HPV-associated head and neck cancer: a virus-related cancer epidemic

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A rise in incidence of oropharyngeal squamous cell cancer—specifically of the lingual and palatine tonsils—in white men younger than age 50 years who have no history of alcohol or tobacco use has been recorded over the past decade. This malignant disease is associated with human papillomavirus (HPV) 16 infection. The biology of HPV-positive oropharyngeal cancer is distinct with P53 degradation, retinoblastoma RB pathway inactivation, and P16 upregulation. By contrast, tobacco-related oropharyngeal cancer is characterised by *TP53* mutation and downregulation of *CDKN2A* (encoding P16). The best method to detect virus in tumour is controversial, and both in-situ hybridisation and PCR are commonly used; P16 immunohistochemistry could serve as a potential surrogate marker. HPV-positive oropharyngeal cancer seems to be more responsive to chemotherapy and radiation than HPV-negative disease. HPV 16 is a prognostic marker for enhanced overall and disease-free survival, but its use as a predictive marker has not yet been proven. Many questions about the natural history of oral HPV infection remain under investigation. For example, why does the increase in HPV-related oropharyngeal cancer dominate in men? What is the potential of HPV vaccines for primary prevention? Could an accurate method to detect HPV in tumour be developed? Which treatment strategies reduce toxic effects without compromising survival? Our aim with this review is to highlight current understanding of the epidemiology, biology, detection, and management of HPV-related oropharyngeal head and neck squamous cell carcinoma, and to describe unresolved issues.

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Introduction

Cancers of the head and neck arise from mucosa lining the oral cavity, oropharynx, hypopharynx, larynx, sinonasal tract, and nasophaynx. By far the most common histological type is squamous cell carcinoma, and grade can vary from well-differentiated keratinising to undifferentiated non-keratinising. An increase in incidence of oropharyngeal squamous cell carcinoma—specifically in the tonsil and tongue base—has been seen in the USA, most notably in individuals aged 40–55 years. Patients with oropharyngeal cancer are mainly white men. Unlike most tobacco-related head and neck tumours, patients with oropharyngeal carcinoma usually do not have a history of tobacco or alcohol use. Instead, their tumours are positive for oncogenic forms of the human papillomavirus (HPV), particularly 16 type. About 60% of oropharyngeal squamous cell cancers in the USA are positive for HPV 16. HPV-associated head and neck squamous cell carcinoma seems to be a distinct clinical entity, and this malignant disease has a better prognosis than HPV-negative tumours, due in part to increased sensitivity of cancers to chemotherapy and radiation therapy. Although HPV is now recognised as a causative agent for a subset of oropharyngeal squamous cell carcinomas, the biology and natural history of oropharyngeal HPV infection and the best clinical management of patients with HPV-related head and neck squamous cell tumours is not well understood.

Epidemiology and risk factors

Head and neck cancer is the sixth most common cancer worldwide, with an estimated annual burden of 563 826 incident cases (including 274 850 oral cavity cancers, 159 363 laryngeal cancers, and 52 100 oropharyngeal cancers) and 301 408 deaths.[1] Although HPV has been long known to be an important cause of anogenital cancer, only in recent times has it been recognised as a cause of a subset of head and neck squamous cell carcinomas.[2] More than 100 different types of HPV exist,[3] and at least 15 types are thought to have oncogenic potential.[4] However, most (>90%) HPV-associated head and neck squamous cell cancers are caused by one virus type, HPV 16, the same type that leads to HPV-associated anogenital cancers.

The proportion of head and neck squamous cell carcinomas caused by HPV varies widely (figure 1),[5–16] largely because of the burden of tobacco-associated disease in this population of tumours. Tobacco, alcohol, poor oral hygiene, and genetics remain important risk factors for head and neck tumours overall, but HPV is now recognised as one of the primary causes of oropharyngeal squamous cell cancers. In the USA, about 40–80% of oropharyngeal cancers are caused by HPV, whereas in Europe the proportion varies from around 90% in Sweden to less than 20% in communities with the highest rates of tobacco use (figure 1).

The incidence of head and neck cancers overall in the USA has fallen in recent years, consistent with the decrease in tobacco use in this region. By contrast, incidence of HPV-associated oropharyngeal cancer seems to be rising, highlighting the increasing importance of this causal association.[17–19] In a US study in which data of the Surveillance, Epidemiology, and End Results (SEER) programme were used, incidence of oropharyngeal tumours (which are most likely to be HPV-associated) rose by 1·3% for base of tongue cancers and by 0·6% for tonsillar cancers every year between 1973 and 2004. By contrast, incidence of oral cavity cancers (not associated with HPV) declined by 1·9% every year during the same period.[17] Increasing incidence of oropharyngeal cancers was noted predominantly in white men (but not in women) in this study, and at young ages (figure 2).

