Eur Arch Otorhinolaryngol (2013) 270:405–416
DOI 10.1007/s00405-012-2086-4

REVIEW ARTICLE

# Human papillomavirus and oropharyngeal squamous cell carcinoma: what the clinician should know

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Received: 16 May 2012 / Accepted: 5 June 2012 / Published online: 30 June 2012


**Abstract** The incidence of oropharyngeal squamous cell carcinoma (OPSCC) is rising in contrast to the decreasing incidence of carcinomas arising in other subsites of the head and neck. The human papillomavirus (HPV) infection has played an increasing role in these epidemiological changes and as the etiology for a significant fraction of head and neck squamous cell carcinomas, OPSCC in particular. Most importantly, many retrospective studies have shown that the prognosis differs significantly between patients with HPV-associated tumors and non-HPV associated tumors. Thus, questions arise on the choices of treatment for patients based on HPV status and the consequences of therapy. Given the recognized relevance of HPV status in OPSCC, many new questions concerning the biology, treatment, and prevention of HPV infection arise. This review is intended to highlight some of the major issues and frequently asked questions relevant for the clinician dealing with patients with OPSCC.

**Keywords** Human papillomavirus · Oropharyngeal cancer · Screening · Detection · Treatment

## Introduction

While rates of laryngeal, oral, and hypopharyngeal squamous cell carcinoma have been decreasing as smoking has decreased in the United States, the incidence of oropharyngeal squamous cell carcinoma (OPSCC) has been rising [1, 2]. Commensurate with this change, there has also been a change in the patient demographics. The demographics for these cancers have shifted from a population of older patients (>60 years of age) with a strong history of tobacco and alcohol use to a younger population (<60 years of age) of patients with no or limited history of tobacco and

This paper was written by members and invitees of the International Head and Neck Scientific Group (http://www.IHNSG.com).

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alcohol use [3, 4]. Interestingly, in spite of the increasing incidence of OPSCC, overall survival has also been improving. Until recently, these trends were poorly understood and not directly connected. However, evidence now suggests that the increased incidence, changing demographics, and improved survival characteristic of OPSCC may be associated with human papillomavirus (HPV) infection and specifically HPV-16, although other high risk HPV types are associated with cancer [1]. Infection with HPV is the most common sexually transmitted infection worldwide. The identification of high risk HPV as causative agents for epithelial carcinoma is not a new finding. The established role of HPV-16 in OPSCC has raised many questions related to public health including prevention, screening, treatment, and patient education.

## History

The relationship between sexually transmitted disease and carcinoma was initially described by the Italian physician, Rigoni-Stern. In 1842, he examined the death certificates of women in Verona during the period 1760–1839 and identified a high incidence of cervical cancer in married women, widows, and prostitutes. He also noted that cervical cancer was rarely observed in virgins and nuns [5]. Rigoni-Stern concluded that the development of cervical cancer was related to sexual contact. Prior to Rigoni-Stern's work, Bafverstedt [6] published evidence that the ancient Greek and Romans had documented skin and genital warts and identified the relationship between sexual promiscuity and the infectious nature of these lesions. It was not until 1934 that the first experimental evidence linking carcinogenesis and HPV was published [7]. This work was done in domestic rabbits and thus had limited implications on the study of human disease. However, it was critical in establishing the relationship between the viral infection and carcinogenesis.

Since the 1930s, HPV-related diseases including cutaneous warts, laryngeal papillomatosis, and anogenital warts have become more common. There was, however, a relative paucity of human research in the area. In 1976, Gissman and zur Hausen [8] employed a series of hybridization studies with in vitro transcribed plantar papillomavirus RNA and DNA from various cutaneous, genital warts, and cervical cancers to identify the heterogeneity of HPV. In 1976 and 1977, zur Hausen [9, 10] published several important papers articulating his hypothesis that HPV plays an important role in the cause of cervical cancer. With his collaborators, he later identified HPV type 16 and HPV type 18 in cervical cancers. zur Hausen's contributions were later recognized and he was awarded the Nobel Prize in 2008 for his work in HPV and human carcinogenesis. The remarkable worldwide rise in HPV-associated oropharyngeal cancer has prompted many questions related to the relationship between transmission of HPV and the development of OPSCC.

## What is the HPV?

HPV is a DNA virus in the papilloma viridae family. HPV is only known to infect the epithelial layers of human hosts, and more than 200 genotypes of the virus have been described. The HPV genome is a short circular double-stranded DNA molecule that codes for up to eight proteins, ranging in function from genomic regulation (E1 and E2) to capsid proteins (L1 and L2), and viral oncoproteins (E6 and E7).

The numerous HPV serotypes are distinguished clinically by anatomic site of preference as cutaneous or mucosal. The cutaneous serotypes are encountered frequently in the general population, and are the agents of benign common warts. These account for roughly 60 % of HPV types. Mucosal strains account for 40 % of HPV strains and have a more diverse spectrum of clinical presentation, ranging from benign mucosal and anogenitial papillomas (typically caused by low-risk strains such as types 6 and 11) to invasive cervical, anogenital, and oropharyngeal carcinomas (caused by high-risk strains such as types 16 and 18). Low-risk and high-risk sub-types are differentiated by the oncogenic potential of their E6 and E7 genes.

