Salivary Diagnostics for Oral Cancer Risk Assessment

Introducing a Game-Changing Breakthrough

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Oral cancer is the sixth most common cancer worldwide, with 90% of oral cancers diagnosed as squamous cell carcinomas.[1-5] The 5-year survival rate is more than 90% if detected in its early stages, but unfortunately this disease is most often recognized in its later stages.[1,5,6] As a result of later diagnoses, the overall 5-year survival rate drops dramatically, to approximately 60%; unfortunately, this number has remained static during the last 50 years.[1,5] Those who do survive after a late-stage diagnosis often have difficulty in tasting, swallowing, speaking, breathing, and chewing, and often require operations for facial reconstruction (Table 1).

Definitive diagnosis often follows a patient's presentation of discomfort in the oral cavity (especially the buccal mucosa, tonsils, lips, tongue, and base of the mouth) which then leads to a physical examination with biopsy and pathologic histologic confirmation. Determining the appropriate patients for biopsy is difficult, as evidenced by the *high rate of negative biopsies* and the *low rate of early detection*.[6] The limitations of physical examination alone in detecting oral cancer are well known and alternatives, such as saliva-based biomarkers, are therefore urgently needed to aid in early detection.[1,7]

Table 1. Oral Cancer—The Grim Statistics

- Of all oral cancers, 90% are squamous cell carcinomas
- In 2014, of the more than 40,000 (estimated) new cases, more than 8,000 will die
- There are 2.5 times as many men as women diagnosed with oral cancer:
  - fourth leading cancer in black men
  - sixth leading cancer in white men
- One person in the United States dies from oral cancer every hour of every day
- The 5-year survival rate is approximately 60%
- If caught early, the survival rate is higher than 90%
- The mortality rate has not improved much in the last 30 years
- There are more than 250,000 men and women who have survived oral cancer in the United States

The 5-year survival rate is more than 90% if detected in its early stages, but this disease is most often recognized in its later stages.

SALIVARY DIAGNOSTICS: AN EASY TEST IS READY FOR PRIME TIME

The use of saliva as a diagnostic fluid has been under development for more than a decade. Because of the frequent late stage of diagnosis for oral cancer, saliva has been recently considered as a diagnostic aid. Saliva is recognized as a convenient body fluid for biomarker evaluation because of its ease of procurement[8] with a range of potential targets, as is the case in plasma.

The elimination of needles makes it a quick and painless procedure. This results in an easy test for the practitioner—whether a physician, dentist, nurse, or dental hygienist—to suggest and administer to patients. Now that stabilization reagents for saliva samples are available to maintain biomarker integrity during the shipping process to a central laboratory for analysis, there is also no need for refrigeration or special storage of the samples.[9,10]

The salivary proteome and transcriptome have been well characterized, and a number of candidate salivary biomarkers have been suggested to aid in the early detection of oral squamous cell cancer (OSCC).[8,11] Single biomarkers have limited diagnostic capability, and this has prompted efforts to determine multiple biomarker panels for improved discriminatory accuracy.[12] Prior studies identified a discriminatory salivary mRNA footprint for OSCC (Table 2).[13] These markers were subsequently prevalidated in additional multiethnic cohorts.[13,14] The National Cancer Institute-Early Detection Research Network (NCI-EDRN) also independently validated these mRNA markers along with discriminatory proteins in a large case-control study.[12] Case-control studies can introduce bias related to control subject selection, study in a setting different from the intended clinical application, and overfitting of the models.[12,15] To overcome these limitations, the NCI recommends the prospective-specimen-collection, retrospective-blinded-evaluation (PRoBE) study design as the most rigorous biomarker validation.[16]

Recently, PeriRx successfully completed a validation of the mRNA markers in such a PRoBE designed trial at the University of Michigan, Michigan State University, and the St. Johns Providence Health System in Detroit. Salivary specimens were collected from the intended use population of patients with suspicious oral lesions before clinically driven biopsy and the biomarkers were evaluated in a central laboratory blinded to the final diagnosis. All 6 prespecified mRNAs were significantly increased in cancer compared with controls, and the performance of a prespecified multimarker panel derived from the EDRN study was validated in this new prospective cohort based on prespecified statistical analysis plan.[17] This validation supports the informative nature of these biomarkers and the strength of the multimarker panel methodology. This intended use population data is now being used to develop additional discriminatory panels that incorporate internal reference genes that are necessary for the quality assurance of individual patient results in clinical practice.