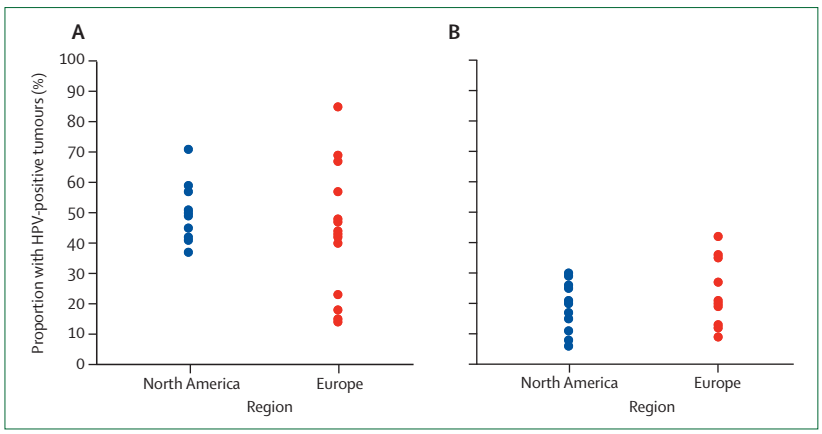

Figure 1: **Proportion of oropharyngeal (A) and head and neck (B) squamous cell carcinomas caused by HPV in North America and Europe**
Only studies with more than 25 oropharyngeal cancers (n=27)[2,5–13] or 50 head and neck tumours (n=30)[5–9,11,14–16] were included.

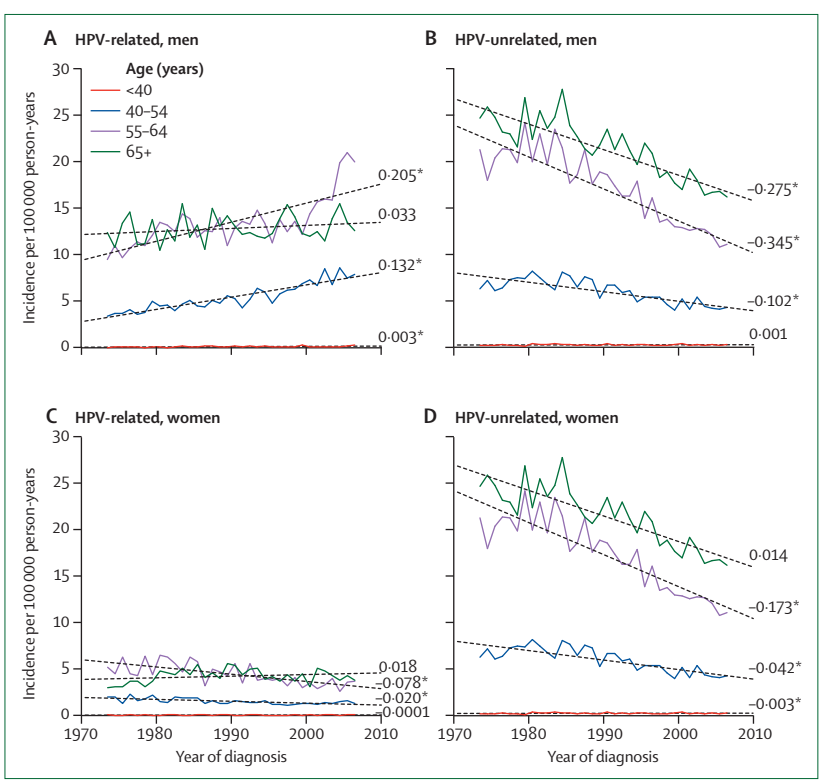

Figure 2: **Age-adjusted incidence of head and neck squamous cell cancers between 1973 and 2006, stratified by age at diagnosis**
The annual percent change in incidence for every age category is shown next to every line. *Slope with p<0·05. HPV-related sites include base of tongue, lingual tonsil, tonsil, oropharynx, and Waldeyer ring. HPV-unrelated sites include other and unspecified areas of the tongue, gum, floor of mouth, palate, and other parts of the mouth. Graph generated with assistance from Anil Chaturvedi.[17]

Similar to the USA, growth in incidence of oropharyngeal cancers has been reported internationally, including in Sweden,[20] the Netherlands,[21] and the UK.[22,23] In a study from the Swedish Cancer Registry during a similar period to the US study (1970–2002),[24] amplified oropharyngeal cancer rates were recorded, but rises were substantially larger than in the US study and happened in both women and men. The age-adjusted incidence of tonsillar cancer increased 3·5-fold in women and 2·6-fold in men between 1970 and 2002.[24] Augmented incidence of HPV-associated oropharyngeal cancers represents an emerging viral epidemic of cancer.

Why is increased incidence of HPV-associated oropharyngeal cancer most pronounced in young individuals? This effect could be attributable to changes in sexual norms (ie, more oral sex partners or oral sex at an earlier age in recent than past generations) combined with fewer tobacco-associated cancers in young cohorts, making the outcomes of HPV-positive cancers more visible. Can the higher rates of HPV-associated oropharyngeal cancers in men compared with women be accounted for solely by differences in sexual behaviour, or are biological differences in viral clearance present that could contribute to the higher burden of these cancers in men? HPV prevalence in cervical rather than penile tissue might boost the chances of HPV infection when performing oral sex on a woman, contributing to the higher rate of HPV-associated oropharyngeal cancer in men.