## What is the basic immunobiology of HPV?

Unlike some viral infections, HPV exposure rarely confers systemic immunity. HPV infection typically evades the immune system because its replication does not induce cytolysis, necrosis, or viremia. It does not appear in the blood stream during usual infections. Viral proteins and particles are released in areas that typically evade immune surveillance. Several studies have also demonstrated that HPV inhibits interferon synthesis [11–13], resulting in a minimal immune response [14]. In the majority of HPV infections, seroconversion is rare. Seroconversion typically occurs in approximately 60 % of women and <30 % of men, is typically not robust and is usually limited to the L1 and L2 capsid proteins and not the early antigens associated with viral replication and persistence [15]. Cell-mediated immunity against the viral proteins clears the infection within 24 months in most individuals with cervical infection [16]. The combination of a weak immune response to the virus and the effect of cell-mediated immunity are reasons why the antibodies developed during natural infection do not provide protection against reinfection in most individuals [17, 18]. This is in contrast to the HPV vaccine that elicits a brisk durable antibody response to the capsid proteins.

## How is HPV contracted?

Reports of HPV DNA isolated from inanimate environmental objects have been published [19, 20]. However, there is no evidence to suggest that these isolates contained sufficient copies of viral particles or adequate virulence to transmit infection. In addition, there are no reported cases of environmentally acquired HPV. The only known mechanism of transmission of the virus is person-to-person contact.

The common modes of HPV transmission have been the subject of some debate. Some have speculated that changes in sexual behavior over the past 2 decades, such as the decreasing age of sexual debut, the increasing number of lifetime sexual partners, and the reported increase in oral sexual partners, may be responsible for the epidemic. Turner et al. [21] reported a progressive rise in the proportion of women who reported having sexual relations before age 18 as <10 % before 1920, 25 % before 1940, 45 % before 1960, and 60 % in the 1970s. Similar trends have been observed in studies documenting oral sexual practices, and multiple sexual partners. The increase in sexual risk-taking behaviors may play an important role in the widespread rise in infection rates but it is not clear that this explains the increase in HPV-related carcinoma. One possibility is that with the overall rise in HPV infection, there may be a disproportionate rise in the high risk types (HPV-16 and 18) relative to the low risk types.

In addition to intercourse and oral sexual practices, open-mouth kissing has been suggested as a mode of viral transmission. D'Souza et al. [22] conducted a study evaluating this hypothesis in college-age men, however, the data were inconclusive. What is known through a variety of case–control studies is that the risk of developing HPV-associated OPSCC is correlated with the number of lifetime sexual partners. It has also been shown that the risk of developing a malignancy is higher in those patients with active oral HPV infection. Recent epidemiologic data suggest that the incidence of HPV oral infections is 6.9 % overall, with men having a higher overall incidence than women (10.1 vs. 3.6 %). Also, HPV-16 positivity in the saliva of 1.6 % of men and 0.3 % of women suggesting that HPV-16 is frequently present and detectable in the population [23]. Whether this represents transmissible virus is unknown [20, 23].

## What do we know about the epidemiology and transmission of HPV?

The life-time risk of cervical HPV infection is 80 % [24]. Ninety percent of infections are transient and only rarely do infections become persistent [25]. It seems that the persistent or chronic infections represent a higher risk for carcinogenesis. The epidemic of HPV-related OPSCC has prompted some to re-evaluate the prevalence of this viral disease in an effort to predict the incidence of new OPSCCs. Although the prevalence of the incidence of HPV in men is not well established, it is known that infection is highest in young women and in newly formed heterosexual couples [26, 27]. Evidence suggests a bimodal distribution of infection in women. It seems that world-wide women between the ages of 25 and 35 are at highest risk. However, in Africa, the Americas, and Europe, a second peak has been documented in women older than 45 years [28]. Determining accurate rates of infection is limited, in that various criteria for defining infection have been used in the literature. Clinically evident lesions, seroconversion, and the immunohistological evidence of viral proteins have all been used to confirm infection. However, it should be emphasized that seroconversion may confirm viral exposure but may not confirm active infection and is not observed in a large fraction of infected women.

## What is the relationship between HPV and carcinogenesis?

Experimental work related to HPV-associated carcinogenesis in the oropharynx is in its infancy and drawing, in large part, from the science of cervical carcinogenesis. Cervical and anal squamous cell carcinomas typically develop at sites of squamous metaplasia such as the transformation zone of the cervix where the external squamous cell-lined epithelium meets internal columnar-lined epithelium [29]. We know that HPV-related OPSCC is most commonly restricted to the palatine and lingual tonsils. HPV targets preferentially the highly specialized reticulated epithelium that lines tonsillar crypts. It is not clear, however, if squamous metaplasia occurs in these areas and why these areas are particularly susceptible to carcinogenesis.

