Human Papilloma Virus and Oropharyngeal Carcinoma – Lessons from History

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The human papilloma virus (HPV) is a common virus that infects epithelium in 10% of the world's population. While most sexually active people become infected, the majority with a healthy natural immunity control their infection. When the infection becomes persistent in cervical mucosa for instance, it is associated with nearly all cervical cancers. Fortunately cervical cancer screening is both sensitive and specific and when accessed has led to significant reductions of this disease. Despite this, cervical cancer still remains one of the leading causes of death from cancer. Oropharyngeal mucosa is becoming persistently infected with HPV in an increasing number of people leading to a potential epidemic of oropharyngeal carcinoma. While only 10% of new oropharyngeal infections persist, those in elderly men who smoke are more likely to do so. Some centres report more than 70% of oropharyngeal cancers are associated with HPV infection, which is different to cancers caused by alcohol and tobacco. Other centres report only a 20% association. Education against high-risk sexual behaviour has been met with limited success. Screening for oropharyngeal HPV infection has been disappointing with a pickup rate of only 40%. Some hope lies in detecting viral DNA in both the saliva and plasma. A HPV vaccine has been available since 2006 but is not yet routinely given to both sexes in many countries. Its effect on the incidence of HPV-positive oropharyngeal carcinomas is currently unknown. Vigilance by dental and medical colleagues in the meantime is essential.

Key words: *cervical carcinoma, human papilloma virus, oropharyngeal carcinoma, screening, vaccination*

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The identification of the human papilloma virus

Our understanding of the role that viruses play in the development of cancer is still evolving, and has been doing so from at least the beginning of the 18th century. In 1713 the Italian doctor Bernardino Ramazzini noticed that nuns did not get cervical cancer and wondered if this was because they never had sexual intercourse[1]. This important observation led towards understanding the relationship of sexually transmitted infections and the risk of cancer. Percival Pott who was an English doctor described scrotum cancer in men who cleaned chimneys in 1775[2]. This led to studies that identified substances that directly caused cancer. For instance, tobacco snuff was linked to nose cancer in 1761, to lip cancer in 1787 and to mouth cancer in 1858. In 1911 Peyton Rous, an American doctor, described a cancer in chickens caused by a virus and showed that the cancer could be transmitted from one chicken to another chicken for which he was awarded the Nobel Prize in 1966[3]. In 1953 Watson and Crick from Cambridge in England also received the Nobel Prize for their description of the structure of DNA, which led to our understanding of how genes worked and how they could be damaged[4]. This answered many complex questions about cancer.

In 1974 the German doctor Harald zur Hausen proposed that the human papilloma virus (HPV) caused cervical cancer. Eventually in 1983 he identified HPV-16 in cervical cancer and in 1984 he identified HPV-18

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in cervical cancer[5,6] for which he too received the Nobel Prize in Physiology or Medicine in 2008. Interestingly he shared the Nobel Prize that year with Luc Antoine Montagnier of the Pasteur Institute in Paris for his identification of the human immunodeficiency virus. The discovery that the HPV virus caused cervical cancer led directly to the development of a HPV vaccine.

The spread and effects of the human papilloma virus

The Human papilloma virus (HPV) has no envelope and so can survive outside of a cell for 20 days. It is a single circular molecule of double-stranded DNA with 8000 base pairs. More than 100 genotypes have been identified by the genetic sequence of the outer capsid protein L1. The virus mainly infects skin and mucosal cells and so is classified according to the site of the disease it affects i.e. cutaneous or mucosal. Most types of HPV infect the skin and cause benign warts while more than 40 types infect mucosa and cause genital warts, of which 15 types are at high risk and 12 types are at low risk[7,8]. The most prevalent HPV types that cause cervical cancer are HPV-16 and HPV-18.

HPV can only infect basal cells which are undifferentiated stem cells of the epidermis[6,9]. Infection occurs through abrasions or crypts on the surface, which allows access to the basal cells. As high-risk HPV is found in up to 80% of tonsil cancers, the microanatomy of the tonsils may explain this. Deep invaginations or tonsil crypts may expose immature basal cells to HPV.

HPV has a reservoir, which is human and its communicability is presumed to be high. The transmission of HPV is by direct contact during sexual activity. HPV infections present with skin lesions, common warts, genital lesions and genital warts.

The sexual revolution of the 1960s was due in part to the contraceptive pill, which provided sexual freedom for women, however was blamed for an increase in sexually transmitted diseases (STD). In the 1960s there were four key STDs, now there are twenty-four. The change in sexual behaviour in the 1960s may have led to increased HPV exposure.

