

# Human papillomavirus prevalence and clinicopathological associations in oropharyngeal squamous cell carcinoma in the Lebanese population

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## Main Article

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## Abstract

**Objectives.** To evaluate for the first time the prevalence of human papillomavirus in oropharyngeal squamous cell carcinoma in a Middle-Eastern population, and to determine associations between human papillomavirus profiles and clinicopathological characteristics.

**Methods.** A retrospective chart review was conducted of all patients treated for oropharyngeal squamous cell carcinoma at the Hotel Dieu de France University Hospital (Beirut, Lebanon) between January 2010 and 2016. Existing formalin-fixed paraffin-embedded tumour samples were analysed. Human papillomavirus DNA viral load and p16 expression were evaluated using polymerase chain reaction and immunohistochemistry respectively.

**Results.** Thirty patients (mean age of 60 years) were included. Twenty-seven per cent of patients were p16-positive/human papillomavirus DNA positive, 53 per cent were p16-negative/human papillomavirus DNA negative and 20 per cent were p16-positive/human papillomavirus DNA negative. Human papillomavirus 16 was the most frequent subtype (75 per cent). Smoking and alcohol consumption were significantly lower in the human papillomavirus positive group compared to the human papillomavirus negative group ( $p = 0.049$  and  $0.004$ , respectively).

**Conclusion.** Human papillomavirus rate was lower than reported rates in Western populations. Possible explanations include differences in social and cultural behaviours.

## Introduction

Head and neck squamous cell carcinomas (SCCs) rank seventh in terms of cancer incidence worldwide, with an estimated 633 000 new cases diagnosed annually.<sup>1</sup> Over the past three decades, their incidence has regressed significantly in Western countries. For example, in the US male population, the incidence of non-oropharyngeal head and neck SCCs has decreased by 20.3 per cent in the 1990s.<sup>2</sup> This decrease paralleled a decrease in tobacco smoking and alcohol consumption. However, during the same time-frame, the overall decrease in head and neck SCC incidence was paradoxically associated with an increase in incidence of oropharyngeal SCCs, especially in a subset of younger patients who were free of the classically established risk factors.<sup>3</sup> In fact, the incidence of oropharyngeal SCCs increased by 10.8 per cent in the US male population during the 1990s.<sup>2</sup> This epidemiological finding led to the search for different risk factors for oropharyngeal SCC. The role of human papilloma virus (HPV) infection in the pathogenesis of oropharyngeal SCC has since been widely validated and confirmed.<sup>4,5</sup>

Approximately 5 per cent of healthy individuals have been diagnosed with oral and oropharyngeal HPV infection.<sup>6,7</sup> This infection has a slightly higher prevalence among men, and among individuals aged 30–34 years and 60–64 years.<sup>8</sup> In a 2013 systematic review, Mehanna *et al.* showed a significant increase of HPV infection prevalence in oropharyngeal SCC patients in North America and Europe: from 40.5 per cent before 2000, to 64.3 per cent between 2000 and 2004, and 72.2 per cent between 2005 and 2009.<sup>9</sup> On the other hand, the prevalence of HPV infection has not changed in non-oropharyngeal head and neck SCC patients, with a mean value of 20 per cent.<sup>9</sup> The currently supported hypothesis to explain this difference in HPV prevalence between head and neck SCC sub-sites is that the Waldeyer's ring acts as a reservoir for the HPV, with HPV inhabiting the normal crypt epithelia.<sup>10</sup>

The oropharyngeal SCC population has some distinct characteristics compared to the classical head and neck SCC population: young age, non-smoker, non-alcoholic and multiple sex partners.<sup>3</sup> Compared to a control group, the oropharyngeal SCC cancer group has a significantly higher prevalence of oral HPV infection, a higher lifetime number of oral sex partners and a higher seropositivity for HPV-16.<sup>11</sup> In fact, certain sexual behaviours, such as a high lifetime number of vaginal sex or oral sex partners, are known to be implicated in the viral transmission.<sup>11–13</sup>

