Brush-based Cytology Screening in the Tonsils and Cervix:
There Is a Difference!
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Abstract
This perspective on the report by Fakhry and colleagues in this issue of the journal (beginning on page 1378) examines the diagnostic accuracy of a "Pap-test equivalent" for screening for human papillomavirus (HPV)-associated cancers in the tonsils. HPV infection is strongly associated with cancer development in the oropharynx (tonsils and base of tongue) and cervix; the data discussed here underscore the differences in screening for cervical versus oropharyngeal malignancies and discuss some of the challenges and limitations associated with screening for tonsillar premalignancy and early cancers. Cancer Prev Res; 4(9); 1350–52. ©2011 AACR.

With an annual incidence of nearly 600,000 cases, squamous cell carcinoma (SCC) of the oral cavity and oropharynx (comprising the tonsils and base of tongue), is the sixth most common malignancy in the world (1). Chronic exposure to tobacco products and alcohol is the classic and most common etiologic factor for this disease (2). Certain high-risk human papillomavirus (HPV) subtypes, however, particularly HPV16, are an increasingly important etiologic factor for oropharyngeal SCC (OSCC), particularly in the tonsils (3–8). Despite numerous advances in treatment, long-term survival remains poor for this disease with the classic etiologies, in part because the disease is often diagnosed at advanced stages when treatment options are limited. Although the prognosis of HPV-associated OSCC seems to be markedly better than that of the classic forms of the disease, HPV-associated OSCC is also often diagnosed at a more advanced stage. Screening and early detection allow early treatment that can decrease morbidity and mortality in some cancer settings.

For example, screening for lung cancer and breast cancer have been shown to decrease mortality by 20% and 15%, respectively (9, 10). Although specific data on screening and early detection are currently lacking for OSCC, it is hoped that these approaches can be effective in reducing OSCC morbidity and mortality as well.

Cancer screening can be defined as the application of a test to high risk but asymptomatic individuals to sort out those who probably have from those who probably do not have a particular disease. The first step of this process for OSCC is to identify a screening test(s) with sufficient diagnostic accuracy to reliably detect both premalignant and early malignant lesions. Therefore, effective cancer screening tests must be sensitive, to detect as many lesions as possible, and they must be specific, to differentiate lesions of interest (possessing biologically or clinically worrisome features) from ones that are not. The conventional visual and tactile exam (CVTE) is the current gold standard for OSCC screening. The CVTE is often confounded, however, because, for example, premalignant lesions and early cancers can be subtle in appearance and may mimic common reactive/inflammatory lesions. Therefore, the addition of adjunctive screening devices/tests to the CVTE may increase both sensitivity and specificity, that is, diagnostic accuracy, for premalignant lesions or early OSCC. The data on the ability of these devices to improve diagnostic accuracy are limited, however (11–14).

As reported in this issue of the journal, Fakhry and colleagues investigated the potential association between HPV16 infections (determined by linear array or real-time PCR), cytopathology, and histopathology in 2 different populations considered to be at a high risk for the development of OSCC (15). The major goal of this work was to determine the diagnostic utility of a "Pap-test equivalent" for detecting premalignancy and early OSCC in high-risk individuals. They conducted 2 studies, which were designated PAP1 and PAP2.

PAP1 was a cross-sectional study using a commercially available cytobrush to collect specimens from patients who presented with obvious clinical abnormalities from any region of the oropharynx. These samples were then used for both a cytologic interpretation and for determination of HPV16 infection via PCR. In addition, an incisional biopsy was also carried out in all but 1 case for definitive diagnosis purposes. PAP2 was a nested case–control study designed to evaluate associations between HPV16 infection and specifically tonsillar cytopathology in an HIV-infected population, which is at a high risk for both HPV infection