The human papilloma virus (HPV) is one of the most common virus groups in the world. 600 million people or 10% of the world's population is infected[10]. According to the Centers for Disease Control and Prevention, at least 20 million people in the United States are infected with HPV and there are approximately 6 million new cases each year. The prevalence of HPV in the United States is 6% and it has an incidence of 2%. By age 50 more than 80% of women will have had genital HPV infection. The lifetime risk for sexually active men and women is > 50%. Of all sexually active women, 1% will have genital warts at any one time. Out of the new infections in women, 75% of them occur in the 15 to 24 year old age group. The overall prevalence in women younger than 25 years old is 46% or 9.2 million women. In the USA the overall prevalence of HPV in females aged 14 to 59 years old was 26.8%. The HPV prevalence was 44.8% amongst women aged 20 to 24 years old. There is a statistically significant trend for increasing HPV prevalence with each year of age from 14 to 24 years old followed by a gradual decline in prevalence through to 59 years old[11]. There is currently no cure for HPV infection.

HPV induces cancer via two oncoproteins that are expressed during the early stages of the lifecycle, E6 and E7, which bind and inactivate host tumour suppressor proteins p53 and pRb, respectively[12]. There is overexpression of p16 in HPV-positive cancer due to the degradation of pRb by the viral oncoprotein E7. Overexpression of p16 correlates with HPV positivity in oropharyngeal cancer. Overexpression of p16 is not seen in HPV-negative cancers and therefore is a surrogate marker for HPV positivity in oropharyngeal cancer. It is usually detected by immunohistochemical (IHC) staining and is an independent prognostic factor of improved locoregional control and disease-free survival in oropharyngeal carcinoma.

The past rise and fall of cervix cancer

George N Papanicolaou (1883 to 1962) was a Greek doctor who earned a PhD in zoology from the Munich Zoological Institute. He then moved to Cornell University in the USA and studied how the sex of guinea pigs was determined. He inserted a small nasal speculum into the vagina of a guinea pig on a daily basis and observed the vaginal discharge. Later he took vaginal smears and correlated the smear with changes in the ovary and uterus. He began taking vaginal smears from his wife in 1920 and subsequently recognised abnormal cells purely by accident. He continued to study vaginal smears for cancer detection. He published 'Diagnosis of Uterine Cancer by the Vaginal Smear' in 1943. Dr Papanicolaou's legacy is the Pap test or Pap smear. In 1946 he published a paper in the Journal of the American Medical Association in which he reviewed the history and development of the vaginal smear as a method of cancer diagnosis and noted that in his own series he only failed to detect malignant cells in 3.2% of cases of cervical cancer[13,14].

Between 1955 and 1992 in the United States, death from cervical cancer fell by more than 60%. This was

Table 1 Two distinct profiles of head and neck cancers based on HPV status.

	HPV-positive	HPV-negative
Age	Younger	Older
Anatomical site	Lingual and lingual tonsil	All sites
Cofactors	Immunosuppression, marijuana	Diet, hygiene
Gender (Men:women)	3:1	3:1
Genetics	P53wt, p16 positive	P53mu, p16 negative
Histology	Poorly differentiated basaloid	Moderately to well differentiated keratinised
Incidence	Increasing	Decreasing
Risk factors	Sexual behaviour	Alcohol and tobacco
Socioeconomic status	High	Low
Survival	Better	Worse

When head and neck cancers are subdivided into HPV-positive and HPV-negative groups, they form two distinct profiles and therefore each should be considered as a distinct cancer or entity. This table is based on data from Gillison et al[20].

due to the success of Pap smear screening programs introduced in the 1940s. In Canada, the age standardised mortality rate from invasive cervical cancer declined by 83% from 1952 to 2006 while the age standardised incidence rate has declined by 58% since 1972. The greatest declines in both mortality and incidence are observed in age groups over 45 years with reductions as high as 74% in mortality and 69% in incidence[15]. This screening test, the Pap smear/test, for the early detection of cancer or lesions that may progress into cancer has reduced the number of deaths from this widespread and devastating disease by over 70% in the United States since it was introduced in the late 1940s. It is the most successful cancer screening test to be developed in the entire history of medicine.