Human papilloma virus is an encapsulated double-stranded DNA with two main carcinogenic types: HPV-16 and HPV-18.<sup>14</sup> Human papilloma virus induced carcinogenesis is different from the tobacco- or alcohol-induced type. Once integrated to the host genome, the viral genes are expressed. This produces two main oncoproteins, E6 and E7, which inactivate the products of tumour suppressor genes p53 and Rb respectively. This allows the infected cell to acquire a malignant phenotype.<sup>15–17</sup> The p16<sup>INK4A</sup> overexpression resulting from this malignant phenotype is currently considered a highly sensitive, indirect marker of HPV infection.<sup>17,18</sup>

Human papilloma virus related oropharyngeal SCCs are associated with a better prognosis.<sup>19,20</sup> In their systematic review, O'Rourke *et al.* found that HPV was an independent good prognosis factor for oropharyngeal SCC.<sup>21</sup> Ang *et al.* demonstrated better overall survival and progression-free survival in 323 HPV-related oropharyngeal SCC patients treated by concomitant radiation and chemotherapy.<sup>19</sup> They further defined three subgroups according to prognosis implication: low risk (HPV-positive non-smokers), intermediate risk (HPV-positive smokers) and high risk (HPV-negative smokers).

Human papilloma virus related oropharyngeal SCC is a distinctive subgroup with different carcinogenesis, risk factors, clinical features and prognosis profile. Furthermore, the association between HPV and oropharyngeal SCC has important implications for prevention, treatment and prognosis.

It is thus crucial to detect HPV infection in oropharyngeal SCC patients. In the absence of a standardised detection technique, a key element in choosing the optimal technique to detect HPV involvement in oropharyngeal SCC is to differentiate between certain highly sensitive tests able to detect the mere presence of the virus without any carcinogenetic involvement and other more specific tests providing proof of viral transcriptional activity and thus evidence of clinically relevant infections.<sup>22–24</sup> The association of viral DNA detection by polymerase chain reaction and p16 detection by immunohistochemistry is a validated diagnostic algorithm, which aims to reliably determine biologically relevant HPV infections in formalin-fixed paraffin-embedded biopsy material taken from oropharyngeal SCC patients.<sup>17,24</sup>

To date, epidemiological data concerning the HPV prevalence in oropharyngeal SCC are mainly limited to North America and Europe. And while the Middle-Eastern population is characterised by different social and sexual behaviours, a study regarding the HPV prevalence in this population has yet to be published. With the present study, and for the first time in a Middle-Eastern population, we aimed to evaluate the prevalence of the p16 overexpression and oncogenic HPV DNA viral load in a homogeneous single-institution oropharyngeal SCC cohort, in Lebanon, and compare it to the available data from a Western population.

## Materials and methods

### Patient selection

A retrospective chart review was conducted on all patients diagnosed or treated for oropharyngeal SCC between January 2010 and 2016 at the Hotel Dieu de France Hospital, Beirut, Lebanon. Only patients with a single and previously untreated primary lesion of the oropharynx were considered eligible for the study. Formalin-fixed paraffin-embedded tumour samples were collected from the pathology

archives and available amounts of tissue were assessed. This work had been reviewed and approved by the institutional ethical committee at the Hotel Dieu de France Hospital.

### Tissue sample preparation and analysis

From each patient's tissue sample, a section was first obtained and reviewed by the pathologist to ensure that the tumour core was reached. The section was used for formalin-fixed paraffin-embedded tissue sample preparation for immunohistochemistry. The rest of the paraffin block was macrodissected with trimming of excessive wax. Serial sections were then collected and directly processed for DNA extractions. Disposable, sterile microtome blades and scalpels were changed before every block to avoid contamination.

The p16 expression determined by immunohistochemistry was evaluated for all samples by two different pathologists blinded to each other's results. There was no inter-rater variability and the results were concordant for all patients. The HPV DNA viral load data obtained by polymerase chain reaction were analysed by a single bioengineer. Both procedures were conducted simultaneously in separate laboratories in a blind fashion, to avoid any bias. For p16<sup>INK4A</sup> expression evaluation, tumours were classified into two groups: p16<sup>INK4A</sup>-positive (strong, diffuse, nuclear and cytoplasmic staining in more than 10 per cent of carcinoma cells) or p16<sup>INK4A</sup>-negative.<sup>25</sup>

The E6 and E7 regions of the oncogenic HPV strains were targeted for the polymerase chain reaction analysis, and tumours were then classified dichotomously as HPV DNA positive or HPV DNA negative. In the positive group, the HPV strain was also determined.