zur Hausen's work was initially focused on cervical lesions although his findings were clearly applicable to nearly all forms of HPV-related disease. The potential conversion of laryngeal papillomatosis into squamous cell carcinoma was initially identified in the 1940s. Interestingly Newell et al. [30] reported a five- to sixfold increased risk of oral cancer in women with cervical cancer. Another report almost 10 years later reported the possible role of HPV infections in oral squamous cell carcinomas [31]. Syrjänen et al. [31] identified HPV antigens in premalignant oropharyngeal lesions suggesting the role of HPV in the development of carcinoma. Löning et al. [32] reported on the relationship between specific HPV types and OPSCCs. More recently, some authors [33–35] confirmed the causal relationship between HPV and OPSCC. These

reports give biological reasons for the observation of many clinicians that the number of tobacco-related OPSCC has declined, and a younger population of non-smokers has become more prevalent.

HPV-associated OPSCC is rising in prevalence worldwide increasing from 40 to 80 % in the US [36], from 22 to 67 % in the UK [37], from 19 to 60 % in Australia [38], and from 29 to 93 % in Sweden [39] over the last two decades. In a European study analyzing data from 15 population-based cancer registries that cover ten European countries (EUROCARE project), incidence rates of squamous cell carcinoma of the head and neck increased more for HPV-related than HPV-unrelated cancer sites during the period 1988–2002. Also, significantly lower age at diagnosis and more improved 3-year survival rates were reported for related subsites [40]. It is now considered the fastest increasing cancer in Scotland according to the Scottish Cancer Registry. Chaturvedi et al. [41] have confirmed a change in the population, incidence, and survival of OPSCCs in the US in a study of 271 cases of OPSCC from 1984 to 2004. The data were collected by the three population-based cancer registries in the Surveillance, Epidemiology, and End Results (SEER), Residual Tissue Repositories Program using polymerase chain reaction (PCR) and genotyping HPV-16 viral load and HPV viral expression. Trends in HPV prevalence were estimated using logistic regression. Chaturvedi et al. [41] found an increase in the prevalence of HPV-positive oropharyngeal tumors from 16.3 % during the 1980s to 72.2 % in the 2000s. Most concerning was that Chaturvedi predicted that if the published trends continue, the annual number of HPV-positive OPSCCs will surpass the annual number of cervical cancers by the year 2020.

These reports highlight the virulence of HPV and the long-term consequences of early infection. The data related to relative risk of developing OPSCC are alarming. The overall risk of developing OPSCC in an alcohol user is 5.5 [42], the overall risk rises in tobacco users to 19.5 [42], and the synergistic risk of alcohol and tobacco combined increases to 56.5 [43]. This compares with a study by Hansson et al. [44] that demonstrated that the risk of developing OPSCC in a patient infected with high risk HPV is 230. When one compares the prevalence of tobacco and alcohol over the past half century and the associated risk (56.5) of developing OPSCC and the prevalence of HPV and its associated risk (230), the projections for new cases of OPSCC may be underestimated. Furthermore, smoking tobacco may increase the risk for development of squamous cell carcinoma of the head and neck even further in HPV-infected cases. A synergistic interaction between smoking and HPV infection in the development of cancer has been reported in several studies [45]. In a large multicenter study on the interaction between smoking and HPV status of patients, odds ratios were determined for the risk of developing OPSCC of smokers who were negative for HPV-16 (OR 11.2; 95 % CI 5.9–21.4), never smokers who were positive for HPV-16 (OR 64.5; 95 % CI 18.3–226.7), and smokers who were positive for HPV-16 E6 or E7 (OR 56.2; 95 % CI 22.5–140.4) when compared with never smokers who were negative for HPV-16 [46].

## How does the molecular pathogenesis of HPV OPSCC differ from smoking-related cancers?

HPV targets the basal cell or stem cell layer of human epithelial tissue. It is hypothesized that the viral particles reach this deep layer either through traumatic micro-tears, or at exposed sites in the cryptic epithelial lining of the oropharynx [47]. Once established in the basal cell nucleus, HPV proteins are transcribed by the host cell. Subsequent replication of viral particles mirrors the development of the host tissue, with low levels of HPV in basal cells and increasing numbers of replicating and shedding virus encountered in the more differentiated cell layers [48].

HPV gains its oncogenic potential when the E6 and E7 proteins become dysregulated following integration into the host cell nucleus. The E6 protein causes substantial degradation of the p53 tumor suppressor protein via ubiquitin-mediated proteolysis [49]. This loss of functional p53 interferes with the cell's intrinsic DNA repair and apoptotic mechanisms, leading to genomic instability. In conjunction with increased telomerase activity seen in response to E6, these genetically unstable cells are able to escape the failsafe checkpoint in G1, and continue to replicate with a prolonged lifespan. This unique oncogenic mechanism of E6-mediated p53 degradation, as opposed to p53 mutation, is a distinct biologic feature of HPV carcinomas when compared to traditional tobacco and alcohol-induced malignancies.