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and tonsillar cancer. Biannual oral rinses (for detecting HPV infection) and annual brush cytology samples (for detecting both HPV infection and cytologic changes; using a commercially available cytobrush) were collected from each individual. It should be noted that although HPV-associated OSCC is observed in both the tonsil and base of tongue, there are several reasons for this study’s focus on the tonsil only. First, there is the practical issue of accessibility. The clinical examination and Pap-test equivalent of the tonsillar region can be easily carried out in a manner analogous to the current cervical Pap-test protocol. However, given difficulty in reaching the base of tongue, the examination and collection of a cytobrush sample from this region would pose considerably greater challenges that are unlikely to be easily translated to everyday clinical practice. Furthermore, the anatomy of the base of tongue contains a mixture of both flat mucosa and tonsillar tissue. This anatomic confounder raises the possibility of the Pap-test equivalent having an unacceptable level of false negatives.

PAP1 found that HPV16 status and abnormal cytology were strongly associated with the presence of OSCC (OR = 20, 95% CI = 4.2–95.4). This high OR is reasonable given that PAP1 patients had clinically obvious lesions identified during their conventional examination. Conversely, in PAP2, the authors were unable to detect either tonsillar dysplasia or OSCC in individuals with HPV16 detected either by the oral rinse or by tonsillar brushings. The data from PAP2 suggest that an oropharyngeal Pap-test equivalent may not be a practical method by itself for screening for tonsillar OSCC. Why is screening for tonsillar abnormalities (dysplasia and/or cancer) via brush cytology not an effective screening method? The inability of the test to identify abnormalities did not seem to be due to inadequacy of the collected samples or of their evaluation by the diagnostic laboratory. More likely, the limitation is due to the anatomy of the tonsil. In general, the cervix is covered by a flat and uniform layer of stratified squamous epithelium. Because of this structure, the detection of cytologic abnormalities via brush-based cytology is highly successful (Fig. 1A) and has directly contributed to the marked decrease in cervical cancer incidence (16–18). Unlike the cervix and the majority of head and neck mucosa, however, the architecture of the tonsillar epithelium is not arranged as a flat mucosal surface. Rather, the tonsillar epithelium contains numerous invaginations that plunge into the lymphoid structures of the tonsil itself, forming deep crypts, and it is believed that the majority of premalignant and malignant mucosal lesions of the tonsil arise within these crypts below the mucosal surface (Fig. 1B; ref. 19). Given this background, it is easy to understand how the diagnostic accuracy of brush-based cytology could have such dichotomous results for the same histologic diagnosis in the 2 different anatomic locations of the oropharynx and cervix.

What are the current alternatives if the Pap-test equivalent lacks the diagnostic accuracy to be a stand-alone screening test for HPV-associated tonsillar SCC? Unfortunately, the options are limited at this time. For example, a commercial diagnostic laboratory has begun offering a saliva-based test for the identification of oral HPV infections. They assert that the test will determine patient risk for developing an HPV-associated OSCC and will aid clinicians in monitoring the patient’s condition. Unfortunately, there are no published data to support this contention.
The lack of a validated HPV-associated OSCC screening test is an important obstacle that must be addressed. The Center for Disease Control (CDC) estimates that there are 6 million new HPV infections each year in the United States and that a total of 20 million U.S. residents are currently infected (21). Furthermore, it is estimated that more than 50% of sexually active adults will be infected with HPV in their lifetime (21). However, the CDC estimates that only 33,000 men and women will develop an HPV-associated malignancy this year and that 12,000 of these cancers will be OSCC (21). These numbers support the concept that the majority of individuals exposed to HPV are successful in clearing the infection while the viral genome is still at the epithelial, rather than integrated, stage; recent work has shown that host-integrated HPV can be detected in saliva, although this detection is in early development (22). A major challenge, therefore, is to develop a screening test that has sufficient diagnostic accuracy to identify only the HPV infections that are derived from the high-risk HPV subtypes and for which the HPV genome has become integrated into the host genome. Such a test could be employed with CVTE and innovative molecular imaging that can help visualize pre-malignant lesions and early cancers lurking within the tonsillar crypts to produce effective screening for the early detection of OSCC or for detecting highest risk individuals (23–26).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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