The Promise of Advance Salivary Molecular Diagnostics

The majority of the patient population does not report having an oral screening exam as part of their routine care, even though such exams are recommended by the American Cancer Society and the ADA.[18] Some may question the value of routine screening for oral cancer, especially in low-risk populations, even though there is a higher incidence in younger people in recent years that may be tied to human papillomavirus (HPV). In high-risk populations, such as those with premalignant lesions, previous incidents of any kind of cancer, a history of alcohol use, tobacco smokers, and "cured" patients, patients would benefit greatly.[18,19] Data gathered from the higher risk populations suggest that routine screening for oral cancer can prevent disfigurement and save lives.[20]

The detection of OSCC on the basis of clinical examination has known limitations because of the location of many of the lesions and, since screening is not routine, the average healthcare provider sees few new cases of oral cancer.[1,8] The initial intended use of oral cancer salivary markers is to aid the primary care providers in the *identification of patients who are in need of an oral lesion biopsy*. As with other multimarker panels (known as multivariate index assays) used for risk stratification of other diseases, the diagnostic scores can be divided into low, moderate, and high risk.[21,22] A low-risk score identifies a group in which biopsy can be

Table 2. Salivary Transcriptome Diagnosis is Better Than Blood Tests for Oral Cancer Detection

	Saliva Transcriptome Diagnosis*	Blood Tests**
Area Under ROC Curve	0.95	0.88

*Li Y, St John MA, Zhou X, et al. Salivary transcriptome diagnostics for oral cancer detection. *Clin Cancer Res*. 2004;10(24):8442-8450.
**Li Y, Elashoff D, Oh M, et al. Serum circulating human mRNA profiling and its utility for oral cancer detection. *J Clin Oncol*. 2006;24(11):1754-1760.

deferred and reserved for lesions that do not resolve. High-risk scores identify patients who should be referred to a cancer specialist for consideration of biopsy. Intermediate scores would identify a group requiring early follow-up and referral for consideration of biopsy, based on clinical features.

Advanced salivary molecular diagnostics holds great promise to provide an objective and quantifiable tool to aid primary caregivers in the early identification of patients with OSCC. The early identification of these patients has the potential to save lives as well as to reduce the cost of care of this deadly disease. The use of these discriminatory markers with efficient laboratory methods has the potential to be cost saving, in part due to fewer unnecessary biopsies. In addition, the cost of oral cancer treatment is substantially reduced when the disease is detected early.[23-25] Once incorporated into routine practice, the use of salivary markers also has the potential to impact the detection and surveillance of other oral diseases and conditions commonly presented and treated or referred for treatment in all dental practices.

Salivary diagnostics can now be readily adopted in primary dental offices. These powerful molecular diagnostic tools can potentially transform the practice of dentistry.

Salivary Markers Identified for Other Diseases and Cancers

Discriminatory saliva marker footprints have been identified for other cancers and systemic diseases. Markers for Sjögren's syndrome have been tested in multiple cohorts and are currently undergoing a multicenter regulatory approval trial.[26-28] Sjögren's syndrome is a debilitating disease that often goes undetected for many years due to nonspecific symptoms of dry eyes and mouth, as well as lethargy and arthralgia.[29] Definitive diagnosis often requires the inconvenience of a salivary gland biopsy.[30] Salivary markers can potentially play a key role in earlier diagnosis and may be available within the next year.

Animal models support the concept that lung cancer can affect the composition of the salivary transcriptome and proteome.[31] Salivary biomarkers for the detection of lung cancer were also reported in prior case-control trials.[32,33] Most of these individual markers have been previously found to be up-regulated in lung and other cancers in tissue or blood.[31] A prospective trial for the validation of salivary markers for lung cancer is presently ongoing. The initial intended use of these markers would be for the identification of at-risk current and former smokers for chest CT scanning.