Tobacco use has fallen in past decades, and the corresponding rise in proportion of head and neck cancers that are oropharyngeal in origin has been striking, both in the USA and internationally. SEER data suggest that about 18% of all head and neck carcinomas in the USA were located in the oropharynx in 1973, compared with 31% of such squamous cell tumours in 2004.[19] Similarly, in Sweden, the proportion of oropharyngeal cancers caused by HPV has steadily increased, from 23% in the 1970s to 57% in the 1990s, and as high as 93% in 2007.[13,25] These data indicate that HPV is now the primary cause of tonsillar malignant disease in North America and Europe.

Despite the recognised importance of HPV in many oropharyngeal cancers, the epidemiology of oral HPV infection is not well understood (table 1). Findings of initial studies suggest that oral HPV frequency increases with age. Prevalent oral HPV infection is detected in 3–5% of adolescents[26–28] and 5–10% of adults.[14,29] We do not yet know whether the natural history of oral HPV or risk factors for persistent HPV infection in the oropharynx differ from those known for anogenital HPV infection (table 1). Data suggest oral HPV prevalence is amplified with number of sexual partners and is more typical in men, in HIV-infected individuals, and in current tobacco users.[26–28,30,31]

In view of the importance of tobacco use in head and neck squamous cell carcinoma, most cases of this malignant disease seen in non-smokers are unsurprisingly HPV-related. However, oral HPV infection is common in smokers and non-smokers and is an important cause of oropharyngeal cancer in both groups. For example, in case series, only 13–16% of individuals with HPV-positive head and neck squamous cell cancer did not smoke or drink alcohol.[32,33] Although a higher proportion of individuals with HPV-positive compared with HPV-negative tumours are non-smokers or neither

smoke nor drink alcohol, many with HPV-positive disease have a history of alcohol and tobacco use. In fact, 10–30% of HPV-positive head and neck squamous cell carcinomas were recorded in heavy tobacco and alcohol users.[32,33] This finding underscores that HPV-associated malignant disease not only arises in people who do not smoke or drink alcohol but also occurs in people with the traditional risk factors of tobacco and alcohol use.

HPV is a sexually transmitted infection, and findings suggest that the number of lifetime sexual partners is an important risk factor for development of HPV-associated head and neck squamous cell carcinoma. In case-control studies, the odds of HPV-positive malignant disease increased two-fold in individuals who reported between one and five lifetime oral sexual partners and five-fold in those with six or more, compared with those recalling no oral sex.[32–34] However, it is noteworthy that HPV-positive head and neck squamous cell cancer is present in individuals reporting few sexual partners. For example, of patients with HPV-positive tumours, more than half reported five or fewer lifetime oral sexual partners and 8–40% said they had never had oral sex.[32,33] Therefore, although sexual behaviour is an important risk factor for HPV-positive head and neck squamous cell carcinoma, the absence of a high number of sexual partners does not exclude the diagnosis.

Biology and clinical presentation

HPV-associated head and neck squamous cell carcinoma arises most commonly in the lingual and palatine tonsils.[35] HPV targets preferentially the highly specialised reticulated epithelium that lines tonsillar crypts; however, the intrinsic properties of this epithelium that render it vulnerable to HPV infection are not yet recognised.[36] Once the virus integrates its DNA genome within the host cell nucleus, it dysregulates expression of the oncoproteins E6 and E7.[37] The E6 protein induces degradation of P53 through ubiquitin-mediated proteolysis, leading to substantial loss of P53 activity. The usual function of P53 is to arrest cells in G1 or induce apoptosis to allow host DNA to be repaired. E6-expressing cells are not capable of this P53-mediated response to DNA damage and, hence, are susceptible to genomic instability. The E7 protein binds and inactivates the retinoblastoma tumour suppressor gene product pRB, causing the cell to enter S-phase, leading to cell-cycle disruption, proliferation, and malignant transformation.[37]

Morphologically, head and neck squamous cell cancers are usually characterised as moderately differentiated keratinising, but HPV-positive carcinomas deviate from this type. Consistent features of these tumours are that they: arise from tonsillar crypts; are not associated with dysplasia of surface epithelium; show lobular growth; are permeated by infiltrating lymphocytes; do not undergo clinically significant keratinisation; and have a prominent basaloid morphology.[38] Clinically, HPV-positive tumours present mostly at an early T stage and advanced nodal stage (table 2).[39] In general, HPV-associated oropharyngeal cancers at presentation are stage III or IV. Nodal metastases are usually cystic and multilevel.[40]

Pathological diagnosis

HPV detection may ultimately serve a more comprehensive role than mere prognostication. Detection of HPV is emerging as a valid biomarker for discerning the presence and progress of disease encompassing all aspects of patients' care, from early cancer detection,[41] to more accurate tumour staging (eg, localisation of tumour origin),[42,43] to selection of patients most likely to benefit from specific treatments,[44] to post-treatment tumour surveillance.[45,46] Consequently, there is a pressing need for a method of HPV detection that is highly accurate, reproducible from one diagnostic laboratory to the next, and practical for universal application in the clinical arena.

