The Role of Human Papillomavirus in Oropharyngeal Squamous Cell Carcinoma

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- **Context.**—Human papillomavirus (HPV) is implicated in the development of oropharyngeal squamous cell carcinomas (OPC), particularly those cancers developing in tonsillar tissue.

- **Objectives.**—To review the prevalence, subtypes, and methods of detecting HPV in OPC and to review the epidemiology, histology, staging, management, and prevention of these cancers.

- **Data Sources.**—The study comprised a review of the literature.

- **Conclusions.**—The incidence of HPV-OPC is rising globally and in the United States, but rates of HPV-positivity vary with the anatomic site(s) and the population studied, as well as the method of detecting HPV infection. These tumors are more common in men. In contrast to HPV-OPC, the rates of smoking and alcohol abuse are lower. The HPV 16 subtype is predominant, and immunohistochemistry staining for p16 and in situ hybridization are the most widely used methods clinically to detect transcriptionally active HPV. Moreover, HPV-OPC has a unique tumor phenotype with predominantly nonkeratinizing morphology and a variety of patterns. These cancers often present with cystic lymph node metastases. The prognosis for HPV-OPC is significantly better than HPV-OPC and has led to differences in grading, staging, and management. Although there are similarities to cervical cancer, there are challenges in preventing such cancers.

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Although an infectious etiology for cervical cancer was postulated for decades, the association between human papillomavirus (HPV) and oropharyngeal carcinoma (OPC) spans only the past 2 decades. The anatomic sites included within oropharynx may vary. The World Health Organization classification of oropharyngeal carcinomas includes the base of the tongue, tonsils, and adenoids. In contrast, the eighth edition of the American Joint Committee on Cancer Cancer Staging Manual includes a broader range of sites in the OPC, including base of the tongue and tonsils (lingual and pharyngeal), the soft palate, uvula, vallecular, and lateral or posterior pharyngeal walls. Recent evidence suggests that the strongest association of HPV with squamous cancer is limited to tonsillar tissue of the OPC, including the base of the tongue, the palate tonsils, and the adenoids.

The incidence of these cancers is rising globally and in the United States. The increase is more marked for men than it is for women. Although several meta-analyses have suggested a rising prevalence of HPV in the setting of OPC over time, differences in the sensitivity of methods used to confirm HPV infection should be considered when reviewing the data. The most common HPV type associated with these cancer is HPV 16. Patients with HPV-OPC tend to be younger than uninfected patients. The behavioral risk factor for HPV-OPC is oral sex. Not surprisingly, a Dutch study found that women with a history of cervical intraepithelial neoplasia 3 had an increased, long-term risk for HPV-OPC compared with matched controls. Patients with HPV-OPC are also less likely to be users of tobacco products and/or alcohol and have a higher functional status. However, smoking and HPV-positivity are each independent risk factors for oropharyngeal carcinoma, and, in fact, tobacco smoking is associated with significantly greater oral HPV prevalence. There are important diagnostic, prognostic, and management implications for HPV-OPC, and all oropharyngeal squamous carcinomas, particularly those associated with tonsillar tissue, should be routinely tested for HPV. The strong association between HPV and OPC carcinoma also has implications for vaccination strategies.

**METHODS FOR DETERMINING HPV-POSITIVITY**

There is no gold standard for HPV-positivity in formalin-fixed, paraffin-embedded tissue, and detection of transcriptionally active virus is needed in the setting of HPV-OPC. One type of testing is detection of HPV DNA or messenger RNA (mRNA) via a variety of methods following polymerase chain reaction (PCR). The most widely used clinical method is immunohistochemistry (IHC) for a surrogate biomarker, p16. Expression of this protein is upregulated by E7 oncogene expression. However, because p16 can be upregulated by other mechanisms, there can be false-positives. It has been suggested that different p16 staining...
patterns have prognostic significance with strong, diffuse nuclear and cytoplasmic positivity conferring a better prognosis whereas cases with only nuclear staining have a worse prognosis. In contrast, HPV–OPC is generally negative for p16 but demonstrates overexpression of p53. Another commonly used technique is in situ hybridization (ISH) for HPV DNA or RNA. Both techniques are easily applied to formalin-fixed paraffin-embedded tissue.

