oral medicine disease (OMD) clinic. In some cases, the dental offices directly referred patients to the OMD clinic. Oral medicine specialists at the OMD clinic determined if a biopsy or further follow-up was warranted.

Statistical analysis
Descriptive statistical analysis was used to describe data on knowledge and baseline screening behavior of participating dentists collected at the initial workshop and on the patient screening forms. These latter forms were imaged and then uploaded directly into a Microsoft Excel study database using Teleform (version 10.1; Vista, CA). Chi-squared tests were used to compare demographic and risk-habit variables (Table 1), logistic regression models were used for Tables 2, 3, and 4, and the ‘Akaike Information Criteria (AIC)’ was also used in Tables 4 and 5. Data analysis was performed with SPSS software, version 16.0 for Windows, 2007 (SPSS Inc., Chicago, IL).

Results
A total of 2404 patients received a white light and FV screening examination and 357 patients with lesions (15% of patients) were identified. Of these lesions, 192 were FV+ (54%), 26 FVE (7%), and 139 FV−/C0 (39%).

Table 1 Demographic information and risk-habit behaviors associated with an oral lesion of 2404 patients

<table>
<thead>
<tr>
<th>Demographic variable</th>
<th>Lesion (%)</th>
<th>No lesion (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (N = 2390)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>165 (46)</td>
<td>857 (42)</td>
<td>0.164</td>
</tr>
<tr>
<td>Female</td>
<td>192 (54)</td>
<td>1176 (58)</td>
<td></td>
</tr>
<tr>
<td>Age at screening (years) (N = 2301)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>58 (17)</td>
<td>458 (23)</td>
<td>0.006</td>
</tr>
<tr>
<td>≥40</td>
<td>288 (83)</td>
<td>1497 (77)</td>
<td></td>
</tr>
<tr>
<td>Family history of oral cancer (N = 2342)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15 (4)</td>
<td>45 (2)</td>
<td>0.040</td>
</tr>
<tr>
<td>No</td>
<td>332 (96)</td>
<td>1950 (98)</td>
<td></td>
</tr>
<tr>
<td>History of smoking (N = 2343)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever smokera</td>
<td>166 (47)</td>
<td>764 (38)</td>
<td>0.002</td>
</tr>
<tr>
<td>Never smoker</td>
<td>186 (53)</td>
<td>1227 (62)</td>
<td></td>
</tr>
<tr>
<td>History of chewing tobacco (N = 1824)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9 (3)</td>
<td>32 (2)</td>
<td>0.277</td>
</tr>
<tr>
<td>No</td>
<td>278 (97)</td>
<td>1505 (98)</td>
<td></td>
</tr>
<tr>
<td>History of chewing tobacco (N = 1824)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever smokerb</td>
<td>234 (67)</td>
<td>1138 (57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Never drinker</td>
<td>115 (33)</td>
<td>865 (43)</td>
<td></td>
</tr>
<tr>
<td>Clinic volume (no. of patients screened per clinic) (N = 2404)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤200</td>
<td>130 (36)</td>
<td>924 (45)</td>
<td>0.002</td>
</tr>
<tr>
<td>&gt;200</td>
<td>227 (64)</td>
<td>1123 (55)</td>
<td></td>
</tr>
</tbody>
</table>

aEver smoker – smoked more than 100 cigarettes and longer than 1 year.

bEver drinker – drinks 2 or more alcohol drinks per week.