The E7 protein targets the retinoblastoma (pRb) tumor suppressor protein. pRb naturally inactivates E2F, a transcription factor that drives cells from G1 to S phase in the cell cycle. Subsequent increased concentrations of active E2F following E7-inactivation of pRb lead to increasing loss of cell cycle control in infected host cells. Loss of functional pRb leads to an increase in p16, a cyclin-dependent kinase inhibitor, as the host cell attempts to restore control to the cell cycle. Increased p16 staining (which is usually inactivated by mutation or promoter hypermethylation in tobacco-induced head and neck cancers) in tumor cells is used as another molecular indicator of HPV-induced malignancy.

### Are there any histopathological differences between HPV-associated OPSCCs and smoking-associated cancers?

Studies have shown that HPV-associated tonsil cancers may arise from the tonsillar crypts where HPV DNA is incorporated [50]. In contrast, HPV-negative tumors or smoking-associated tumors were felt to originate from the surface epithelium [51]. The growth patterns of these tumors may also be different with HPV-related tumors demonstrating an invaginated growth pattern, while HPV-negative tumors tend to be more fungating with polypoid or tentacular growth. Consistent histopathological features of these tumors are that they are not associated with dysplasia of surface epithelium, show lobular growth, are permeated by infiltrating lymphocytes, do not undergo significant keratinization, and have a prominent "basaloid-like" morphology although they should not be confused with the highly aggressive basaloid squamous cell carcinomas. However, there are no specific histologic characteristics that distinguish HPV-positive from HPV-negative tumors.

### Is there a test available to screen for HPV infection or a premalignant lesion?

There is currently no simple and reliable screening tool for oropharyngeal HPV infection. Unfortunately, there is no clinically useful serologic test of HPV exposure or infection risk. However, just as pap-smears lead to early diagnosis of cervical cancer, dramatically reducing the mortality of the disease, similar localized screening exams are being sought in the head and neck. Serologic testing has identified responses to HPV early antigens in patients with HPV-positive tumors. DNA assays of mouthwash specimens have been used to successfully identify patients with oral HPV infection [52, 53].

There is also no widely accepted screening test for premalignant oropharyngeal lesions. One study investigated the use of a pap-test equivalent using tonsillar brush biopsies to determine an association between high risk (HPV-16 positive) samples and atypical cytopathology [54]. In this study, HIV-positive patients, who are considered at high risk for HPV infection and tonsil cancer, received brush biopsies of the tonsils at 1-year intervals. Although there was a cumulative HPV-16 infection rate of 11 %, there were no detected cases of dysplasia. Similarly, there was no statistically increased likelihood of HPV positive cases developing atypical squamous cells of undetermined significance (ASCUS) when compared to HPV-negative controls. The authors concluded that a screening test may not be feasible. In cervical cancers, the pap-smear is a good tool for detecting squamous cell carcinomas as these arise from the superficial ectocervix, whereas, it is inadequate for detecting adenocarcinomas as these arise from the deeper glandular cells of the endocervix. The authors rationalize that pap-smears for cervical cancer adequately sample the abnormal epithelium whereas a similar screening tool for tonsil cancer does not sample the abnormal epithelium of the tonsillar crypts where HPV-associated cancers arise.

### Who is at risk for HPV-induced OPSCC?

The risk of oral HPV infection and subsequent OPSCC has been linked to sexual practices by multiple studies [35, 55]. OPSCC incidence is increased in people with increasing numbers of lifetime vaginal sexual partners or oral sexual partners, age younger than 18 at first intercourse and lack of condom use. In a case–control study of sexual practices, lifetime numbers of vaginal sexual partners >26 and oral sexual partners >6 resulted in odds ratio of 3.1 and 3.4, respectively, for the development of OPSCC [55]. However, it is noteworthy that HPV-positive head and neck squamous cell cancer is present as well in more individuals reporting few sexual partners. Therefore, although sexual behavior is an important risk factor for HPV-positive head and neck squamous cell carcinoma, it is only a small population with OPSCC and higher numbers of partners, and the absence of a high number of sexual partners does not exclude the diagnosis.

Another study examining college-aged men compared HPV infection rates with sexual practices and tobacco use [22]. Associations were shown between oral HPV infection and increasing lifetime oral sexual partners and current use of tobacco. Of additional concern, common practices such as open-mouth kissing demonstrated a statistical trend toward HPV infection. Further elevated rates of tonsil cancer have been reported in husbands of women with cervical carcinoma [56], in addition to synchronous oropharyngeal carcinomas reported in a husband and wife infected with the same sub-type of HPV-16. Immunosuppression, as encountered with HIV infection or post-transplant immunosuppression, has also been shown to increase oral HPV risk.

### What are the odds of developing OPSCC related to HPV?

Oral sex has been shown to increase the odds of developing HPV-positive HNSCC. Studies have also shown that oropharyngeal infection with HPV increases the risk even more [55]. Oropharyngeal HPV infection is associated with 12 times the risk of OPSCC. When limiting analysis to

only those patients infected with high risk HPV-16 strain specifically, patients are 14 times more likely to develop OPSCC.

