Head and neck cancer is a broad term that encompasses a large number of tumour entities that originate from different sub-sites, such as the nasal cavity, nasopharynx, oral cavity, oropharynx, larynx, hypopharynx, and salivary glands. It is the sixth most common cancer worldwide. In Belgium, in the year 2011, 2628 people were diagnosed with head and neck cancer. Of these, 536 cancers originated in the oropharynx; 404 in male patients and 132 in female patients (Belgian Cancer Registry). In the United States, there will be an estimated 55,070 new cases of head and neck cancer in the year 2014. By far, head and neck squamous cell carcinoma (HNSCC) is the most common histological subtype (85%).

Exposures to tobacco and/or alcohol are well-known, important risk factors for developing HNSCC. With the decreasing prevalence of smoking in the western world, the incidence of HNSCC at most subsites has stabilized, or even decreased, over recent years. On the other hand, the incidence of oropharyngeal cancer (particularly tonsillar cancer and the base of tongue cancer) appears to be increasing. This trend has been attributed to an increase in oncogenic human papilloma virus (HPV) infections, another important risk factor for the development of HNSCC. HPV has long been known to be an important cause of cervical, penile, and anal cancer and of genital warts. In recent years, the importance of HPV in the development of oropharyngeal cancer has become elucidated. In particular, HPV 16 and 18 play important roles in oropharyngeal cancer.

In the United States, about 40-80% of oropharyngeal cancers are caused by HPV. In Europe, this proportion varies from around 90%, in Sweden, to less than 20% in communities with high rates of tobacco use. In Belgium, a recent analysis at our centre showed that 24.78% of oropharyngeal cancer cases were associated with HPV.

The increased incidence of HPV-associated oropharyngeal cancer is most pronounced among young individuals. This effect could be attributable to changes in sexual behaviour. The risk factors for HPV include multiple oral sex partners, oral sex at a young age, unprotected sex, and a history of sexually transmitted diseases (particularly genital warts). Findings have suggested that two
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lines the tonsillar crypts; however, the intrinsic properties of this epithelium that render it vulnerable to HPV infection have not been identified.

Once the virus integrates its DNA genome within the host cell nucleus, it dysregulates expression of the oncoproteins E6 and E7 (Figure 1) (1). Dysregulation of E6 and E7 plays an important role in the development of cervical cancer as well. The E6 protein induces degradation of p53 through ubiquitin-mediated proteolysis, which leads to substantial loss of p53 activity. The function of p53 is to arrest cells in G1 to allow host DNA repair or induce apoptosis. E6-expressing cells are not capable of this p53-mediated response to DNA damage, and thus, they are susceptible to genomic instability.

Pathophysiology of HPV-positive head and neck tumours

HPV-associated head and neck squamous cell carcinoma commonly arises in the base of tongue and the palatine tonsils. HPV preferentially infects the highly specialised, reticulated epithelium that important risk factors are a large number of sexual partners over a lifetime and practicing anal intercourse. Transmission of HPV, however, cannot be fully prevented by the use of a condom. The infection can be transmitted through saliva or during birth, when the mother is infected.

At least 80% of sexually active men and women become infected with HPV at some point during their lifetime. However, most of these infections disappear spontaneously, after 1 or 2 years. An infection that persists can transform into a malignant lesion.

Figure 1
HPV pathogenesis

Transmission of HPV, however, cannot be fully prevented by the use of a condom. The infection can be transmitted through saliva or during birth, when the mother is infected. HPV preferentially infects the highly specialised, reticulated epithelium that lines the tonsillar crypts; however, the intrinsic properties of this epithelium that render it vulnerable to HPV infection have not been identified.

Once the virus integrates its DNA genome within the host cell nucleus, it dysregulates expression of the oncoproteins E6 and E7 (Figure 1) (1). Dysregulation of E6 and E7 plays an important role in the development of cervical cancer as well. The E6 protein induces degradation of p53 through ubiquitin-mediated proteolysis, which leads to substantial loss of p53 activity. The function of p53 is to arrest cells in G1 to allow host DNA repair or induce apoptosis. E6-expressing cells are not capable of this p53-mediated response to DNA damage, and thus, they are susceptible to genomic instability. The E7 protein binds the 2 ubiquitin ligase complex and inactivates the retinoblastoma (RB) tumour suppressor gene product, pRB, which causes the cell to enter S-phase; the lack of pRB activity leads to cell-cycle disruption, proliferation,
and malignant transformation. Elevated expression of E7 also leads to inactivation of negative feedback mechanisms, which ultimately leads to upregulation of p16. This upregulation can be used in clinical practice as a surrogate marker for HPV infection.

Ultimately, the genomic instability caused by dysregulation of E6 and E7 can lead to carcinogenesis.

**Biological and clinical presentation**

HPV-positive HNSCC has a distinct risk profile and pathophysiology. This disease is also characterized by a specific biological and clinical presentation, compared to HPV-negative tumours. The most important differences are summarized in Table 1.