Of the 357 patients with a lesion, age, gender, tobacco consumption (either smoking or chewing), alcohol consumption, lesion appearance, and risk of site were not associated with FV+ status (Table 2). Only the presence of color and texture were associated with FV+ status. Red, or mixed red and white lesions had a 5.6-fold (95% CI: 3.5–10.4) increased risk of being FV+. Lesions that were brown, black, or purple (common confounders such as amalgam tattoos, melanotic macules, nevi, and vascularities) had a 2.8-fold (95% CI: 1.2–6.7) increased risk of being FV+. Many of these benign conditions are dark and hence will provide a positive FV result. This emphasizes the importance of training in the use of FV and awareness of possible confounders. Lesions with a rough [RR = 0.5 (95% CI: 0.2–0.9)] or ‘other’ [RR = 0.3 (95% CI: 0.1–0.8)] texture

Figure 1 Map of the oral cavity.
**Table 2** Demographic and clinical factors associated with FV+ status in 357 patients with an oral lesion

<table>
<thead>
<tr>
<th>Gender (N = 357)</th>
<th>FV+ (%)</th>
<th>FV- and FVE (%)</th>
<th>RR of a FV+ lesion (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>106 (55)</td>
<td>86 (52)</td>
<td>1</td>
</tr>
<tr>
<td>Male</td>
<td>86 (45)</td>
<td>79 (48)</td>
<td>0.883 (0.582–1.341)</td>
</tr>
<tr>
<td>Age at screening (years) (N = 346)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>28 (15)</td>
<td>10 (19)</td>
<td>1</td>
</tr>
<tr>
<td>≥40</td>
<td>158 (85)</td>
<td>130 (81)</td>
<td>1.302 (0.740–2.291)</td>
</tr>
<tr>
<td>Family history of oral cancer (N = 347)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>174 (94)</td>
<td>158 (98)</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>11 (6)</td>
<td>4 (3)</td>
<td>2.497 (0.779–8.001)</td>
</tr>
<tr>
<td>History of smoking (N = 352)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>95 (50)</td>
<td>91 (57)</td>
<td>1</td>
</tr>
<tr>
<td>Ever smoker</td>
<td>96 (50)</td>
<td>70 (44)</td>
<td>1.314 (0.862–2.002)</td>
</tr>
<tr>
<td>History of chewing tobacco (N = 287)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>152 (97)</td>
<td>126 (97)</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>5 (3)</td>
<td>4 (3)</td>
<td>1.036 (0.272–3.941)</td>
</tr>
<tr>
<td>History of drinking alcohol (N = 349)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never drinker</td>
<td>54 (28)</td>
<td>61 (38)</td>
<td>1</td>
</tr>
<tr>
<td>Ever drinker a</td>
<td>134 (71)</td>
<td>100 (62)</td>
<td>1.514 (0.967–2.371)</td>
</tr>
<tr>
<td>Visible clinical lesion (N = 353)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>25 (13)</td>
<td>32 (20)</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>166 (87)</td>
<td>130 (80)</td>
<td>1.634 (0.923–2.894)</td>
</tr>
<tr>
<td>High-risk site (N = 308)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-risk site</td>
<td>114 (63)</td>
<td>89 (70)</td>
<td>1</td>
</tr>
<tr>
<td>High-risk site b</td>
<td>66 (37)</td>
<td>39 (31)</td>
<td>1.321 (0.815–2.142)</td>
</tr>
<tr>
<td>Appearance (N = 229)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homogeneous</td>
<td>102 (75)</td>
<td>70 (76)</td>
<td>1</td>
</tr>
<tr>
<td>Non-homogeneous</td>
<td>35 (26)</td>
<td>22 (24)</td>
<td>1.092 (0.591–2.017)</td>
</tr>
<tr>
<td>Color (N = 271) (P &lt; 0.001)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>20 (13)</td>
<td>50 (43)</td>
<td>1</td>
</tr>
<tr>
<td>Red or red and white</td>
<td>117 (76)</td>
<td>52 (44)</td>
<td>5.625 (3.048–10.382)</td>
</tr>
<tr>
<td>Other</td>
<td>17 (11)</td>
<td>15 (13)</td>
<td>2.833 (1.191–6.740)</td>
</tr>
<tr>
<td>Texture (N = 257) (P = 0.021)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smooth</td>
<td>93 (65)</td>
<td>52 (46)</td>
<td>1</td>
</tr>
<tr>
<td>Rough</td>
<td>25 (17)</td>
<td>30 (27)</td>
<td>0.466 (0.248–0.875)</td>
</tr>
<tr>
<td>Ulcer</td>
<td>17 (12)</td>
<td>16 (14)</td>
<td>0.594 (0.277–1.273)</td>
</tr>
<tr>
<td>Other</td>
<td>9 (6)</td>
<td>15 (13)</td>
<td>0.335 (0.137–0.820)</td>
</tr>
<tr>
<td>Lesion risk (N = 357)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low risk c</td>
<td>175 (91)</td>
<td>150 (91)</td>
<td>1</td>
</tr>
<tr>
<td>Intermediate and high risk</td>
<td>17 (9)</td>
<td>15 (9)</td>
<td>0.971 (0.469–2.011)</td>
</tr>
<tr>
<td>Clinic volume (number of patients screened per office) (N = 357)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤200</td>
<td>67 (35)</td>
<td>63 (38)</td>
<td>1</td>
</tr>
<tr>
<td>&gt;200</td>
<td>125 (65)</td>
<td>102 (62)</td>
<td>1.079 (0.858–1.355)</td>
</tr>
</tbody>
</table>