## What is the epidemiological data concerning HPV OPSCC?

In US, the overall incidence of HNSCC has steadily declined over the past few decades, likely due to decreasing rates of tobacco use. However, despite this progress, tongue base and tonsil cancers have continued to increase at rates of 2 and 4 % per year, respectively [10]. In addition, the majority of new cases of OPSCC occur in non-smoking and non-drinking Caucasian males between the ages of 40 and 55 years old [1, 57]. In the EUROCARE Working Group study, this increase was observed only for the age group 50+, suggesting delay in the risk of developing an HPV-related tumor in Europeans as compared to the US [40]. A Swedish study showed a sustained increase over time in HPV DNA isolated from archived tonsil cancer specimens over the past 40 years: 23 % in the 1970s, 28 % in the 1980s, 57 % in the 1990s and 68 % in the 2000s [58]. The figures from Denmark are comparable with reported prevalence of p16-poitive OPSCC of 41 % in the 90s, 58 % between 2001 and 2005 and 72 % in the period from 2006 to 2009 [59]. In the US, about 40–80 % of OPSCCs are caused by HPV, whereas in Europe the proportion varies from around 90 % in Sweden to <20 % in communities with the highest rates of tobacco use [60].

HPV has long been studied in cervical cancer, where >90 % of cases are caused by serotypes 16 and 18. Elevated HPV serotype concordance among infected couples has been demonstrated, supporting the sexually transmitted nature of this disease. In parallel with the sexually transmitted nature of HPV in cervical and anogenital cancers, multiple studies have examined person-to-person spread of HPV in OPSCC.

## What HPV types are associated with OPSCC?

Although most cervical cancers are associated with the high-risk types 16 and 18, the literature suggests that the majority of HPV-related OPSCCs are caused by type 16 alone. In one large study of 432 tonsillar cancers, 84 % were attributable to type 16 which was supported by a smaller study of 52 tonsillar cancers in which 87 % were caused by HPV-16. Another clinical study reported a 96 % rate for HPV-16. Other high risk types such as 18, 33, 35, and 58 were also responsible for a small percentage. Interestingly, 3 % of HPV-related tumors may be caused by low-risk types such as 6 and 11 [61].

## Why is HPV-associated OPSCC seen more frequently in younger, male patients?

With the relaxation of sexual norms in the 1970s and the emergence of HIV in the early 1980s, oral sex has become a far more common sexual practice. Although the incidence of active oral HPV infection in the general population has increased from 3–5 % in adolescence to 5–10 % in adulthood [62], the incidence of HPV-positive OPSCC in patients over 65 years of age has remained relatively constant over time. This is in contrast to the increasing incidence of OPSCC noted in patients 40–64 years of age. A leading theory is that the differing sexual practices exercised amongst these age groups over time contribute to this discrepancy, however, there are no studies available to prove this.

The discrepancy in cancer incidence between male and female patients, however, remains unresolved. The prevalence of genital HPV infection in males ranges from 29 to 65 %, compared to 24.5 % in females [62, 63]. However, it is reported that up to 25 % of cervical HPV infections are caused by highly oncogenic HPV-16 strain. A recent population-based study suggests that the incidence of oral HPV infection is higher in men than in women (10.1 vs. 3.6 %). This is also true of HPV-16 oral infections (1.6 % in men vs. 0.3 % in women) [23]. A host of theories have been proposed to account for the discrepancy of carcinoma incidence between men and women. These include possible gender differences in immunity, local tissue environments, the propensity for a higher number of lifetime sexual partners amongst males and general changes in sexual practice. However, currently there is no definitive evidence to support any of these proposed hypotheses.

## My significant other is HPV positive yet has no history of cervical cancer, can I get OPSCC by kissing her or having oral sex with her?

Longitudinal studies of partners examining transmission of oral HPV have never been performed. However, some retrospective reviews suggest that transmission between infected partners is possible and frequent. A Swedish retrospective review examined the effect of cervical carcinoma in situ and invasive cervical carcinoma on development of head and neck cancers in both patients and their husbands [56]. A significant statistical risk for the development of head and neck malignancies was seen in both female patients and their male husbands. This suggests that cervical HPV infection in sexually active couples increases both the male and female's risk for oral infection and subsequent OPSCC development. An additional study of HPV in spouses showed that failure of one partner to clear an oral HPV infection led to a 10-fold increase in the persistence of HPV in their spouse [64].

### Is there an advantage to vaccination and when is this indicated?

Currently, there are two FDA-approved vaccines in clinical use. Cervarix is a bivalent vaccine that prevents infection from HPV types 16 and 18, which cause most types of HPV-induced cancers, including OPSCC and cervical cancer. Gardasil is a quadrivalent vaccine with protection against HPV-16 and 18, in addition to HPV 6 and 11, which are linked to genital warts and their associated cutaneous malignancies. These vaccines were licensed in 2006 for use female patients between the ages of 9 and 26. In 2009, indications for Gardasil were expanded to include males after protection against genital warts in both sexes was demonstrated [65]. These vaccines have up to 97 % efficacy among HPV naïve patients [66]. Vaccination has no effect, however, against existing HPV infections or their viral-induced malignancies.