In the 1950s, cervical cancer was the top cancer in women. Cervical cancer remains the commonest cancer in women in the developing world and the second most common female cancer worldwide[8,16,17]. In the US, it was the commonest cancer in young woman aged between 25 and 29, between 1973 and 1978; the second commonest cancer in woman aged between 15 and 34; and the second commonest cause of death from cancer in 30- to 34-year-old women[18]. Today, uterine cancer is the second leading cause of cancer death in young women aged 30 to 34 and the third leading cause in the 25- to 29–year-old age group[19]. With regard to cancer incidence overall, it is the second most common cancer (after breast cancer). Mortality rates have decreased from a rate of 25 per million in 1950 to eight per million in 1978. Survival rates in this age group are high with a 5-year survival rate of 93% for cancer of the corpus of the uterus and 72% for cancer of the cervix[18].

The present continuing rise of oropharynx cancer

Gillison et al[20] have noted that HPV-16-positive and HPV-16-negative head and neck squamous cell carcinomas have different risk factor profiles which indicate that they should be considered as distinct cancers (Table 1).

Chaturvedi et al[21] reports that in the USA the HPV prevalence in oropharyngeal cancers has significantly increased over time, from 16.3% during the period between 1984 and 1989 to 71.7% during the period between 2000 and 2004. The incidence of HPV-positive oropharyngeal cancers has therefore increased by 225% in this population from 1988 to 2004. If these trends continue, the annual number of HPV-positive oropharyngeal cancers will surpass the annual number of cervical cancers by the year 2020. Increases in survival of oropharyngeal cancers in the United States since 1984 are due to the fact that a greater proportion of them are caused by HPV infection which carries a better prognosis (Figs 1 and 2)[21].

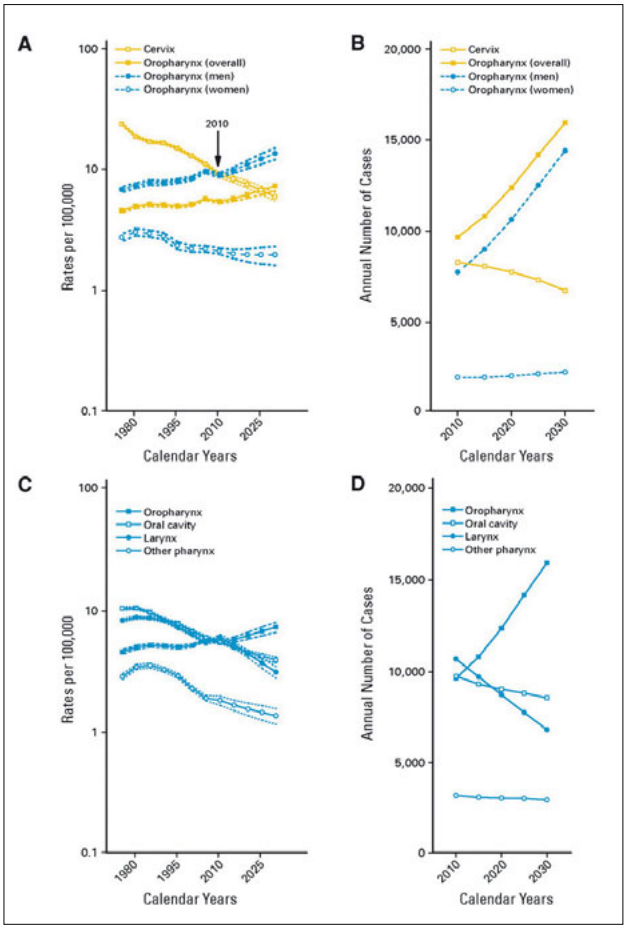

Fig 1 Observed and projected incidence rates (A) and projected annual number of cases (B) for increasing oropharyngeal cancers overall (solid squares), increasing oropharyngeal cancers in men (solid circles), decreasing oropharyngeal cancers in women (open circles) and decreasing cervical cancers (open squares). Observed and projected incidence rates (C) and projected annual number of cases (D) for increasing oropharyngeal cancers (solid squares), decreasing oral cavity (open squares), decreasing larynx (solid circles) and decreasing other pharynx (open circles) cancers. From reference 21: Chaturvedi A et al. Human Papillomavirus and Rising Oropharyngeal Cancer Incidence in the United States. J Clin Oncol 2011;29:4294–4301[21]. Reprinted with permission. © 2011 American Society of Clinical Oncology. All rights reserved.