CLOSING COMMENTS

Salivary diagnostics can now be readily adopted in primary dental offices. In addition to the above diseases, there is the potential for the detection of other cancers as well as the detection and surveillance of other chronic conditions, including diabetes and periodontal disease.[34] These powerful molecular diagnostic tools can potentially transform the practice of dentistry.✦

References
1. Rethman MP, Carpenter W, Cohen EE, et al; American Dental Association Council on Scientific Affairs Expert Panel on Screening for Oral Squamous Cell Carcinomas. Evidence-based clinical recommendations regarding screening for oral squamous cell carcinomas. *J Am Dent Assoc*. 2010;141:509-520.
2. Brocklehurst P, Kujan O, O'Malley LA, et al. Screening programmes for the early detection and prevention of oral cancer. *Cochrane Database Syst Rev*. 2013 Nov 19;11:CD004150.
3. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. *Oral Oncol*. 2009;45(4-5):309-316.
4. American Cancer Society. *Cancer Facts & Figures 2011*. Atlanta, GA: American Cancer Society; 2011.
5. National Cancer Institute. *SEER Stat Fact Sheets: Oral Cavity and Pharynx Cancer*. Bethesda, MD: National Cancer Institute; 2012.
6. McLeod NM, Saeed NR, Ali EA. Oral cancer: delays in referral and diagnosis persist. *Br Dent J*. 2005;198:681-684.
7. Epstein JB, Güneri P, Boyacioglu H, et al. The limitations of the clinical oral examination in detecting dysplastic oral lesions and oral squamous cell carcinoma. *J Am Dent Assoc*. 2012;143:1332-1342.
8. Wong DT. Salivary diagnostics: Amazing as it might seem, doctors can detect and monitor diseases using molecules found in a sample of spit. *Am Sci*. 2008;96:37-43.

A Breakthrough in Oral Cancer Detection?

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Given the bleak outlook of late-stage oropharyngeal squamous cell carcinoma (OSCC) detection, and the fact that more than 65% of OSCCs aren't detected until later stages, there is a need to improve the earlier detection and diagnosis of OSCC. The 5-year survival for late and later stages is about 50%.[1] Even if one survives the initial diagnosis and treatment, 51% become disabled.[2] Currently, OSCC is the most expensive cancer in America to treat. In fact, it is double the cost of most cancers.[3] The high costs are due to late detection, subsequent rehabilitation costs, and high reoccurrence.

The recently completed validation study of salivary biomarkers is promising. When coupled with the emerging point-of-care technology, the potential of salivary biomarkers is even more compelling. An inexpensive and simple chairside procedure to facilitate the referral of the appropriate high-risk patient for a definitive biopsy would go a long way in the early detection of OSCC, significantly reducing the costs of care.

One might question the value proposition of this new disruptive screening technology to the patient/consumer, treating dentist, healthcare payer, and society in general. Given that more than 60% of the US population sees a dentist every year, and the emerging evidence of human papillomavirus-associated cancers increasing OSCC in younger individuals and women, salivary biomarkers to facilitate early detection would be greatly valued by all stakeholders. Further, the opportunity to monitor individuals who have previously been diagnosed and treated would be enhanced by a simple, inexpensive, "spit in a cup" technology.

In 2014, it is estimated that 42,440 new cases of oral cavity and pharyngeal cancer will be diagnosed, 65% in a later stage, which will result in 8,390 deaths. If we could move the needle, even a little, on earlier detection through this promising salivary biomarker technology, the impact could be enormous in terms of lives and medical costs saved.That alone should compel us to continue to validate this simple, inexpensive "spit in a cup" technology.

References
1. SEER Stat Fact Sheets: Oral cavity and pharynx cancer. Bethesda, MD: National Cancer Institute; 2014. seer.cancer.gov/statfacts/html/oralcav.html. Accessed September 10, 2014.
2. Taylor JC, Terrell JE, Ronis DL, et al; University of Michigan Head and Neck Cancer Team. Disability in patients with head and neck cancer. *Arch Otolaryngol Head Neck Surg*. 2004;130:764-769.
3. Jacobson JJ, Epstein JB, Eichmiller FC, et al. The cost burden of oral, oral pharyngeal, and salivary gland cancers in three groups: commercial insurance, Medicare, and Medicaid. *Head Neck Oncol*. 2012;4:15.