### Statistical analysis

Associations between the clinicopathological features of patients and both p16 expression and HPV DNA viral load status (positive or negative) were evaluated using the Fisher exact test and the two-sample *t*-test. A *p*-value of less than 0.005 was considered statistically significant. All statistical analyses were performed with SPSS® Statistics Desktop software, version 22.0.

### Ethical considerations

The institutional review board gave its approval on the study protocol and patient confidentiality was protected.

## Results

### Patients' characteristics

Thirty patients met the criteria for eligibility and were included in the study: 21 were men and 9 were women. Mean age at diagnosis was 60 years (range, 36–89 years), and no significant difference was found between the sexes. Seventeen patients (56.67 per cent) were smokers. Thirteen patients (43.33 per cent) consumed alcohol; all of them were concomitant smokers. Thirteen patients (43.33 per cent) did not use either carcinogen.

### Tumour characteristics

Twenty-three tumours (76.67 per cent) were found in lymphoid areas: 12 in the tonsillar fossa and 11 in the base of the tongue. The remaining seven tumours were found in the soft palate. All of the tumours were SCCs. On histopathology, 14.81 per cent of the lesions were well differentiated, 55.56 per cent were moderately differentiated and 29.63 per cent were poorly differentiated. Twenty-three per cent of patients were diagnosed with early stage disease: tumour–node–metastasis (TNM) stages I (11.54 per cent) and II (11.54 per cent). Seventy-seven per cent of patients were diagnosed with advanced stage disease: TNM stages III (19.23 per cent) and IV (57.69 per cent). These findings are summarised in Table 1.

### Immunohistochemistry and polymerase chain reaction findings

Based on the predefined criteria, 14 patients (46.67 per cent) tested positive for the p16<sup>INK4A</sup> protein on immunohistochemistry. Eight patients (26.67 per cent) were found to have HPV DNA on polymerase chain reaction. Human papilloma virus 16 was responsible for the majority of infections (75 per cent); other HPV subtypes were less frequently involved.

As discussed earlier, an oncologically relevant HPV infection in an oropharyngeal SCC patient is defined by the presence of HPV DNA in the tumour as well as overexpression of p16<sup>INK4A</sup>. Hence, the HPV-positive group comprised patients with a p16-positive/HPV DNA positive profile. The prevalence of this profile was 26.67 per cent, while 53.33 per cent and 20 per cent of patients were p16-negative/HPV DNA negative and p16-positive/HPV DNA negative, respectively.

Gender, age, tumour location and tumour TNM stage at diagnosis were not significantly associated with HPV status. Tobacco exposure was significantly lower in the HPV-positive group than the HPV-negative group (25 per cent vs 68 per cent;  $p = 0.049$ ). None of the HPV-positive patients consumed alcohol, whereas 59 per cent of HPV-negative patients did ( $p = 0.004$ ; Table 2).

## Discussion

### Human papilloma virus prevalence

In this study sample, the prevalence of HPV-related oropharyngeal SCCs was only 26.67 per cent. The majority of oropharyngeal SCCs in the Lebanese population were not HPV-related tumours, but rather secondary to tobacco or alcohol consumption.

In a recent study from the Gustave Roussy Institute in France, the prevalence of HPV-related oropharyngeal SCC was as high as 48 per cent.<sup>17</sup> In another multi-institutional French study, published in 2011, St Guily *et al.* reported a prevalence of 46.5 per cent for HPV-related oropharyngeal SCCs.<sup>26</sup> Moreover, in the 2005 meta-analysis by Kreimer *et al.*, the overall reported prevalence of HPV infection in oropharyngeal SCCs was 35.6 per cent.<sup>27</sup> This difference in prevalence values could in part be due to the variability of detection techniques between studies or a result of geographic differences in the distribution of risk factors.<sup>17</sup>