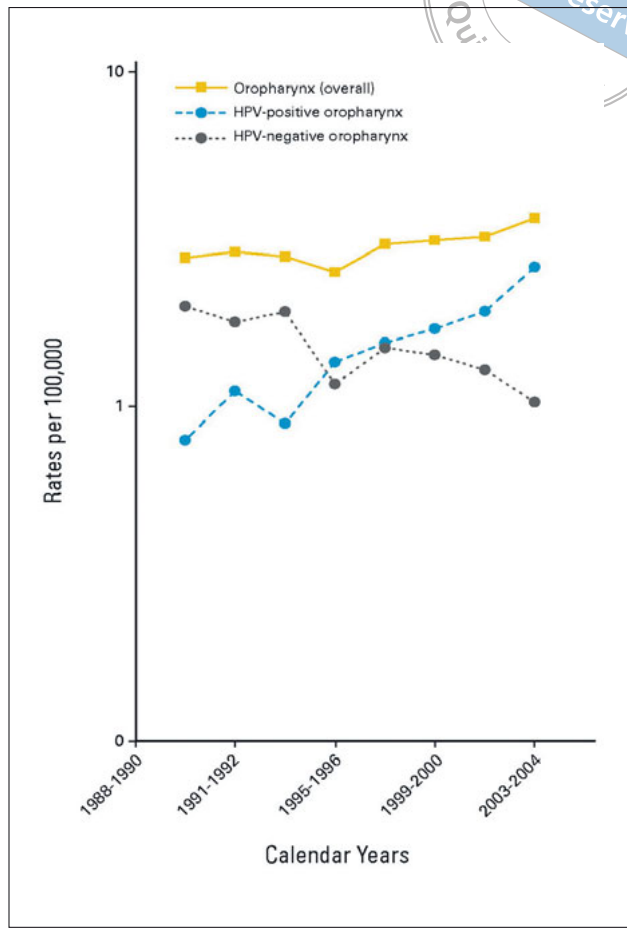

Fig 2 Incidence rates for all oropharyngeal cancers (yellow), HPV-positive oropharyngeal cancers (blue), and HPV-negative oropharyngeal cancers (gray) from 1988 to 2004. HPV-positive oropharyngeal cancers increased from 0.8 to 2.6 per 100,000 during this period. HPV-negative oropharyngeal cancers decreased significantly from 2.0 to 1.0 per 100,000 during this period. The incidence of all oropharyngeal cancers increased from 2.8 to 3.6 per 100,000 during this period, mainly due to an increase in HPV-positive cancers. From reference 21: Chaturvedi A et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol 2011;29:4294–4301. Reprinted with permission. © 2011 American Society of Clinical Oncology. All rights reserved.

Ang et al[22] analysed patients with oropharyngeal carcinoma and found that patients with HPV-positive tumours had significantly better 3-year rates of overall survival than patients with HPV-negative tumours and a 58% reduction in the risk of death.

They used recursive-partitioning to classify patients as having a low, intermediate or high risk of death on the basis of four factors: HPV status, pack years of tobacco smoking, tumour stage and nodal stage. The low-risk group were those with HPV-positive tumours except smokers with a high nodal stage who were intermediate risk. The high-risk group were patients with HPV-negative tumours except those of non-smokers with tumours of stage T2 or T3, who were considered to be intermediate risk (Fig 3). They concluded that the tumour HPV status is a strong and independent

prognostic factor for survival amongst patients with oropharyngeal cancer.

D'Souza et al[23] performed a multivariate analysis on risk factors in patients with oropharyngeal cancer and found it to be independently associated with HPV-16 seropositivity (odds ratio: 32.2; 95% CI: 14.6 to 71.3), poor dentition (odds ratio: 4.1; 95% CI: 1.6 to 10.6), infrequent tooth brushing (odds ratio: 6.9; 95% CI: 1.6 to 30.3), a family history of squamous cell carcinoma of the head and neck (odds ratio: 5.4; 95% CI: 1.0 to 30.8) and heavy tobacco use (odds ratio: 2.5; 95% CI: 1.1 to 6.0). These factors were collectively estimated to be responsible for 90% of cases of oropharyngeal cancers, with 55% of cases attributable to HPV-16 exposure alone.

The percentage of oropharyngeal cancers in which HPV-16 genomic DNA was detected by in situ hybridization was 72%. This established oral HPV infection as having a strong association with oropharyngeal cancer even without the established risk factors of tobacco and alcohol use.

Beachler et al[24] showed that the incidence of oral HPV was related to HIV infection, decreased CD4 (which is found on the surface of immune cells e.g. T-helper cells) and the increased number of oral sex partners, while persistence of oral HPV was related to being male, older age and current smokers.