Clinically, HPV-positive tumours present mostly with an early tumour (T) classification; however, with an advanced nodal (N) classification. Consequently, the majority of these tumours are diagnosed in a locally advanced stage. Patients with HPV-positive HNSCC tend to be younger than patients with HPV-negative tumours, with a mean age difference of 4-10 years. This age difference may explain the observation that patients with HPV-positive HNSCC tend to have less comorbidity than those with HPV-negative tumours. Moreover, patients with HPV-positive tumours generally have a higher socio-economic status and higher education than those with HPV-negative tumours. Smoking and alcohol misuse are less common in patients with HPV-positive tumours than in those with HPV-negative tumours.

HPV-positive tumours have some consistent features. For example, they tend to originate from the tonsillar crypts. Mostly, they are not associated with dysplasia of the surface epithelium. This could explain why secondary tumours are less common in HPV-positive HNSCC than in head and neck tumours related to alcohol and smoking.

HPV-positive and HPV-negative tumours have similar rates of distant metastases; however, they show different patterns of metastases. Patients with HPV-positive tumours are more at risk for brain and liver metastases, and those with HPV-negative tumours are more at risk for lung and bone metastases. Another important finding is that patients with HPV-positive HNSCC remain at risk of developing metastases for a longer time period than those with HPV-negative tumours. Thus, in cases of disease dissemination, metastasis occurs within the first 2 years after a HPV-negative tumour diagnosis and within the first 5 years after a HPV-positive tumour diagnosis. From an imaging point of view, the borders of HPV-positive HNSCC lesions are more well-defined than those of HPV-negative HNSCC lesions. Also, nodal metastases are more often cystic in a HPV-positive HNSCC than in a HPV-negative HNSCC. Therefore, radiologists should be alert for underlying malignancies, when evaluating cystic neck lesions in adult patients that lack significant tobacco or alcohol exposure.

**Pathological diagnosis**

HPV detection may serve a more extended purpose than merely prognosis. For example, several studies have shown that, among patients with head and neck cancer, HPV positivity was strongly associated with primary tumour location in the oropharynx.
Thus, detection of HPV is emerging as a valid biomarker for early cancer detection, accurate tumour staging, and selection of patients most likely to benefit from specific treatments.16

Consequently, there is a pressing need for a method of HPV detection that is highly accurate, reproducible, and sufficiently practical for wide application.18 HPV testing is most worthwhile for oropharyngeal tumours, because the causal link is weaker between HPV and tumours in other subsites of the head and neck region.2 Importantly, HPV is present both in the tumour and in nodal and distant metastases; thus, it is possible to test for HPV in a nodal metastasis.2

Unfortunately, the best method for HPV detection remains to be established. Various techniques are currently in use.2,6 Although PCR-based detection of HPV E6 oncogene expression in fresh-frozen tissue samples is generally regarded the gold standard for establishing the presence of HPV, there are concerns about reproducibility, cost, and feasibility.19

Both in-situ hybridisation and PCR are commonly used for detecting HPV. Most PCR-based methods are best used with fresh-frozen samples. However, expertise is essential in performing these methods. Alternatively, in-situ hybridisation permits direct visualisation of the HPV distribution in tissue samples. Moreover, localisation of the HPV genome in tumour cell nuclei allows a distinction between aetiologically-relevant HPV and HPV contamination.2

In HPV-positive oropharyngeal carcinomas, the viral oncoprotein, E7, is known to inactivate the RB gene product. Without RB activity, other key components are perturbed in the retinoblastoma pathway. One result is the upregulation of p16 expression to levels that can be detected readily with immunohistochemistry. Therefore, p16 is used as a surrogate marker of HPV infection for oropharyngeal cancers. E6 and E7 mRNA expression are generally taken as conclusive evidence of HPV involvement. By comparison, immunostaining for p16 in HNSCC tissues is 100% sensitive, but only 79% specific for detecting HPV infection.15 This 21% false positivity might be due to non-HPV-related p16 upregulation, which may occur when the pRB pathway is defective. Immunohistochemical staining is easy to perform in daily clinical practice, and therefore, it is widely available.3

**Treatment and prognosis**

HPV-positive oropharyngeal cancer is recognised as a distinct subset of HNSCC, which has a relatively favourable prognosis. Patients with HPV-positive tumours had better 5-year survival rates, higher loco-regional control rates, and better responses to therapy compared to those with HPV-negative tumours.1,3,6-11 Differences in survival rates between HPV-positive and HPV-negative cases have varied by up to 30% among different studies. Most studies were based on treatment with concomitant chemoradiotherapy. HPV-positive tumours were also reported to show better outcomes after surgical treatment compared to HPV-negative tumours; however, most patients were also treated with postoperative radiotherapy.12,18-20

The prognosis in HPV-positive cancer is dependent on tobacco use.20 The risk of tumour progression in HPV-positive HNSCC increases by 1% per year in patients that habitually smoke tobacco. On the other hand, smoking cessation is associated with a decrease in the oral HPV viral load.20 Furthermore, studies suggest that prognosis is the same for both genders. Racial differences have been described; Caucasians have been found to have the best prognosis among several different races.5