While some inter-study variability in prevalence rates is found in Western publications, these rates remain in a somewhat narrow range.<sup>26,27</sup> In contrast, the results of the current study show that the prevalence of HPV-related oropharyngeal SCCs in the Lebanese population is considerably lower and

**Table 1.** Summary of patient and tumour characteristics

Characteristic	Value
Age (mean (range); years)	60 (36–89)
Sex (n (%))	
– Female	9 (30)
– Male	21 (70)
Risk factors (n (%))	
– Tobacco use	17 (56.67)
– Alcohol use*	13 (43.33)
– No tobacco, no alcohol	13 (43.33)
Tumour location (n (%))	
– Lymphoid areas	23 (76.67)
– Non-lymphoid areas	7 (23.33)
Cancer stage <sup>†</sup> (n (%))	
– Stage I	3 (11.54)
– Stage II	3 (11.54)
– Stage III	5 (19.23)
– Stage IV	15 (57.69)
Tumour differentiation <sup>‡</sup> (n (%))	
– Poorly differentiated	8 (29.63)
– Moderately differentiated	15 (55.56)
– Well differentiated	4 (14.81)
P16 IHC status (n (%))	
– P16+	14 (46.67)
– P16–	16 (53.33)
HPV DNA PCR status (n (%))	
– Positive	8 (26.67)
– Negative	22 (73.33)
Tumour HPV profile (n (%))	
– PCR+/p16+	8 (26.67)
– PCR–/p16–	16 (53.33)
– PCR–/p16+	6 (20)
– PCR+/p16–	0 (0)

\*All alcohol drinkers also smoked. <sup>†</sup>Staging information was missing for four patients.

<sup>‡</sup>Tumour differentiation details were missing for three patients. IHC = immunohistochemistry; HPV = human papilloma virus; PCR = polymerase chain reaction

accounts approximately for half of that found in Western countries.

The primary risk factor for developing HPV-related oropharyngeal SCC is oral HPV infection. Oral sex and multiple sexual partners are the major causes of oral HPV infections.<sup>28</sup> A study conducted among Lebanese university students showed that only 20 per cent of them were engaged in regular sexual activity, while an additional 15 per cent had had only one sexual encounter.<sup>29</sup> These numbers, while similar to those seen in other countries witnessing a health transition, such as China,<sup>30</sup> are lower than those reported in Western countries.<sup>29,31</sup> These differences in sociocultural and sexual behaviours between the Middle-Eastern Lebanese population and the Western population may constitute a potential explanation for the lower rates of HPV-related oropharyngeal SCCs seen in the Lebanese population when compared to Western countries.

**Table 2.** Clinical associations with HPV-positive oropharyngeal SCC

Clinical characteristic	HPV+	HPV–	P-value
Mean age (years)	64	58	ns
Sex ratio (M:F; n)	6:2	15:7	ns
Tumour in lymphoid areas (n)	6	17	ns
Early stage tumours (n)	1	5	ns
Advanced stage tumours (n)	6	14	ns
Tobacco use (n)	2	15	0.049
Alcohol use (n)	0	13	0.004

HPV = human papilloma virus; SCC = squamous cell carcinoma; ns = non-significant; M = males; F = females

### Associations with clinical features

Some previously published studies found that oropharyngeal SCC patients who are HPV-positive tend to be predominantly male and younger than HPV-negative patients.<sup>32–34</sup> In the current study, however, age and sex showed no significant associations with HPV positivity. In line with previously published reports,<sup>33</sup> HPV positivity was associated with the absence of both tobacco and alcohol consumption. In fact, it is now well established that while tobacco and alcohol consumption are the primary risk factors for HPV-negative head and neck SCC, sexual habits have become the primary risk factors for HPV-positive oropharyngeal SCCs.<sup>34</sup>

Human papilloma virus positive oropharyngeal SCCs are preferentially located to the lymphoid areas of the oropharynx. Although tumour location and HPV status were not associated in our results, the widely accepted theory is that Waldeyer's ring acts as a natural habitat and reservoir for the virus.<sup>35,36</sup> In several published reports, there was a significant association between HPV positivity and a more advanced TNM stage at diagnosis.<sup>36,37</sup> In the vast majority of these reports, the advanced stage at diagnosis is attributed to the positive nodal status, rather than a large tumour size or local aggressiveness.<sup>17</sup> However, this finding was not reproduced in our results, probably because of the smaller sample size of the study.