Fortunately there is a natural clearance of HPV with most new oral HPV infections (93%) clearing within 2 years. Clearance of oral HPV infections is similar to anogenital HPV infections, where only a small minority (10%) of new infections persist for 2 years[25].

While the persistence of anogenital infection is an accepted indicator for subsequent risk of anogenital dysplasia, we do not yet know whether persistent oral HPV infection can be used to identify people at risk of oropharyngeal cancer[26]. Factors associated with the reduced clearance of oral HPV infection are cigarette smoking, older age and being male[24].

Not all new oropharyngeal HPV infections are acquired through sex. Some may be acquired through autoinoculation or may be reactivated latent infections. In sexually abstinent individuals, there is a strong association between oral HPV incidence and immunosuppression. The increased risk of oral HPV infection amongst immunosuppressed people may be due to poor immune surveillance[24].

The ability of HPV-16 to establish persistent infection might be responsible for its prominence in cancers. In young adults most infections clear within 1 to 2 years, while infections at older ages are uncommon. In old adults the increased prevalence of oral HPV might be caused by the increased duration of infection rather than increased incidence.

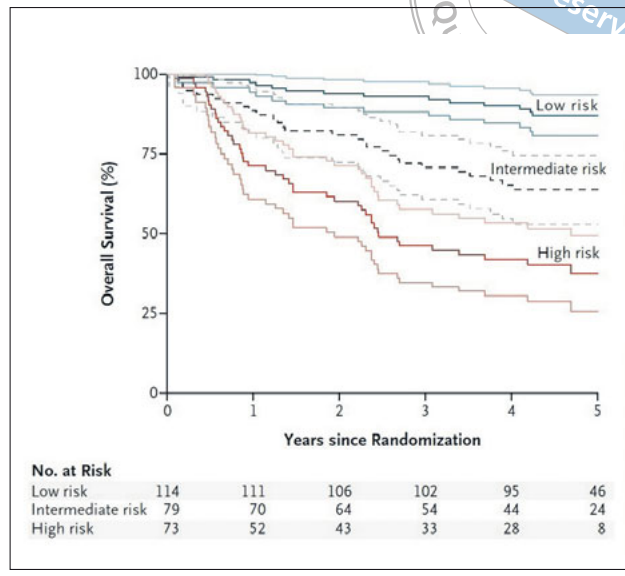

Fig 3 Kaplan-Meier estimates of OS of patients with oropharyngeal carcinoma according to three risk of death categories, based on HPV status, pack years of tobacco smoking, tumour stage and nodal stage. Low-risk (mainly non-smokers with HPV-positive tumours) 3-year OS: 93.0%; intermediate-risk 3-year OS: 70.8% (HR: 3.54; 95% CI: 1.91 to 6.57); and high-risk (mainly smokers with HPV-negative tumours) 3-year OS: 46.2% (HR: 7.16; 95% CI: 3.97 to 12.93). OS = overall survival; HR = hazard ratio. From reference 22: Ang KK et al.Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363:24–35. Copyright © 2010 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

HPV infection is classified as a carcinogen causing cancer of the anus, cervix, oropharynx and penis. HPV-16 is a cause of multiple cancers in men including oropharyngeal, penile and anal, as well as in women including oropharyngeal, cervical, vaginal, vulvar and anal[27]. Five percent of all cancers worldwide are due to HPV infection[28]. Of all adults worldwide 5% to 8% have oral HPV infection at any given time. In 2008, approximately 610,000 of the 12.7 million new cancer cases were attributable to HPV. Currently, 12% to 70% of oropharyngeal cancer is caused by HPV[29]. The fraction of the population attributable to HPV in OPC is estimated to be 26% globally, but HPV prevalence amongst OPC cases rises to 50% in North America, Japan and Australia[28]. Eighty-four percent of HPV-associated head and neck cancers are associated with HPV-16 only.

The future fall of oropharynx cancer - HPV quo vadis?

Education

A Cochrane Review of interventions for encouraging sexual behaviours intended to prevent cervical cancer found the effects of interventions on sexually transmitted infections (STIs) were limited. While there was some impact in the first 6 months, they suggest a longer intervention was needed to be sustained beyond a year, with booster sessions, to help young women to continue to protect themselves as they mature and become sexually active[30].

In a review of the effectiveness of behavioural interventions for the prevention of STIs in young people, Shepherd et al found few significant differences between the interventions and comparators, in terms of changes in behavioural outcomes such as condom use[31].