Despite growing calls for routine HPV testing of all oropharyngeal carcinomas, the best method for HPV detection is not established. Various techniques are currently in use, ranging from consensus and type-specific PCR methods, real-time PCR assays to quantify viral load, type-specific DNA in-situ hybridisation, detection of serum antibodies directed against HPV epitopes, and immunohistochemical detection of surrogate biomarkers (eg, P16 protein). Although PCR-based detection of HPV E6 oncogene expression in frozen tissue samples is generally regarded as the gold standard for establishing the presence of HPV, selection of assays for clinical use

	Unanswered questions
HPV causes a subset of oropharyngeal cancers	Does HPV cause cancer at any other head and neck squamous cell carcinomas subsites?
Oral HPV is sexually transmitted	Which exact sexual behaviours are associated with transmission?
HPV-positive and HPV-negative head and neck squamous cell carcinomas are distinct cancers	How common is prevalent and persistent oral HPV infection in the general population?
Anogenital HPV infections are common but most clear on their own	What is the natural history of oral HPV? What is the median time from oral HPV infection to cancer? Which factors affect oral HPV persistence and progression to cancer?
Incidence of HPV-positive oropharyngeal cancer is increasing in some groups	Why is the increase in incidence of oropharyngeal cancer: Seen in men but not women? Most apparent in younger cohorts?
P16 immunohistochemistry is strongly associated with tumour HPV 16 status of oropharyngeal cancers	Do patients with HPV-negative, P16-positive and HPV-positive, P16-positive oropharyngeal cancers have similar survival outcomes?
HPV-positive head and neck squamous cell carcinomas have better median survival than HPV-negative tumours	Should treatment of people with HPV-positive and HPV-negative head and neck squamous cell carcinomas be different?
Of people with HPV-positive head and neck squamous cell carcinomas, non-smokers have better median survival than smokers	What is the biological mechanism for different survival rates in people with HPV-positive head and neck squamous cell carcinomas who use tobacco versus non-users?
Oropharyngeal cancers are generally detected at a late stage	Do precancerous oropharyngeal lesions exist (ie, which could be detected by oral Pap)? Can testing for persistent oral HPV infection be a useful screening method?
A cure for established HPV infection is not known, but HPV vaccines can prevent new cervical HPV infections	What is the effectiveness of HPV vaccines against oral HPV infection?

Table 1: **Summary of knowledge about HPV-related head and neck squamous cell carcinoma**

	HPV-positive tumours	HPV-negative tumours
Anatomical site	Tonsil and base of tongue	All sites
Histology	Non-keratinised	Keratinised
Age	Younger cohorts	Older cohorts
Sex ratio	3:1 men	3:1 men
Stage	Tx, T1–2	Variable
Risk factors	Sexual behaviour	Alcohol and tobacco
Incidence	Increasing	Decreasing
Survival	Improved	Unchanging

Table 2: **Differences between HPV-positive and HPV-negative head and neck squamous-cell carcinomas**

will ultimately be influenced by concerns relating to sensitivity, specificity, reproducibility, cost, and feasibility.

Development of non-fluorescent chromogens has enabled visualisation of DNA hybridisation by conventional light microscope; furthermore, adaptation of in-situ hybridisation to formalin-fixed and paraffin-embedded tissues has made this technique compatible with standard tissue-processing procedures and amenable to retrospective analysis of archival tissue blocks. Most PCR-based methods, on the other hand, need a high level of technical skill and are best used with fresh-frozen samples. In-situ hybridisation permits direct visualisation of HPV distribution in tissue samples (figure 3). Localisation of the HPV genome to tumour cell nuclei allows us to distinguish between etiologically relevant HPV detection (clonal presence in all tumour cells) and virus or contamination (low copy detection in only a few cells). By contrast, mere detection of virus by non-quantitative PCR-based methods does not distinguish transcriptionally active (ie, clinically relevant) from transcriptionally inactive (ie, clinically irrelevant) HPV infections. The superior sensitivity of in-situ hybridisation does not compromise its specificity. Introduction of various signal amplification steps has greatly boosted sensitivity of this technique, even to the point of viral detection down to one viral copy per cell.