One study compared PCR followed by mass array with ISH and p16 IHC. In that study, PCR with sequencing was used as the gold standard for discrepant cases. The PCR–mass array was the most sensitive and specific method at almost 100% sensitivity and specificity in that study followed by p16 IHC which was quite sensitive (94%) but not entirely specific (86%). Moreover, ISH was the least sensitive (83%) and specific (81%) testing. Clinical guidelines call for either detection of p16 or ISH to stage oropharyngeal carcinomas as HPV+. Recently, several studies have suggested that the Roche cobas 4800 (Roche Diagnostics USA, Indianapolis, Indiana) platform be used to determine HPV-positivity in formalin-fixed, paraffin-embedded tissue, although that would require further validation.

PREVALENCE AND TYPES OF HPV IN OROPHARYNGEAL CARCINOMAS

The authors of a large international study stringently defined the criteria for HPV-positivity in head and neck cancer as a combination of positivity for HPV-DNA and either HPV E6 mRNA or p16 as biomarkers. Using those criteria, they found that 22% of oropharyngeal carcinomas (anatomic site not further defined) were HPV+. With the requirement that all 3 markers be positive, that rate dropped to 19% for OPC. Single infections were much more common than multiple infections and HPV 16 was the most common type followed by HPV 33, HPV 26, HPV 35, and HPV 18. The HPV positive rates were highest in South America, central and eastern Europe, and northern Europe and were lowest in southern Europe.

A large European case-control study which used serology for antibodies directed against HPV 16 L1 as the biomarker found 30.2% positivity in patients with OPC compared with 0.8% of controls without cancer. Others have suggested that HPV 16 seropositivity is strongly correlated with HPV-DNA status in patients with HPV-OPC. A Danish study that defined HPV-OPC as being positive for both p16 and HPV DNA by PCR and genotyping found that 62% of the base of the tongue and tonsillar carcinomas were HPV+ without a significant difference between those sites. The most common types were HPV 16 (86%) followed by HPV 33 (6%) and HPV 35 (4%) with a variety of other types contributing to the remainder.

A recent study looked at HPV-positivity in tonsillar versus nontonsillar sites, including soft palate and posterior pharyngeal wall with p16 and ISH. The rate of HPV positivity in nontonsillar tissues of the palate and pharynx was concordant with that of other head and neck sites (ie, <10%) compared with greater than 92% in primary tonsillar locations. Finally, there are ethnic differences in the percentage of oropharyngeal carcinomas that are HPV+ with whites having the highest prevalence, followed by Hispanics and African Americans having the lowest. However, African American patients of high socioeconomic status were more likely to have HPV+ tumors than were those of low socioeconomic status. In the United States, the western states had the highest prevalence of HPV+ OPC whereas the southern states had the lowest.

Thus, significant differences in HPV+ rates for OPC differ in studies in part because of the composition of the study population, the distribution of anatomic sites considered as part of the oropharynx, and the biomarkers used for calling a tumor HPV+. Whatever the true rate of HPV-positivity, there is a strong association between HPV infection and tonsillar tissues. In contrast, rigorous studies have shown HPV+ rates of less than 5% in laryngeal and oral cavity squamous carcinoma.

HISTOLOGY OF HPV+ OROPHARYNGEAL CARCINOMAS

The HPV-OPC tumors are often small and clinically occult and patients with those tumors frequently present with lymph node metastases as compared with HPV+ tumors that commonly present with pain. Such tumors are often diagnosed by fine-needle aspiration biopsy, although false-negative rates are possible with extensive cystic degeneration. At present, there is no consensus for HPV testing on cytologic material in this setting. When no primary can be found clinically, the tonsils are often removed. Because HPV-OPC arises from tonsillar crypt epithelium, it grows beneath the surface of the epithelium as nests and lobules often with lymphocytes interspersed and with central necrosis. Features of invasion such as stromal desmoplasia are seldom seen, so the distinction between in situ and invasive squamous carcinoma may not be possible. Given the small size of such tumors and the lack of stromal reaction, all grossly negative tonsils in this setting should be serially sectioned across the short axis and submitted in total. Because tumor without features of invasion can metastasize, all such tumors should be called squamous carcinoma without the designation of in situ. However, unlike HPV+ squamous carcinomas, there is rarely dysplasia of the surface epithelium.

The most common HPV-OPC histologic manifestation is a nonkeratinizing squamous cell carcinoma. Although less differentiated on histology, the nonkeratinizing squamous carcinoma paradoxically has a more favorable prognosis than keratinizing squamous carcinoma. For that reason, HPV-OPC should not receive a histologic grade. Keratinizing squamous carcinomas are less likely to be associated with HPV, but if a keratinizing squamous cell carcinoma is p16+, it should be considered HPV-OPC.