In a review of teenage sexual attitudes and behaviour in China, Yu confirmed that the prevalence of STIs/HIV had increased in recent years. The author found that the sexual knowledge of young people was poor and that the media was the main source of information, therefore advocated the need to develop more comprehensive sex education programs and to make sexual and reproductive health services available to teenagers and unmarried young people[32].

Vaccination

The high proportion of non-cervical HPV-related cancers due to HPV-16 infection (63% to 95%) shows the potential for prevention of the majority of oropharyngeal cancers through prophylactic HPV vaccination. In 2006 a preventive cancer vaccine was approved for cervical cancer. In the same year a prophylactic HPV vaccine that prevented uninfected people from getting HPV-6, HPV-11, HPV-16 and HPV-18 was released. A year later in 2007 a prophylactic HPV vaccine that prevented uninfected people from getting HPV-16 and HPV-18 was released. A candidate 9-valent vaccine (currently in clinical trials) could have the potential to prevent virtually all (> 97%) HPV-associated oropharyngeal cancers[33].

Screening

Screening refers to tests and examinations used to find cancer in people without symptoms. The first screening test to be widely used for cancer was the Pap test in the 1960s. Cervical cancer screening is the most cost-effective screening of all cancers. Mammography was introduced in the 1960s and was first officially recommended in 1976. The American Cancer Society Guidelines[34] advocate screening for cancers of the cervix, breast, colon, rectum, endometrium, lung and prostate, and a cancer-related check-up depending on age and gender for cancers of the thyroid, mouth, skin, lymph nodes, testes and the ovaries. One suggestion would be adjunctive screening aids that detect precursor or pre-malignant lesions, if they exist, especially when conventional visual and tactile examinations are not sensitive enough to identify small and early lesions. This would broadly include light-based handheld devices, cytology with or without additional analyses (such as loss of heterozygosity), *in vivo* imaging with molecular probes/paints and salivary diagnostics. The biomarker set for oropharyngeal screening will need to cover HPV-related and tobacco/alcohol-related neoplasias, both of which can occur in smokers. One future avenue of study may be to examine methylation markers in HPV-related oropharyngeal lesions[35].

Early detection

A test that uses polymerase chain reaction (PCR) and nucleic acid hybridization is currently available that can specifically detect HPV-16 and HPV-18 in a sample of cervical cells while concurrently detecting 12 other types of high-risk HPVs. The future application of this for the detection of HPV in oropharyngeal cells is one line of research to pursue, as an increasing percentage of oropharyngeal cancers follow an infection by oncogenic HPV types and so screening opportunities using alternative HPV-based strategies give further hope to expanding and simplifying screening strategies[36].

Wang et al were able to detect HPV DNA in the plasma of 86% of patients with a HPV-positive oral cavity or oropharyngeal carcinomas but in only 40% of the saliva from these patients[19]. Given that no false-positives are expected, the assays can be combined, increasing sensitivity without compromising specificity. The combination of saliva and plasma assays could allow more of the cancers to be detected than with either saliva or plasma alone. This could also be valid for screening and surveillance after treatment[19]. Using a combination of pre-treatment plasma and saliva can increase the sensitivity of pre-treatment HPV-16 status, as a tool for screening patients with HPV-16–positive oropharyngeal carcinomas compared to using either sample alone[37].

Surveillance

In addition, analysis of HPV-16 DNA in saliva and plasma after primary treatment may allow for early

detection of recurrence in patients with HPV-16–positive oropharyngeal carcinomas. Thus, quantitative PCR detection of HPV DNA in post-treatment surveillance saliva and plasma samples can function as a valuable prognostic biomarker of relapse-free survival and overall survival in patients with HPV-positive oropharyngeal carcinomas[37].

Treatment

Chemoradiation is the treatment of choice for the majority of patients with HPV-positive oropharyngeal carcinomas and gives an excellent prognosis. To reduce the morbidity of late toxicity in survivors, without compromising the high rates of survival currently enjoyed, and simultaneously addressing the poor prognosis of those with recurrence, it is critical to capitalise on the viral aetiology of the cancer and translate discoveries in genomics, target/drug discovery, viral oncogenesis and immunobiology to ensure improved outcomes with but not limited to molecularly targeted and immunomodulatory therapies[34].

Until education, screening and perhaps vaccination significantly impact the incidence of HPV-related cancer of the oropharynx, as it did for cancer of the cervix, vigilance by dental and medical colleagues will remain essential.

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