In HPV-positive oropharyngeal carcinomas, as described previously (See Biology and clinical presentation), transcription of the viral oncoprotein E7 is known to inactivate function of the *RB* gene product, causing perturbation of other key components of the retinoblastoma pathway, and to induce upregulation of P16 expression, reaching levels that can be detected readily by immunohistochemistry.[37,47] Accordingly, P16 immunohistochemistry is sometimes advocated as a surrogate marker of HPV infection for oropharyngeal cancers.[48,49] In our experience, comparison of P16 immunohistochemical staining and HPV 16 in-situ hybridisation for large numbers of head and neck squamous cell carcinomas shows that these methods are discordant in 7% of cases (unpublished observation). Discrepancies are consistent for cancers that are negative by HPV 16 in-situ hybridisation but positive by P16 immunohistochemistry. Since the P16 assay cannot discern HPV type, the higher rate of positivity might indicate detection of non-HPV 16 types that comprise 5–10% of HPV-positive oropharyngeal cancers. Alternatively, P16 overexpression could suggest pRB pathway disturbances unrelated to HPV (eg, mutational inactivation of retinoblastoma protein). Using E6 and E7 mRNA levels as conclusive evidence of HPV involvement, P16 immunostaining of head and neck squamous cell carcinomas is 100% sensitive but only 79% specific as a surrogate marker of HPV infection.[50] Although data suggest that P16 overexpression could predict clinical outcomes independent of HPV status,[49,51] replacement of HPV in-situ hybridisation by P16 immunohistochemistry is premature and awaits further confirmation of similar survival outcomes for patients with HPV-negative, P16-positive and HPV-positive, P16-positive oropharyngeal cancers.

Limitations of any one detection assay can be offset by algorithms that combine the strengths of complementary assays.[50] A highly feasible strategy incorporates P16 immunohistochemistry and HPV in-situ hybridisation. In view of sensitivity that approaches 100%, P16 immunostaining is a good first-line assay for elimination of HPV-negative cases from any additional analysis. Since specificity is almost 100%, a finding positive for HPV 16 on in-situ hybridisation reduces the number of false-positive cases by P16 staining alone. A P16-positive, HPV 16-negative result singles out a subset of tumours that qualifies for rigorous analysis for other (ie, non-HPV 16) oncogenic virus types. This third-tier analysis could include wide-spectrum in-situ hybridisation probes that detect an extended panel of HPV types, or PCR-based methods for detection of transcriptionally active virus.[50] Whichever the method used to establish the presence of non-HPV 16 virus types, upfront use of P16 immunostaining and HPV 16 in-situ hybridisation accurately establishes the HPV status of most oropharyngeal cancers.

HPV in-situ hybridisation and P16 immunostaining as a practical diagnostic approach to discernment of HPV status can be applied readily to cytological preparations, including fine-needle aspirates from patients with cervical lymph-node metastases.[41,52] Further expansion of HPV testing to blood and other body fluids would advance the role of HPV as a clinically relevant biomarker, but these specimens would need other detection platforms. PCR-based detection of HPV DNA in blood[53] and saliva[54] of patients after treatment of their HPV-positive cancers suggests a future role in tumour surveillance. Detection of serum antibodies to HPV-related epitopes can predict the HPV status of head and neck cancers, and this method has been advocated as a way to project clinical outcomes and guide treatment without the constraints of tissue acquisition.[53,55]

Although HPV in-situ hybridisation could serve as a starting point for routine and universal analysis of oropharyngeal carcinomas, HPV detection alone might not exploit fully its potential as a biomarker. A more advanced understanding of HPV-induced tumorigenesis—

including the complex interaction of HPV infection with interconnecting molecular genetic pathways—will inevitably drive implementation of increasingly elaborate and comprehensive assays. Disruptive *TP53* mutations,[56] aberrant *BCL2* expression,[57] overexpression of epidermal growth factor receptor (*EGFR*),[58] and other pathway disturbances can act individually or in concert to modulate the prognostic effect of HPV detection, needing expanded biomarker profiling in conjunction with HPV analysis. Moreover, the finding that therapeutic responses can correlate with HPV copy number suggests a future role for quantitative measurement of viral load.[58]

Management

The standard of care for locally advanced (T3–T4 or N2–N3) oropharyngeal cancer is either surgery and adjuvant radiotherapy with or without concurrent cisplatin, as indicated, or more usually, concurrent chemoradiation for preservation of speech and swallowing function, which is especially applicable to management of disease at the base of the tongue or tonsil. This approach became the standard of care after publication of a multicentre, randomised controlled trial of 226 patients with stage III or IV squamous cell cancer of the oropharynx that was undertaken in France.[59] Patients were randomly assigned to either radiotherapy alone (70 Gy, 35 fractions) or the same radiotherapy regimen with concomitant carboplatin and fluorouracil.[59] 3-year survival (51% *vs* 31%; p=0·02) and disease-free survival (42% *vs* 20%; p=0·04) rates were raised significantly with addition of chemotherapy to radiotherapy. Rates of local-regional recurrence and death from oropharyngeal cancer were also reduced significantly with combined treatment; however, occurrence of distant metastases did not differ between treatments. These results were maintained at 5 years.[60] It is noteworthy that the concomitant treatment group showed greater acute toxic effects, including mucositis-related weight loss, feeding-tube dependency, and myelosuppression, but a significant difference in late effects was noted only for dentition.[61] Also of note is that the low survival outcomes, relative to current data from the USA, relate to the population enrolled and traditional risk factors of tobacco and alcohol.