### Prognosis, survival and therapeutic repercussions

Human papilloma virus positive status has served as a significant prognostic factor for survival in oropharyngeal SCCs.<sup>38,39</sup> In fact, HPV-related oropharyngeal SCCs have better treatment response rates, which translate into improved survival rates that are persistent regardless of the treatment modality.<sup>39,40</sup> This is mainly explained by the inherent immunogenicity, increased radiosensitivity and absence of field cancerisation in the HPV-related oropharyngeal SCCs.<sup>17,41,42</sup>

In a report published in 2008, Fakhry *et al.* showed that locoregional failure rates at three years were significantly lower in HPV-related oropharyngeal SCC patients compared to HPV-negative patients (13.6 per cent vs 35 per cent, respectively).<sup>38</sup> In addition, the three-year progression-free survival rates have shown important differences between HPV-positive and HPV-negative oropharyngeal SCCs, with rates ranging from 75 to 82 per cent and from 45 to 57 per cent, respectively.<sup>17,19,39,43–45</sup> Conversely, the rates of distant metastasis in HPV-positive oropharyngeal SCCs are similar to those in the HPV-negative oropharyngeal SCCs, with lung metastasis being the most common.<sup>46</sup> However, even in a scenario of

recurrent or metastatic disease, an HPV-positive status is still a favourable prognostic factor.<sup>39</sup>

The striking difference in clinical presentation and treatment response between HPV-negative and HPV-positive oropharyngeal SCCs has led authors to consider the latter as a distinct biological and clinical entity.<sup>39</sup> Therefore, new staging systems have been validated to better take into account the disease severity and prognostic differences between HPV-positive and HPV-negative oropharyngeal SCCs, although none of these staging systems are universally adopted at this time.<sup>47</sup>

Different therapeutic and preventive approaches are currently being developed for the HPV-related oropharyngeal SCCs. While the present treatment guidelines do not recommend or take into consideration treatment modifications based on HPV status, this will probably change in the future.<sup>39</sup> In fact, several clinical trials targeting HPV-positive oropharyngeal SCC patients are currently underway to evaluate radiation de-escalation, modified surgical interventions and alterations in systemic therapy for this particular patient group. Decreasing radiation dose, replacing cisplatin with rituximab, revisiting the need for chemotherapy added to radiotherapy for adjuvant therapy, employing less invasive surgery using transoral robotic surgery, and introducing immunotherapy to the treatment arsenal are among the proposed de-intensification strategies presently being assessed.<sup>48</sup> Based on the inherent better prognosis of the HPV-related oropharyngeal SCCs, these carefully planned strategies aim to maximise disease control, while minimising both long- and short-term toxicity.<sup>49,50</sup> Unfortunately, there is currently not sufficient evidence to validate the introduction of de-intensification protocols according to HPV status in treatment guidelines.<sup>17</sup>

- Human papilloma virus (HPV) is an established risk factor for oropharyngeal squamous cell carcinoma (SCC)
- It accounts for more than 50 per cent of newly diagnosed oropharyngeal SCCs in Western populations
- To date, no data have been published on the prevalence of HPV involvement in oropharyngeal SCC in a Middle-Eastern population
- In this first Middle-Eastern study, HPV-positive oropharyngeal SCC prevalence was 27 per cent
- This rate is much lower compared to that in Western populations