The effects of vaccination have not been studied in people over the age of 26, and therefore, its use in older patients is not currently licensed. More expanded studies need to be performed on the effects of vaccination in older patients before this may become a treatment option. There is currently no role for vaccination in partners of effected patients as they are undoubtedly already exposed to the virus prior to the development of cancer in the effected partner.

### How do patients with HPV-associated OPSCC present clinically?

Early stage OPSCC rarely causes symptoms, and many patients present with advanced stage disease. A painless, enlarging neck mass is the most common symptom at presentation, encountered in 30 % of patients [67]. Other concerning symptoms that warrant further investigation include persistent sore throat, dysphagia, otalgia, globus, dysarthria, hemoptysis and weight loss. Although adequate visualization of the oropharynx can be limited for the non-otolaryngologist, careful trans-oral inspection and digital palpation of the tonsils and base of tongue can be of value in detecting masses.

When compared with HPV-negative patients, HPV-associated OPSCCs create a distinct clinical group that is characterized with a younger age and better performance status at diagnosis, small primary tumor but an advanced and often cystic neck metastases, no-smoking history and mild-to-moderate alcohol use. Histopathologically, these tumors are often poorly differentiated and an inverse correlation with EGFR expression has also been observed [59]. Frequently patients will receive antibiotics and the symptoms and neck masses may wax and wane early in the course of presentation.

### What tests are available to confirm HPV status in HPV-associated OPSCC?

HPV status for HPV-mediated tumors can be tested using one or a combination of several assays. Assays to test for HPV status include p16 immunohistochemistry (IHC), in situ hybridization (ISH), and either DNA or RNA PCR. The p16 assay is a surrogate assay for HPV as it measures levels of p16, a cell cycle regulator and tumor suppressor gene that are produced in excess as a result of the HPV incorporation into the genome. Although currently unclear, this is felt to be a compensatory mechanism to E7-mediated degradation of the retinoblastoma tumor suppressor gene. This protein can also be produced in excess by undetermined non-HPV mediated mechanisms and as such p16 can be positive in cases where HPV is negative (5–10 %) and negative in cases where HPV is positive (8 %) although in the later it may indicate a disconnection of HPV carcinogenesis from the cancer [68]. The assay is typically performed using IHC and is simpler, and less costly than other methods. Although generally a very sensitive test, this assay can also have false positives due to lack of standardized criteria in reporting as well as non-viral mechanisms for increased expression.

PCR and ISH, on the other hand, directly measure HPV DNA. PCR is generally a very sensitive test but may be limited in that it cannot differentiate between viral contamination and clinically relevant HPV infection that has incorporated into the host genome. PCR is more sensitive than ISH, however, it requires extensive controls and careful performance characteristics. ISH relies on hybridization signals in the nuclei in the tumor. It is generally less sensitive but more specific. Both PCR and ISH are costly and time consuming in comparison to p16 IHC. In a comparative study of several assays on 108 cases of oropharyngeal squamous cell carcinoma, the authors found that PCR had the highest sensitivity when compared to p16 IHC and ISH (97 vs. 94 vs. 88 %) [69]. However, ISH had the highest specificity when compared to p16 IHC and PCR (88 vs. 82 vs. 87 %, respectively). The authors noted that a combination of p16 IHC and DNA PCR provides excellent sensitivity and specificity (97 and 94 %, respectively). In this way, an algorithm was developed for HPV detection in paraffin material that combines satisfactory performance with the option for high-throughput analysis. This is based on the combination of two tests: a p16 immunostaining upfront, followed by reflex HPV-DNA PCR (e.g. GP5+/6+) on the p16-positive cases [70]. In this published series, 100 % sensitivity and 100 % specificity could be reached, taking an aberrant LOH profile and the mRNA expression of E6 on fresh-frozen material as reference. There is a need for standardized diagnostic testing particularly for

inclusion of patients in trials and determination of prognosis in HPV-positive patients.

Another important aspect of HPV testing is those cases with enlarged neck node(s) and no primary tumor found at clinical examination or imaging studies. As the oropharynx is the prevailing site of a hidden primary, there are strong arguments to support the testing all fine-needle biopsy specimens obtained from the cervical nodes of patients with an unknown primary tumor for the presence of HPV. In these patients, HPV-positivity is highly predictive for the index cancer in tonsillar fossa or base of tongue. ISH for HPV-16 testing and determination of p16 expression in fine-needle aspirates were successfully used in this setting [71].

## How does HPV status affect prognosis?

It is clear from multiple retrospective studies that HPV-positive OPSCC has a significantly improved prognosis compared to HPV-negative OPSCC. This improvement is seen in patients treated with radiotherapy, surgery, chemoradiotherapy, and sequential therapy. In the largest trials, which are primarily combined modality studies, retrospective analysis indicates that this prognosis is improved by several additive factors. First, these patients have fewer second primary tumors, they do not die from other competing causes and they have much better local–regional control when compared to patients with HPV-negative OPSCC. In several trials of advanced cancers, the improvement in survival is two- to threefold for HPV-positive cancers compared to HPV-negative cancer and about 75 % of this improvement is attributable to the biology of the cancer and 25 % to the health characteristics of the patient.