*aEver smoker – smoked more than 100 cigarettes and longer than 1 year.
*bEver drinker – drinks 2 or more alcohol drinks per week.
*cHigh-risk site – floor of mouth, round or latal tongue, soft palate.
*dConfounders – trauma, candidiasis, geographic tongue, amalgam tattoo, varicosity, aphthous lesion, herpetic ulcer, melanotic macule.

were found to be significantly less likely to be FV+ than smooth lesions.

**Impact of reassessment**

Demographic, risk habit, and clinical factors were also examined for an association with lesion persistence in the 141 patients called back for reassessment at 3 weeks. Six FV+ patients were referred directly to the oral medicine specialist without reassessment. Gender, age, tobacco consumption, alcohol consumption, risk of site, lesion appearance, and color were not found to be associated with lesion persistence in these patients. Only a rough lesion texture was associated with lesion persistence [RR = 3.7 (95% CI: 1.2–11.2)] (Table 3). Lesions that the dental professionals assessed as high risk (N = 16) at the initial visit were also more likely to still be present at the 3-week reassessment visit than lesions assessed as low risk (N = 121) [RR = 2.7 (95% CI: 1.4–5.1)].

To see if FV and lesion risk assessment have the potential to predict lesion persistence, four different prediction models were compared (Table 4). Model 1 included all variable except for FV and lesion risk assessment, model 2 included FV, model 3 included lesion risk assessment and model 4 included both FV and lesion
AIC, Akaike Information Criteria.  

---

**Table 4** Persistence modelling\(^{a,b}\)

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FV+</strong></td>
<td>–</td>
<td>3.407 (0.749–15.507)</td>
<td>–</td>
<td>2.771 (0.580–13.237)</td>
</tr>
<tr>
<td>Lesion risk (IR and HR)</td>
<td>–</td>
<td>–</td>
<td>7.560 (1.688–33.861)</td>
<td>8.208 (1.592–42.354)</td>
</tr>
<tr>
<td>−2 Log likelihood (df)</td>
<td>104.289 (11)</td>
<td>90.484 (12)</td>
<td>96.486 (12)</td>
<td>83.488 (13)</td>
</tr>
<tr>
<td>AIC</td>
<td>115.289</td>
<td>102.484</td>
<td>108.486</td>
<td>96.488</td>
</tr>
</tbody>
</table>

AIC, Akaike Information Criteria. 

\(^{a}\)All models included gender, age at diagnosis, history of smoking, history of drinking alcohol, lesion appearance, color and texture.  

\(^{b}\)Model 1 included all variables except for FV and lesion risk assessment, model 2 included FV, model 3 included lesion risk assessment, and model 4 included both FV and lesion risk assessment.