In the face of the growing interest in preventive medicine, more attention has been given to the potential of HPV vaccines for preventing oropharyngeal SCC. To our knowledge, the three available prophylactic HPV vaccines are only approved by the US Food and Drug Administration for the prevention of anogenital cancer, pre-cancer and warts, but not for oropharyngeal SCC.<sup>51</sup> To the best of our knowledge, a limited number of studies have been conducted to date to evaluate the impact of the HPV vaccine on oral HPV infection or oropharyngeal SCC.<sup>51</sup> There is, however, some promising biological evidence regarding vaccine efficacy for oral HPV infection. In animal studies, passive antibody transfers and vaccination for canine oral papillomavirus were shown to provide protection against oral infection and the development of oral pre-cancerous lesions.<sup>52</sup> While the epidemiology of oropharyngeal SCCs may be eventually altered by the long-term effects of the female anti-HPV vaccination, a similar

systematic male vaccination protocol should be implemented to efficiently prevent transmission of oncogenic HPV subtypes.<sup>17</sup> The current body of evidence is, however, insufficient to determine the efficacy of these vaccines exclusively within the context of oral HPV infection or oropharyngeal SCC.<sup>51</sup>

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**Competing interests.** None declared

## References

- Rettig EM, D'Souza G. Epidemiology of head and neck cancer. *Surg Oncol Clin N Am* 2015;**24**:379–96
- Ernst JA, Sciotto CG, O'Brien MM, Finch JL, Robinson LJ, Willson T et al. Rising incidence of oropharyngeal cancer and the role of oncogenic human papilloma virus. *Laryngoscope* 2007;**117**:2115–28
- Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer. *Lancet* 2008;**371**:1695–709
- Mehanna H, Jones TM, Gregoire V, Ang KK. Oropharyngeal carcinoma related to human papillomavirus. *BMJ* 2010;**340**:c1439
- Van Monsjou HS, Balm AJ, Van Den Brekel MM, Wreesmann VB. Oropharyngeal squamous cell carcinoma: a unique disease on the rise? *Oral Oncol* 2010;**46**:780–5
- D'Souza G, Agrawal Y, Halpern J, Bodison S, Gillison ML. Oral sexual behaviors associated with prevalent oral human papillomavirus infection. *J Infect Dis* 2009;**199**:1263–9
- Hansson BG, Rosenquist K, Antonsson A, Wennerberg J, Schildt EB, Bladström A et al. Strong association between infection with human papillomavirus and oral and oropharyngeal squamous cell carcinoma: a population-based case-control study in southern Sweden. *Acta Otolaryngol* 2005;**125**:1337–44
- Gillison ML, Broutian T, Pickard RK, Tong Z, Xiao W, Kahle L et al. Prevalence of oral HPV infection in the United States, 2009–2010. *JAMA* 2012;**307**:693–703
- Mehanna H, Beech T, Nicholson T, El-Hariry I, McConkey C, Paleri V et al. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer—systematic review and meta-analysis of trends by time and region. *Head Neck* 2013;**35**:747–55
- Syrjänen S. HPV infections and tonsillar carcinoma. *J Clin Pathol* 2004;**57**:449–55
- D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM et al. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* 2007;**356**:1944–56
- Vidal L, Gillison ML. Human papillomavirus in HNSCC: recognition of a distinct disease type. *Hematol Oncol Clin North Am* 2008;**22**:1125–42
- Rosenquist K, Wennerberg J, Schildt EB, Bladström A, Göran Hansson B, Andersson G. Oral status, oral infections and some lifestyle factors as risk factors for oral and oropharyngeal squamous cell carcinoma. *A population-based case-control study in southern Sweden*. *Acta Otolaryngol* 2005;**125**:1327–36
- Allen CT, Lewis JS, El-Mofty SK, Haughey BH, Nussenbaum B. Human papillomavirus and oropharynx cancer: biology, detection and clinical implications. *Laryngoscope* 2010;**120**:1756–72
- Braakhuis BJ, Snijders PJ, Keune WJ, Meijer CJ, Ruijter-Schippers HJ, Leemans CR et al. Genetic patterns in head and neck cancers that contain or lack transcriptionally active human papillomavirus. *J Natl Cancer Inst* 2004;**96**:998–1006
- Pyeon D, Newton MA, Lambert PF, den Boon JA, Sengupta S, Marsit CJ et al. Fundamental differences in cell cycle deregulation in human papillomavirus-positive and human papillomavirus-negative head/neck and cervical cancers. *Cancer Res* 2007;**67**:4605–19
- Melkane AE, Auperin A, Saulnier P, Lacroix L, Vielh P, Casiraghi O et al. Human papillomavirus prevalence and prognostic implication in oropharyngeal squamous cell carcinomas. *Head Neck* 2014;**36**:257–65