The survival benefit conferred by HPV status is thought to extend from the molecular differences encountered between virally induced and carcinogen-induced cancers. While smoking and alcohol abuse are readily known to mutate p53 and other intrinsic tumor suppressor genes, HPV-positive tumors maintain wild-type protein sequences. Consequently, when stressed by treatment modalities such as chemotherapy and radiation, HPV-positive tumor cells are more able to undergo controlled cell death and attack by immune system surveillance. In patients who are both HPV-positive and have a significant smoking history, a survival benefit is still observed compared to HPV-negative cancers; however, this is less robust.

Superior performance status of HPV-positive OPSCC patients at diagnosis (with better compliance to applied therapies) and reduced risk of second primary tumors (as a consequence of less pronounced smoking history) should not be neglected when looking for sources of improved survival in HPV-positive group of tumors.

## What are the non-surgical treatment options for HPV-related OPSCC?

Although many studies have echoed the national statistics that demonstrate a dramatic improvement in OPSCC 5-year survival rates, at the moment the routine clinical practice in patients with OPSCC are not influenced by the knowledge on HPV status of the tumor. Furthermore, in the absence of reproducible and standardized testing of HPV status, decision-making can be overly risky. While some have suggested that observed survival advantage in HPV-positive group may be a result of multimodality chemoradiotherapy, these trends have not been observed in other sites such as laryngeal, hypopharyngeal or oral cavity cancers. Interestingly, HPV-related carcinoma of the oropharynx seems to respond to treatment significantly better than HPV-negative disease to virtually any therapy.

These findings have prompted some to ask if "is it time to change our treatment paradigm" [72]. According to knowledge accumulated until now, de-intensification strategies should be limited exclusively to HPV-positive non-smokers with OPSCC and should only be recommended in the setting of a clinical trial. The European Cooperation Oncology Group (ECOG) has completed enrollment in a phase II study (1308) to investigate de-intensification of radiotherapy for stage III/IV resectable HPV-positive OPSCCs. Patients enrolled in the study received three cycles of induction chemotherapy followed by reduced dose chemoradiotherapy. The induction arm consists of weekly paclitaxel (90 mg/m$^2$) and cetuximab (250 mg/m$^2$) and cisplatin every third week (75 mg/m$^2$). Patients with a complete responses were treated with dose-reduced IMRT (54 Gy/27 fractions) with weekly cetuximab, while those with less than a complete response will receive 69.3 Gy in 33 fractions with weekly cetuximab (250 mg/m$^2$) [8]. This trial does not compare outcomes to standard therapy. The RTOG is currently conducting a phase III randomized control trial (1016) in which patients with advanced OPSCC (excluding T1, 2 and N0, 1 tumors) will be randomized to two arms. The first arm consists of accelerated IMRT (70 Gy in 6 weeks) with cisplatin (100 mg/m$^2$ on day 1, 22). The second arm consists of the same radiation schedule followed by weekly cetuximab (starting 1 week prior to radiotherapy) [73]. Mount Sinai Medical Center will be treating patients with induction chemotherapy with reduced dose TPF and then randomizing responders to standard chemoradiotherapy with 70 Gy or reduced dose chemoradiotherapy with 56 Gy.

## Is there a place for surgery in HPV-positive OPSCC?

Although nonsurgical de-intensification trials are showing great promise, trans-oral surgery is emerging as a viable

treatment option for early T and N stage OPSCC. Transoral surgery can be performed either by transoral robotic surgery (TORS) or transoral laser microsurgery (TLM). Historically, open surgery for OPSCC was plagued by perioperative morbidity, including tracheostomy and gastric-tube dependence in a large number of patients. Consequently, non-surgical treatment modalities for early-stage OPSCC became the mainstay of treatment, although these modalities give rise to specific morbidity as well. However, much of the morbidity associated with previous large-scale, external approach operations was obviated with transoral surgery. In 2009, the FDA approved the use of transoral robotic surgery for T1 and T2 tumors of the oropharynx.

Although the data for TORS are still young, early oncologic results are promising [74]. In a recent series of patients with selected OPSCCs, as many as 50 % of patients did not require adjuvant therapy [75]. Patients with single modality surgical therapy have radiotherapy or CRT available as a second-line option. More long-term studies are needed comparing the initial oncologic and long-term control and morbidity of TORS compared to primary radiation for early stage OPSCC.

To date, there have been few surgical trials investigating the role of HPV-mediated OPSCC. Cohen et al. [76] retrospectively studied differences in oncologic outcomes in patients who underwent TORS surgery stratified by HPV status. In this study, 50 patients with OPSCC underwent TORS of which 37 were HPV positive and 13 HPV negative. There were no significant differences in overall survival and locoregional control. The authors concluded that TORS surgery was suitable for both HPV-positive and negative groups. The Mount Sinai group similarly demonstrated no differences in overall survival or locoregional control in patients stratified by smoking status with the assumption that patients without a smoking history are predominantly HPV positive [77]. The failure to show statistical differences in HPV-positive and HPV-negative tumors in TORS surgical trials for early T stage differences is unclear. These studies have been small and may lack statistical power to show survival differences. Another possible explanation is that the survival advantage in HPV-positive tumors does not apply to early T-stage tumors or early T-stage tumors that are surgically resected. Lastly, one may argue that HPV-negative tumors are less radioresponsive and surgical resection confers a better prognosis in the cohort being studied. Further multi-institutional studies are needed to confirm that there is in fact no difference in survival for (surgically treated) HPV-positive and negative early stage tumors.