---

**Table 5** Persistence modelling results when using only the final 75% of patients in each clinic\(^{a,b}\)

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion risk (IR and HR)</td>
<td>–</td>
<td>–</td>
<td>36.834 (2.576–526.717)</td>
<td>1166.582 (1.765–770871.390)</td>
</tr>
<tr>
<td>−2 Log likelihood (df)</td>
<td>44.900 (11)</td>
<td>38.310 (12)</td>
<td>41.429 (12)</td>
<td>24.657 (13)</td>
</tr>
<tr>
<td>AIC</td>
<td>55.900</td>
<td>50.310</td>
<td>53.429</td>
<td>37.657</td>
</tr>
</tbody>
</table>

AIC, Akaike Information Criteria.  

\(^{a}\)All models included gender, age at diagnosis, history of smoking, history of drinking alcohol, lesion appearance, color and texture.  

\(^{b}\)Model 1 included all variables except for FV and lesion risk assessment, model 2 included FV, model 3 included lesion risk assessment, and model 4 included both FV and lesion risk assessment.

---

risk assessment. The 4 models were fitted with logistic regression, and the relative risk was checked; this did not change substantively across the different models. For each model, −2Log likelihood was reported and the AIC was generated. All three models (2, 3, and 4) are an improvement over model 1. There were significant differences between models 1 and 2, models 1 and 3, and models 1 and 4. The use of FV and assessing lesion risk are added to the model, both alone and together, increased the prediction of lesion persistence. Of the models 2, 3, and 4, model 4 has the lowest AIC and hence better predicts lesion persistence.

Of the five lesions that were biopsied, all were persistent lesions, including two low-grade dysplasias. Only the melanotic macule was found to be FV+ at reassessment; hence, the brown color of these lesions can be a confounding factor.

**Experience of clinician training effect**

To assess if the results changed after the clinician became more experienced using the autofluorescence imaging device, the first 25% of all patients screened were considered a training set and removed from the total patients screened. The analyses were then repeated. Smoking and alcohol consumption were still significantly associated with the presence of a lesion; however, patient’s age and dental practice clinic volume were no longer significant. FV+ status was associated with alcohol consumption and lesion color (red, or red and white), while a rough texture was still associated with a FV− status. Both a rough texture and an intermediate- or high-risk lesion assessment were associated with the lesion persisting at the 3-week reassessment appointment. The results of the modelling remained the same (Table 5).

**Discussion**

In this study, we began the process of evaluating new technology in community dental practices, to ensure that such a technology transfer would be integrated into the conventional oral examination. Practitioners were introduced to FV during the workshop and supplied with a device for use in their practice. They were instructed to conduct an FV examination at the completion of each conventional white light exam and to record observations made. As this was the first study to introduce FV technology into a community screening framework, our questions mainly focused on whether positive FV results were associated with persisting lesions identified through a step-by-step procedure as described in the oral cancer screening guidelines (9). With training and experience, we hypothesized that this device would add support to the reassessment and referral decision-making of community dental professionals.

The appearance of FV relies on three principles, the scattering of light as it interacts with tissue, the reflection of light from the tissue surface, and the absorption of the light by the tissue components and re-emission as fluorescing light. How the light is absorbed, reflected, or scattered depends on the biochemical composition of the tissue (14). Variation in light scattering may differ between individuals and by site (for example, the thicker epithelium of the buccal mucosa may reduce the back scattering of light as compared to the non-keratinized epithelium of the floor of the mouth) (12,15). Fluorophores are components of the tissue which absorb light at particular wavelengths and re-emit the light at longer wavelengths. They quickly become unstable and release the energy in the form of fluorescence, which is very sensitive to cellular and tissue changes. Three fluorophores that react to the wavelength of
light used with the FV autofluorescence imaging device are collagen and elastin found in the connective tissue and flavin adenine dinucleotide (FAD), a coenzyme involved with cellular metabolism (16). During carcinogenesis, the fluorescence intensity of collagen, elastin, and FAD decreases (16).