- Psyrris A, Gouveris P, Vermorken JB. Human papillomavirus-related head and neck tumors: clinical and research implication. *Curr Opin Oncol* 2009;**21**:201–5
- Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* 2010;**363**:24–35
- Weinberger PM, Yu Z, Kountourakis P, Sasaki C, Haffty BG, Kowalski D et al. Defining molecular phenotypes of human papillomavirus-associated oropharyngeal squamous cell carcinoma: validation of three-class hypothesis. *Otolaryngol Head Neck Surg* 2009;**141**:382–9
- O'Rourke MA, Ellison MV, Murray LJ, Moran M, James J, Anderson LA. Human papillomavirus related head and neck cancer survival: a systematic review and meta-analysis. *Oral Oncol* 2012;**48**:1191–201
- Braakhuis BJ, Brakenhoff RH, Meijer CJ, Snijders PJ, Leemans CR. Human papilloma virus in head and neck cancer: the need for a standardised assay to assess the full clinical importance. *Eur J Cancer* 2009;**45**:2935–9
- Venuti A, Paolini F. HPV detection methods in head and neck cancer. *Head Neck Pathol* 2012;**6**:63–74
- Melkane AE, Mirghani H, Aupérin A, Saulnier P, Lacroix L, Vielh P et al. HPV-related oropharyngeal squamous cell carcinomas: a comparison between three diagnostic approaches. *Am J Otolaryngol* 2014;**35**:25–32
- Lassen P, Eriksen JG, Hamilton-Dutoit S, Tramm T, Alsner J, Overgaard J. Effect of HPV-associated p16INK4A expression on response to radiotherapy and survival in squamous cell carcinoma of the head and neck. *J Clin Oncol* 2009;**27**:1992–8
- St Guily JL, Jacquard AC, Prétet JL, Haesebaert J, Beby-Defaux A, Clavel C et al. Human papillomavirus genotype distribution in oropharynx and oral cavity cancer in France—the EDITH VI study. *J Clin Virol* 2011;**51**:100–4
- Kreimer AR. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2005;**14**:467–75
- Taberna M, Mena M, Pavón MA, Alemany L, Gillison ML, Mesía R. Human papillomavirus-related oropharyngeal cancer. *Ann Oncol* 2017;**28**:2386–98
- Salameh P, Zeenny R, Salamé J, Waked M, Barbour B, Zeidan N et al. Attitudes towards and practice of sexuality among university students in Lebanon. *J Biosoc Sci* 2015;**48**:233–48
- Zhang D, Pan H, Cui B, Law F, Farrar J, Ba-Thein W. Sexual behaviors and awareness of sexually transmitted infections among Chinese university students. *J Infect Dev Ctries* 2013;**7**:966–74
- Lally K, Nathan-V Y, Dunne S, McGrath D, Cullen W, Meagher D et al. Awareness of sexually transmitted infection and protection methods among university students in Ireland. *Ir J Med Sci* 2015;**184**:135–42
- Mellin H, Friesland S, Lewensohn R, Dalianis T, Munck-Wikland E. Human papillomavirus (HPV) DNA in tonsillar cancer: clinical correlates, risk of relapse, and survival. *Int J Cancer* 2000;**89**:300–4
- Gillison ML, D'Souza G, Westra W, Sugar E, Xiao W, Begum S et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. *J Natl Cancer Inst* 2008;**100**:407–20
- Smith EM, Ritchie JM, Summersgill KF, Klusmann JP, Lee JH, Wang D et al. Age, sexual behavior and human papillomavirus infection in oral cavity and oropharyngeal cancers. *Int J Cancer* 2004;**108**:766–72
- Hobbs CG, Sterne JA, Bailey M, Heyderman RS, Birchall MA, Thomas SJ. Human papillomavirus and head and neck cancer: a systematic review and meta-analysis. *Clin Otolaryngol* 2006;**31**:259–66
- Paz IB, Cook N, Odom-Maryon T, Xie Y, Wilczynski SP. Human papillomavirus (HPV) in head and neck cancer. *An association of HPV 16 with squamous cell carcinoma of Waldeyer's tonsillar ring*. *Cancer* 1997;**79**:595–604
- Hafkamp HC, Manni JJ, Haesevoets A, Voogd AC, Schepers M, Bot FJ et al. Marked differences in survival rate between smokers and nonsmokers with HPV 16-associated tonsillar carcinomas. *Int J Cancer* 2008;**122**:2656–64
- Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst* 2008;**100**:261–9
- Fundakowski CE, Lango M. Considerations in surgical versus non-surgical management of HPV positive oropharyngeal cancer. *Cancers Head Neck* 2016;**1**:6
- Dayyani F, Etzel CJ, Liu M, Ho CH, Lippman SM, Tsao AS. Meta-analysis of the impact of human papillomavirus (HPV) on cancer risk and overall survival in head and neck squamous cell carcinomas (HNSCC). *Head Neck Oncol* 2010;**2**:15
- Agrawal Y, Koch WM, Xiao W, Westra WH, Trivett AL, Symer DE et al. Oral human papillomavirus infection before and after treatment for human papillomavirus 16-positive and human papillomavirus 16-negative head and neck squamous cell carcinoma. *Clin Cancer Res* 2008;**14**:7143–50