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9. Jiang J, Park NJ, Hu S, et al. A universal pre-analytic solution for concurrent stabilization of salivary proteins, RNA and DNA at ambient temperature. *Arch Oral Biol*. 2009;54:268-273.
10. Cussenot O, Sighar K, Mohammed M, et al. Detection of specific chromosomal aberrations in urine using BCA-1 (oligo-CGH-array) enhances diagnostic sensitivity and predicts the aggressiveness of non-muscle-invasive bladder transitional cell carcinoma. *World J Urol*. 2014;32:551-557.
11. Nagler R, Bahar G, Shpitzer T, et al. Concomitant analysis of salivary tumor markers—a new diagnostic tool for oral cancer. *Clin Cancer Res*. 2006;12:3979-3984.
12. Elashoff D, Zhou H, Reiss J, et al. Prevalidation of salivary biomarkers for oral cancer detection. *Cancer Epidemiol Biomarkers Prev*. 2012;21:664-672.
13. Li Y, St John MA, Zhou X, et al. Salivary transcriptome diagnostics for oral cancer detection. *Clin Cancer Res*. 2004;10:8442-8450.
14. Brinkmann O, Kastratovic DA, Dimitrijevic MV, et al. Oral squamous cell carcinoma detection by salivary biomarkers in a Serbian population. *Oral Oncol*. 2011;47:51-55.
15. Wei F, Patel P, Liao W, et al. Electrochemical sensor for multiplex biomarkers detection. *Clin Cancer Res*. 2009;15:4446-4452.
16. Pepe MS, Feng Z, Janes H, et al. Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: standards for study design. *J Natl Cancer Inst*. 2008;100:1432-1438.
17. Martin JL, Gottehrer N, Zalesin H, et al. Prospective blinded validation of transcriptome markers for oral cancer detection. *J Dent Res*. 2014;93 (online supplement 6).
18. Moyer VA; US Preventive Services Task Force. Screening for oral cancer: US Preventive Services Task Force recommendation statement. *Ann Intern Med*. 2014;160:55-60.
19. Nagler RM. Saliva as a tool for oral cancer diagnosis and prognosis. *Oral Oncol*. 2009;45:1006-1010.
20. Sankaranarayanan R, Ramadas K, Thara S, et al. Long term effect of visual screening on oral cancer incidence and mortality in a randomized trial in Kerala, India. *Oral Oncol*. 2013;49:314-321.
21. Zhang Z. An In Vitro Diagnostic Multivariate Index Assay (IVDMIA) for ovarian cancer: harvesting the power of multiple biomarkers. *Rev Obstet Gynecol*. 2012;5:35-41.
22. Bristow RE, Smith A, Zhang Z, et al. Ovarian malignancy risk stratification of the adnexal mass using a multivariate index assay. *Gynecol Oncol*. 2013;128:252-259.
23. Lang K, Menzin J, Earle CC, et al. The economic cost of squamous cell cancer of the head and neck: findings from linked SEER-Medicare data. *Arch Otolaryngol Head Neck Surg*. 2004;130:1269-1275.
24. Kim K, Amonkar MM, Högberg D, et al. Economic burden of resected squamous cell carcinoma of the head and neck in an incident cohort of patients in the UK. *Head Neck Oncol*. 2011;3:47.
25. National Cancer Institute. Cancer prevalence and cost of care projections. costprojections.cancer.gov. Accessed August 18, 2014.
26. Hu S, Wang J, Meijer J, et al. Salivary proteomic and genomic biomarkers for primary Sjögren's syndrome. *Arthritis Rheum*. 2007;56:3588-3600.
27. Hu S, Gao K, Pollard R, et al. Preclinical validation of salivary biomarkers for primary Sjögren's syndrome. *Arthritis Care Res (Hoboken)*. 2010;62:1633-1638.
28. Hu S, Vissink A, Arellano M, et al. Identification of autoantibody biomarkers for primary Sjögren's syndrome using protein microarrays. *Proteomics*. 2011;11:1499-1507.
29. Fox RI. Sjögren's syndrome. *Lancet*. 2005;366:321-331.
30. Vitali C, Bombardieri S, Jonsson R, et al; European Study Group on Classification Criteria for Sjögren's Syndrome. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis*. 2002;61:554-558.
31. Gao K, Zhou H, Zhang L, et al. Systemic disease-induced salivary biomarker profiles in mouse models of melanoma and non-small cell lung cancer. *PLoS One*. 2009;4:e5875.
32. Zhang L, Xiao H, Zhou H, et al. Development of transcriptomic biomarker signature in human saliva to detect lung cancer. *Cell Mol Life Sci*. 2012;69:3341-3350.
33. Xiao H, Zhang L, Zhou H, et al. Proteomic analysis of human saliva from lung cancer patients using two-dimensional difference gel electrophoresis and mass spectrometry. *Molecular & Cellular Proteomics*. 2010;11:M111.012112.
34. Giannobile WV, Beikler T, Kinney JS, et al. Saliva as a diagnostic tool for periodontal disease: current state and future directions. *Periodontology 2000*. 2009;50:52-64.

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