TLM is another less morbid surgical option, which has demonstrated good oncologic outcomes. In a prospective study of 204 patients with stage III and IV OPSCC across all T stages, survival and locoregional control were studied as primary endpoints [78]. Disease-free survival was 82 % over 49 months of follow-up, and local control was achieved in 97 % of patients. All failures occurred in patients with either advanced primary or nodal disease. It should be noted, however, that patients were not further sub-divided by HPV status.

In other surgical trials, the survival advantage of HPV-associated tumors has been confirmed. One retrospective study examined the role of HPV status in patients who underwent surgery with or without adjuvant radiotherapy in 124 patients with stage III/IV OPSCC [79]. In multivariate analysis, HPV-positive patients had better locoregional control (HR 0.38; 95 % CI 0.14–0.91) and overall survival (HR 0.11; 95 % CI 0.04–0.28) than those who were HPV negative, independent of whether they received postoperative radiation. The data have been confirmed by other investigators, although using HPV status determined by p16 staining only [80].

## How does HPV impact survival?

There is high-level evidence demonstrating improved prognosis in patients with HPV-positive OPSCC. In a meta-analysis of the case series, a reduction in risk of disease failure by 15 % and of death by 38 % was found in HPV-positive SCCHN compared to HPV-negative tumors, whereas these figures were 49 and 28 %, respectively, in patients with oropharyngeal primaries [81]. These results have recently been confirmed in several prospective studies, which further clarify the importance of tobacco exposure.

In the series by Ang et al. [82], smoking emerged as a further independent prognostic factor. HPV status was combined with smoking history, T and N stage to construct three categories of risk: low, intermediate and high. Patients with HPV-positive tumors and no history of smoking were considered low risk. These patients demonstrated nearly a 90 % overall survival. Conversely, HPV-negative patients with more than a 10 pack year smoking history were considered high risk. Overall survival in this group was estimated to be approximately 50 %. This adverse effect on prognosis of smoking in HPV-positive patients has been found in other studies as well [83, 84]. The survival benefit conferred with HPV-positive OPSCC was again demonstrated in other recent publications that reported on treatment results from prospective ECOG phase II protocol 2399 as well as TROG 02.02 and TAX 324 phase III clinical trials [85].

HPV tumors also have improved survival despite a higher prevalence of more advanced nodal disease. The Mayo Clinic group demonstrated 35 % of HPV-positive patients who underwent surgical resection of OPSCC had

nodal disease in comparison to 11 % who were HPV negative [86]. Despite more aggressive nodal disease, there is some early evidence to suggest that HPV status reduces the overall prognostic significance of nodal disease. That is, in HPV-positive patients, nodal disease is not as strong a prognostic factor for recurrence and survival as it is for HPV-negative patients. Furthermore, prognostic power of extracapsular tumor spread (ECS) seems to be also diminished in surgically treated p16-positive OPSCC. After matching the patients with and without ECS for T-stage, surgical margins and adjuvant therapy, no significant reduction in disease-free survival was observed for the presence of ECS versus the absence of ECS or for the administration of adjuvant radiotherapy alone versus chemoradiotherapy in ECS-positive patients. The authors conclude that de-escalation adjuvant therapy should be considered for patients with p16-positive surgically treated OPSCC, and that reports on the presence of ECE should not justify concomitant administration of chemotherapy with postoperative radiotherapy [87].

Currently, HPV status and smoking history were recognized as important stratifying parameters for categorizing patients into distinct prognostic groups.

## Conclusion

HPV is a ubiquitous sexually transmitted DNA virus. Its role has been clearly established in both benign and malignant processes. High-risk strains such as HPV-16 have been associated with OPSCC in a younger, non-smoking, sexually active population unique to the traditional older, smoking population. Widespread vaccination campaigns with Gardasil and Cervarix for boys and girls present a good primary prevention strategy, but currently secondary prevention strategies to identify premalignant lesions are unavailable. Diagnosis of these tumors should consist of a low index of suspicion and a good clinical exam in patients presenting with a neck mass, dysphagia, or sore throat. Younger age and favourable performance status at diagnosis, advanced and often cystic neck metastases, no-smoking history and mild-to-moderate alcohol use define a distinct clinical profile of HPV-positive patients. Once diagnosed, confirmation of a viral etiology may require one or, better, a combination of available assays. Both non-surgical and surgical treatment options exist, and generally the prognosis of HPV-associated tumors is significantly better than smoking-associated tumors. Future trials are being directed towards tailoring treatment stratified for HPV status in order to maximize quality of life and maintain equivalent oncologic outcomes.

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