The increasing prevalence of oropharyngeal cancer in young populations and substantially amplified survival rates with current treatment approaches stands in contrast to survival achieved in older individuals with comorbid disorders associated with tobacco and alcohol history. Several characteristics of patients with head and neck cancer have been linked with favourable prognosis, including non-smoker, minimum exposure to alcohol, good performance status, and no comorbid disorders, all of which are related to HPV-positive tumour status. Findings of retrospective analyses suggest that individuals with HPV-positive oropharyngeal cancer have higher response rates to chemotherapy and radiation and increased survival[62–65] compared with those with HPV-negative tumours. Augmented sensitivity to chemotherapy and radiotherapy has been attributed to absence of exposure to tobacco and presence of functional unmutated *TP53*.[63,64,66] Increased survival of patients with HPV-positive cancer is also possibly attributable in part to absence of field cancerisation related to tobacco and alcohol exposure.[67] HPV-positive

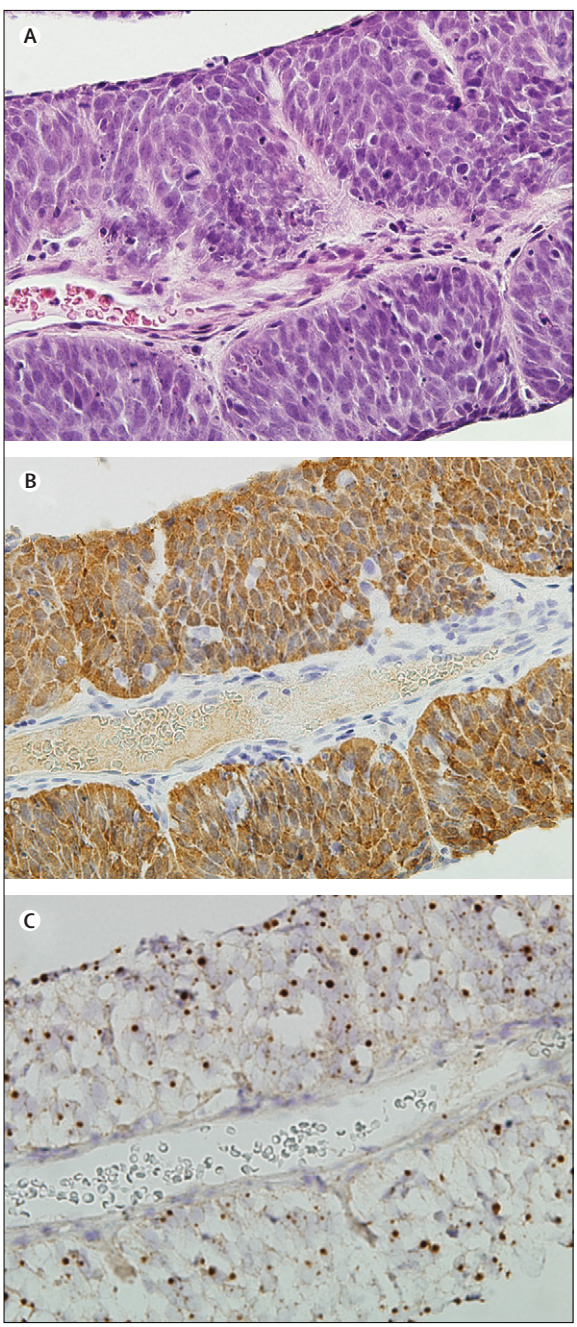

Figure 3: **Strips of metastatic non-keratinised squamous cell carcinoma aspirated from a cystic neck mass**
(A) Haematoxylin and eosin staining. Presence of HPV is visualised as strong cytoplasmic and nuclear staining for P16 by immunohistochemistry (B) and as dot-like hybridisation signals within nuclei of tumour cells by HPV 16 in-situ hybridisation (C).

tumours are more sensitive to cytotoxic chemotherapy and DNA damage-induced apoptosis secondary to incorporation of the viral oncoproteins E6 and E7.[68,69]

In 2008, a prospective clinical trial in patients with head and neck squamous cell carcinoma was published that correlated tumour HPV status with outcome.[70] The US National Cancer Institute-funded Eastern Cooperative Oncology Group (ECOG) undertook a phase 2 trial testing non-surgical management of individuals with clinical stage III or IV squamous cell carcinoma of the oropharynx or larynx. All tumours were assessed for HPV 16 by in-situ hybridisation and P16 status by immunohistochemistry. Treatment consisted of induction chemotherapy with two cycles of carboplatin and paclitaxel followed by weekly paclitaxel concurrent with standard-fractionation radiation therapy (total dose 70 Gy in 35 fractions over 7 weeks). HPV 16 was detected by in-situ hybridisation in 63% (38/60) of oropharyngeal tumour specimens and all samples showed high expression of P16.[70] These patients were predominantly white men with fewer than 20 pack-years of cigarette use, and histology of specimens was poorly differentiated squamous cell carcinoma with basaloid features. HPV status correlated with treatment response, progression-free survival, and overall survival and all outcomes were better in the HPV-positive versus the HPV-negative population. Respective response rates to induction chemotherapy were 82% versus 55% (difference 27% [95% CI 9·3–44·7%]; p=0·01); response at completion of chemoradiation was 84% versus 57% (27% [9·7–44·3%]; p=0·007); progression-free survival at 2 years was 86% versus 53% (33% [12·7–53·3%]; p=0·02 [log-rank test]); and overall survival at 2 years was 95% versus 62% (33% [18·6–47·4%]; p=0·005 [log-rank test]).[70]