The finding that HPV-positive HNSCC had a higher sensitivity to chemotherapy and RT than HPV-negative HNSCC has been attributed to the different characteristics of the corresponding patient populations. The characteristics linked to a favourable prognosis among patients with head and neck cancer included non-smoking (and thus, less in field cancerisation), minimum exposure to alcohol, good performance status, and no comorbidity. All of these characteristics are more prevalent among patients with HPV-positive tumours than among patients with HPV-negative tumours. However, age, tobacco-use, and overall health only contributed to 10% of the difference in progression-free survival between these groups.3 Therefore, other factors must play a role. These findings suggested that HPV-positive tumours may have a higher sensitivity to RT and chemotherapy than HPV-negative tumours; however, the underlying biological mechanisms remain unclear. Several hypotheses have been suggested recently, including an association with a distinct immune response (activation of viral antigens during
therapy), the absence of p53 mutations, and the elevated p16 expression (5). Indeed, HPV-positive HNSCCs have shown few or no mutations in the tumour suppressor gene, p53. Tumours with intact p53 genes are known to be more susceptible to radiation-induced apoptosis. In our lab, we recently found an association between p16-positive tumours and reduced DNA repair mechanisms. This difference could contribute to a high sensitivity to RT.21

The difference in prognosis between HPV-positive and HPV-negative HNSCCs has given rise to the opportunity to investigate less intense treatment strategies for patients with HPV-positive HNSCC. These strategies would not compromise survival outcomes, but could lower the risk of potentially debilitating late side effects.22-23 For the most part, patients with HPV-positive oropharyngeal cancer are young and in good health. Thus, it is important to consider new strategies that might provide a better quality of life and fewer treatment complications.22 Chemoradiotherapy is often the treatment of choice in locally advanced HNSCC. Potential long-term side-effects of this treatment include dysphagia, xerostomia, feeding-tube dependency (due to fibrosis and scarring of the pharyngeal muscles), chronic aspiration, and chronic fatigue. Currently, the following less-intense treatment strategies are now under investigation for HPV-positive tumours.

1) Induction chemotherapy with modification of the RT dose: Currently two trials are investigating this strategy. The RT dose is being modified according to the response. In the ECOG 1308 study, 54 Gy has been applied with good response; in the study of Mehrotra et al., 66 Gy is being applied.23

2) Replacing Cisplatin with Cetuximab®: This strategy aims to reduce side effects. Currently, two large studies are being conducted: the De-ESCALaTE study and the RTOG 1016 study.25

3) The use of transoral robotic surgery (TORS): This strategy also aims to reduce side effects in this patient group.21

4) An immunotherapeutic approach: This strategy employs vaccines intended to stimulate an antitumour immune response. An ongoing study at the Sidney Kimmel Comprehensive Cancer Centre (Johns Hopkins University) aims to employ a HPV-16 DNA vaccine. Currently, this study is recruiting patients, and no data are available. However, in a previous study, good results were shown in women with HPV-16-positive, grade 3, vulvar intraepithelial neoplasia. Those patients were vaccinated three to four times with a mixture of long peptides based on sequences from the HPV-16 viral oncoproteins E6 and E7. In the future, for better treatment efficiency, these vaccination approaches may be combined with traditional therapy modalities, like surgery or chemoradiation.24

We must await the results of these trials before we can change the current treatment paradigm for HPV-positive head and neck cancer.

Prevention

Currently, there is no treatment for HPV infections. Therefore, prevention is a very important strategy for reducing the number of patients with HPV-positive HNSCC. Gardasil® and Cervarix® are two vaccines that are currently used to prevent HPV-related cervical cancer. The former prevents infection with HPV-6, -11, -16, and -18; the latter prevents infection with HPV subtypes 16 and 18. At the moment, only young girls are receiving vaccinations to prevent cervical HPV infections. The value of this vaccine in head and neck cancer and in anogenital cancer must be confirmed in both sexes.

Currently, there are no clear data on the use of these vaccines to prevent infection or on the use of therapeutic vaccines in the adjuvant setting for locoregional recurrence and distant disease. Therefore, opportunities for primary and secondary prevention should be investigated in clinical trials. Moreover, there are no clear data on vaccinating the partner of a patient with an HPV infection. However, this strategy may not be worthwhile, because the patient was most likely infected with HPV several years before detection.

Conclusion

In summary, HPV-associated oropharyngeal cancer represents a distinct clinical and biological entity with many unresolved issues that must be investigated in future translational and clinical research. Current studies have shown that tumour
HPV status is a prognostic factor for overall survival and loco-regional control. In addition, HPV detection might be a predictive marker for the response to treatment. Smoking and tobacco exposure might modify survival and recurrence of HPV-positive tumours, and these factors should be considered in future trials for risk stratification of patients with HPV-positive malignant disease. The p16 protein could also be useful as a surrogate marker for HPV. Immunohistochemical staining for p16 could be a feasible method for determining HPV status.

Opportunities for both primary and secondary prevention of HPV infections should be assessed. Currently, we face the challenge of designing clinical trials with appropriate risk stratification that will lead to the identification of a treatment that leads to a good outcome, but also preserves the quality of life in this patient group.

References


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