- 42 Lassen P, Eriksen JG, Krogdahl A, Therkildsen MH, Ulhøi BP, Overgaard M *et al.* The influence of HPV-associated p16-expression on accelerated fractionated radiotherapy in head and neck cancer: evaluation of the randomised DAHANCA 6&7 trial. *Radiother Oncol* 2011;**100**:49–55
- 43 Licitra L, Perrone F, Bossi P, Suardi S, Mariani L, Artusi R *et al.* High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. *J Clin Oncol* 2006;**24**:5630–6
- 44 Lindel K, Beer KT, Laissue J, Greiner RH, Aebbersold DM. Human papillomavirus positive squamous cell carcinoma of the oropharynx: a radiosensitive subgroup of head and neck carcinoma. *Cancer* 2001;**92**:805–13
- 45 Heiduschka G, Grah A, Oberndorfer F, Kadletz L, Altorjai G, Kornek G *et al.* Improved survival in HPV/p16-positive oropharyngeal cancer patients treated with postoperative radiotherapy. *Strahlenther Onkol* 2015;**191**:209–16
- 46 Fakhry C, Zhang Q, Nguyen-Tan PF, Rosenthal D, El-Naggar A, Garden AS *et al.* Human papillomavirus and overall survival after progression of oropharyngeal squamous cell carcinoma. *J Clin Oncol* 2014;**32**:3365–73
- 47 O'Sullivan B, Huang SH, Su J, Garden AS, Sturgis EM, Dahlstrom K *et al.* Development and validation of a staging system for HPV-related oropharyngeal cancer by the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S): a multicentre cohort study. *Lancet Oncol* 2016;**17**:440–51
- 48 Kofler B, Laban S, Busch CJ, Lörincz B, Knecht R. New treatment strategies for HPV-positive head and neck cancer. *Eur Arch Otorhinolaryngol* 2014;**271**:1861–7
- 49 Brockstein BE, Vokes EE. Head and neck cancer in 2010: maximizing survival and minimizing toxicity. *Nat Rev Clin Oncol* 2011;**8**:72–4
- 50 Givens DJ, Karnell LH, Gupta AK, Clamon GH, Pagedar NA, Chang KE *et al.* Adverse events associated with concurrent chemoradiation therapy in patients with head and neck cancer. *Arch Otolaryngol Head Neck Surg* 2009;**135**:1209–17
- 51 Guo T, Eisele DW, Fakhry C. The potential impact of prophylactic human papillomavirus vaccination on oropharyngeal cancer. *Cancer* 2016;**122**:2313–23
- 52 Suzich JA, Ghim SJ, Palmer-Hill FJ, White WI, Tamura JK, Bell JA *et al.* Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. *Proc Natl Acad Sci U S A* 1995;**92**:11553–7