The HPV-OPC nonkeratinizing histology encompasses a wide variety of subtypes, including basaloid (Figure 1), lymphoepithelial (without an association with Epstein–Barr virus), papillary, anaplastic, spindle, and adenosquamous variants. Anaplastic features and multinucleated cells are an indicator of poor prognosis (Figure 2). Another less-common HPV-OPC variant is small cell (high-grade neuroendocrine carcinoma) which has a less-favorable prognosis, despite often being positive for transcriptionally active HPV. The morphology and IHC findings are similar to that of small cell carcinoma elsewhere.

Another interesting feature of HPV-OPC is that it often presents with cystic lymph node metastases, as a cancer of unknown origin. In fact, that clinical scenario is increasing. These metastases can contain bland-appearing morphology as well as cancer (Figure 3, A) and can be confused with a diagnosis of cancer arising in a branchial cleft cyst; p16
staining can be helpful (Figure 3, B), especially because the primary tumor can be very small or occult. Even more confusingly, these cystic metastases may occasionally have columnar lining cells with cilia, representing either adenosquamous carcinoma or another interesting manifestation of HPV-OPC.25

PROGNOSTIC IMPLICATIONS OF HPV-OPC

Patients with HPV-OPC generally have a better prognosis than those with HPV− OPC, even with more advanced disease.27 In one study, HPV status was the most important determinant of overall survival, followed by pack-year smoking history, nodal stage, and tumor stage.27 Patients with HPV-OPC were found to be at low risk. However, smoking and high-nodal status elevated HPV-OPC to an intermediate risk, whereas patients with HPV− tumors were generally at high risk.27 A recent study compared results of radiation therapy in p16+ patients to p16− patients and found that the p16+ patients had significantly better progression-free and overall survival.28 Smoking negatively affected survival in p16+ patients, and never-smokers had significantly better progression-free survival.28 In acknowledgment of these differences, the newest version of the American Joint Committee on Cancer’s Cancer Staging Manual created a separate staging system for HPV-OPC.2 To be staged in this classification, the tumor results need to be positive for either p16 or ISH.2 Some of the differences for the HPV-OPC staging system include lack of histologic grading, lack of size criteria for nodal staging, and no N3 nodal stage.2

PREVENTION STRATEGIES

The success of screening and vaccination programs for decreasing cervical cancer raises the hope that similar strategies could be adopted for HPV-OPC.24 Because HPV 16 accounts for the predominance of such tumors, and HPV-OPC is more common in males, vaccination of young males in addition to females should, in theory, decrease the

Figure 1. Low-power view of basaloid squamous carcinoma arising in the base of the tongue. Basaloid tumors are one of the more common variants of squamous carcinoma associated with human papillomavirus–positive status. This tumor had positive immunohistochemistry staining for p16 (hematoxylin-eosin, original magnification ×4).

Figure 2. Anaplastic spindle cells in a nonkeratinizing squamous cell carcinoma of the tonsil. Such cells portend a worse prognosis in tumors that usually have a better prognosis than HPV− cancers (hematoxylin-eosin, original magnification ×40).

Figure 3. A, Low-power view of a cystic lymph node metastasis with both malignant and bland epithelium. Staining for p16 is required in such cases. B, The same field with positive p16 staining (hematoxylin-eosin, original magnification ×2 [A]; original magnification ×2 [B]).
incidence of HPV-OPC.25 However, there are challenges for both screening and vaccination.20 In addition to the lack of preneoplastic lesions (such as cervical intraepithelial neoplasia 3 in cervix) as targets for early intervention, the more advanced age at diagnosis for HPV-OPC will make it harder to gauge success of a vaccination program over the short term.26 Clearly, ongoing study is needed.

SUMMARY

The pathologist should suspect HPV-OPC in all oropharyngeal squamous carcinomas, particularly those associated with tonsillar tissue and in the setting of cystic lymph node metastases. Either p16 IHC or ISH must be used in such cases, and p16, as the more sensitive and specific marker is more widely used and recommended. Although HPV-OPC has unique demographics, histology, and prognosis, such patients respond very well to radiation. A new staging algorithm exists for HPV-OPC, and it is different from that for HPV-OP. Future studies will shape additional approaches to diagnosis, management, and prevention.

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