Hemoglobin absorbs light and is more abundant during carcinogenesis as a result of increased microvascularization. However, hemoglobin also increases as a result of trauma or inflammation and is the main confounding factor, along with pigmented tissue, for FV (15, 17). Table 6 summarizes the tissue and cellular alterations that influence FV during carcinogenesis.

Previous studies with FV have been carried out in high-risk clinics. In a proof-of-concept study, FV was compared to histology. FV was able to distinguish high-grade dysplasia and squamous cell carcinoma (SCC) from normal tissue with a sensitivity of 98% and a specificity of 100% (1). High sensitivity in the detection of SCC has also been found by others, ranging from 84 to 100% (18, 19). The sensitivity in the detection of dysplasia is much lower, particularly in discriminating between benign and premalignant or malignant lesions (18, 19). A high rate of false positives has also been reported; however, all data were collected at an initial visit. There was no follow-up visit to reduce the number of false positives that may have healed within a 3-week period, and FV+ lesions were not followed longitudinally to see if there was progression. Hence, if FV reflects tissue alterations associated with progression, low-grade dysplasias that are FV− may not be at risk for progression (20).

In one study, FV was compared to white light examination by a nurse in patients with a history of head and neck cancer; a head and neck surgeon reviewed any abnormalities. No advantage was found for FV over a conventional white light examination. Autofluorescence identified the true positives; however, it had an increased number of false positives as compared to a white light examination. Approximately 25% of the lesions were not found in the oral cavity or oropharynx and hence may be difficult to visualize directly. It is unknown whether known confounders were excluded. Without follow-up, some of these lesions may be attributable to trauma or other temporary conditions and were not given an opportunity to heal (21).

The value of experience using the FV autofluorescence imaging device along with reassessing patients was shown in our study. The strength of the models were increased when the training set was excluded and only the final 75% of screenings were analyzed from each clinic.

Table 6  Tissue and cellular alterations that influence FV during carcinogenesis (23)

| Increased breakdown of collagen cross-links and the basement membrane by MMPs, including collagenase, causing less collagen fluorescence | Increased nuclear scattering due to changes to the cell nuclei resulting in less back scatter |
| Increased metabolism alters FAD and hence less fluorescence intensity | Increased microvascularity leads to more absorption by hemoglobin |
| Increased thickening of the epithelium leads to less reflectance and back scatter |

While the use of FV has not, to date, validated itself within the general dental practice setting, it may increase the desire of oral health professionals to perform oral screening examinations and follow-up of patients with suspicious lesions.

There are several limitations of this study. Firstly, FV data were missing for the reassessment appointment. Clinicians may not have used FV at the reassessment appointment if the clinical lesion had resolved. Secondly, we were not able to biopsy all lesions for a definitive diagnosis due to ethical considerations. Thirdly, a clinical examination is subjective and varies with the experience of the clinician.

One of the most difficult decisions a clinician may face is when to refer a lesion for further investigation and biopsy. Recent evidence suggests high rates of clinical misdiagnosis by general oral health practitioners (22). For those clinicians in general practice without the experience and expertise of a specialist, an imaging device to aid in the decision to refer would be very helpful. At the community level, the critical decision is not whether or not the lesion is cancer but whether or not the lesion should be referred for further investigation. Reassessment at a 3-week follow-up appointment is critical to improving the specificity of the FV autofluorescence imaging device.

References


Acknowledgements

We would like to thank the dental professionals and their patients who participated in this study; Ms Lorna Lee for her help in organizing the workshops; and the research staff of the BC Oral Cancer Prevention Program. This research was funded by the National Institutes of Health and the National Institute of Dental and Craniofacial Research (R01DE13124 and R01DE17013) and a Senior Graduate Studentship from the Michael Smith Foundation for Health Research/BC Cancer Foundation (DML).

Conflict of interest

The project received 10 VELScope units as a donation from LED Dental Inc., Burnaby, British Columbia, Canada.