When the analysis was restricted to patients with oropharyngeal cancer,[70] those with HPV-positive tumours had a significantly better outcome compared with HPV-negative oropharyngeal carcinomas: overall survival at 2 years was 94% and 58% (p=0·004), respectively, and progression-free survival at 2 years was 85% and 50% (p=0·05), respectively. In the same study, acute toxic effects were reported as acceptable with this regimen: 49% of patients with oropharyngeal cancer had moderate-to-severe swallowing impairment 3 months after treatment and only 3% were still dependent on a feeding tube after 12 months. These good survival results, which suggest increased sensitivity to chemotherapy and radiotherapy in HPV-positive patients, have generated interest into assessment of the association between HPV 16, P16, and tobacco exposure and into design of clinical trials with less toxic regimens for HPV-positive patients.

The association between HPV status, P16, tobacco exposure, and survival was investigated by retrospective analysis of a large phase 3 trial, in which standard fractionation radiotherapy and cisplatin were compared with accelerated fraction radiotherapy and cisplatin.[71] In this study, more than 400 patients with oropharyngeal cancers were enrolled, of whom 61% (198/323) had tumours that were positive for HPV 16 by in-situ hybridisation. P16 was positive in 96% of HPV-positive patients and 22% of HPV-negative patients. The results of the analysis were consistent with findings of the ECOG prospective trial.[70] At median follow-up of 4·4 years, patients with HPV-positive oropharyngeal cancer had significantly better 2-year overall survival (87·5% [82·8–92·2] *vs* 67·2% [58·9–75·4]; p<0·0001) and 2-year progression-free survival (71·9% [65·5–78·2] *vs* 51·2% [42·4–59·9]; p<0·0001), compared with HPV-negative patients. Survival outcomes for individuals with HPV 16-positive and P16-positive oropharyngeal tumours were similar. Failure data indicated significantly diminished rates of locoregional failure and second primary tumour in patients with HPV-positive oropharyngeal cancer compared with those with HPV-negative tumours; distant metastases did not differ between the two groups. When survival was assessed after adjustment for tobacco exposure, in individuals who smoked, those with HPV-positive oropharyngeal tumours and fewer than 20 pack-years had 2-year overall survival of 95%, compared with 80% in those with HPV-positive cancers and 20 pack-years or more, and 63% in HPV-negative cancers and 20 pack-years or more. By comparison with people with HPV-positive oropharyngeal tumours who smoked and had fewer than 20 pack-years, the hazard of death was raised for those with HPV-negative tumours and 20 pack-years or more (hazard ratio 4·33) and those with HPV-positive cancers and 20 pack-years or more (1·79). These data indicate clearly that tobacco exposure alters the biology of HPV-positive oropharyngeal tumours and is an important prognostic factor.

An association between HPV-positive, P16-positive oropharyngeal tumours and survival outcomes was reported in another retrospective analysis of a large phase 3 trial of chemoradiation, which included more than 800 patients enrolled from international sites.[72] This substudy analysis looked at 195 available tumour samples in patients with an oropharyngeal primary cancer, of which 28% were HPV-positive and 58% were P16-positive. Individuals with HPV-positive cancers had 2-year overall survival of 94% and 2-year failure-free survival of 86% compared with 77% (p=0·007) and 75% (p=0·035), respectively, in those with HPV-negative tumours. When co-expression of HPV and P16 was correlated with survival outcomes, individuals with HPV-positive, P16-positive tumours had 2-year overall survival of 95% compared with 88% in those with HPV-negative, P16-positive cancers and 71% (p=0·003) in those with HPV-negative, P16-negative tumours. Similar results were noted for 2-year failure-free survival (89%, 86%, and 69%, respectively; p=0·002) and time to locoregional failure (93%, 95%, and 84%, respectively; p=0·051). By multivariable analysis, HPV 16 and P16 were identified as independent prognostic factors. After median follow-up of 27 months, locoregional failure rates were reduced substantially in patients with

HPV-positive or P16-positive tumours, and no difference was seen in distant failure compared with individuals with HPV-negative, P16-negative cancers. This study concluded that patients with HPV-positive and P16-positive tumours have better prognosis than those with HPV-negative, P16-negative cancers.

Investigators from the University of Michigan analysed oropharyngeal tumour specimens from two sequential phase 2 chemoradiation trials for presence of HPV 16.[73] HPV DNA was detected by PCR analysis that could detect 15 high-risk subtypes. About 81% (102/124) of patients had HPV-positive tumours. HPV status and tobacco use were correlated with local, regional, or distant failure, development of second primary tumours, and survival outcomes. Of the individuals with HPV-positive tumours, 32% were never smokers, 45% were former smokers, and 23% were current smokers. The investigators reported that never smokers with HPV-positive cancers were a more favourable group—with augmented survival outcomes and time to recurrence—than current or former smokers with HPV-positive tumours. Of the never smokers, 88% remained alive with no evidence of disease recurrence at median follow-up (76 months in the first trial and 36 months in the second trial). Data of this study highlight the need to investigate further the effect of tobacco exposure on biology of HPV-positive tumours.

More than 90% of head and neck cancers express *EGFR*, and high expression of EGFR and *EGFR* gene copy number is associated with poor prognosis.[74] Kumar and colleagues[58] investigated the correlation between EGFR expression, P16, BCL2L1, P53, HPV titre, and response to treatment (induction chemotherapy, chemoradiation) in 50 patients with oropharyngeal tumours positive for HPV 16. The combination of low EGFR and high P16 expression correlated highly with better clinical outcome compared with high EGFR expression and low HPV titre or high EGFR and low P16 expression, after adjustment for age, sex, smoking status, TN stage, and primary site. The findings of this study emphasise the need to include EGFR status in addition to HPV status in future clinical trials of oropharyngeal cancers. This additional prognostic factor would help to identify high-risk patients with HPV-positive tumours.

Future direction of treatment

On the basis of prospective and retrospective analyses of data from clinical trials, HPV-positive oropharyngeal cancer is recognised as a distinct subset of head and neck squamous cell carcinoma with a favourable outcome. In future clinical trials, researchers will, at the very least, need to stratify for HPV status. An opportunity now exists to investigate less intense treatment strategies that do not compromise survival outcomes but lower the risk of potentially debilitating late effects. For the most part, patients with HPV-positive oropharyngeal cancer are young and in good health. Thus, provision of a high level of quality of life and the fewest treatment complications are important considerations. Potential long-term side-effects of concurrent chemoradiation include dysphagia, xerostomia, feeding-tube dependency from fibrosis and scarring of the pharyngeal muscles, chronic aspiration, and chronic fatigue.

The National Cancer Institute's head and neck steering committee and task forces met in November, 2008, to consolidate data available on the epidemiology, natural history, and diagnosis of HPV-associated head and neck squamous cell carcinoma, and they reviewed all completed and ongoing clinical trials that have assessed HPV status.[75] Two major issues discussed in this review are statistical and design concerns and their effect on development of future clinical trials based on HPV status.

ECOG and the Radiation Therapy Oncology Group are planning phase 2 clinical trials in patients with HPV-positive tumours. ECOG proposes induction chemotherapy with a triple drug regimen to reduce tumour burden to subclinical disease (clinical complete response at primary site) followed by lower dose radiation (total dose 54 Gy) and concurrent cetuximab. Overall survival and progression-free survival outcomes will be assessed and compared with results of the 2008 ECOG study.[70] The main aim of this planned study is to assess potential for a lower dose of radiation to control disease and to investigate toxic effects and quality-of-life variables.

Concluding remarks

In summary, tumour HPV status is a prognostic factor for overall survival and progression-free survival and might also be a predictive marker of response to treatment. The method of in-situ hybridisation provides a feasible approach for implementation in most diagnostic pathology laboratories, and immunohistochemical staining for P16 could be useful as a surrogate marker for HPV status. Seemingly, locoregional recurrence—but not the rate of distant disease—is diminished in patients with HPV-positive tumours. Smoking and tobacco exposure might modify survival and recurrence of HPV-positive tumours and should be considered in future trials for risk stratification of patients with HPV-positive malignant disease.

HPV-associated oropharyngeal cancer represents a distinct clinical and biological entity with many unresolved issues that will be studied in future translational, clinical research. We need to further investigate and understand why the disease arises predominantly in men and whether the natural history of oral HPV infection differs in men and women. The best tests are needed for HPV diagnosis, and use of HPV DNA copy number for outcome and early relapse needs to be looked into. Opportunities for primary and secondary prevention should be assessed, including use of HPV vaccines against infection and therapeutic vaccines in the adjuvant setting for locoregional recurrence and distant disease. Finally, we face the challenge of designing clinical trials with appropriate risk stratification that will lead to identification of the least morbid treatment

Search strategy and selection criteria

Data for this review were identified by searches of Medline, Current Contents, PubMed, and references from relevant articles, with the search terms "head and neck squamous cell cancer", "oropharynx", "HPV16", and "p16". Abstracts and reports from meetings were included only when they related directly to previously published work. Only papers published in English and French between January, 1980, and November, 2009, were included.

that can cure patients with this malignant disease. Extended follow-up is essential to better understand the natural history and failure patterns.

Contributors

All authors contributed equally to the planning, writing, and editing of this manuscript, and to production of figures.

Conflicts of interest

The authors declared no conflicts of interest.

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