# HPV-related squamous cell carcinoma of oropharynx: a review

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Received 12 May 2020
Accepted 14 May 2020
Published Online First 4 June 2020



**To cite:** Rahimi S. *J Clin Pathol* 2020;**73**:624–629.

## ABSTRACT

In early 1930, R. E. Shope paved the way for the recognition of human papillomavirus (HPV) as a causative agent of some types of cancers. In early 2000, the relationship between HPV and a subset of head and neck cancers, mostly located in the oropharynx, was discovered. In the last 20 years, we have made great progress in the recognition and treatment of HPV-positive head and neck cancers. However, there are still grey areas that leave room to subjective interpretation and need to be addressed. The majority of high risk (HR) HPV-positive oropharyngeal squamous cell carcinoma (OPSCC) shows a 'basaloid' morphology, and despite the variegated morphological spectrum of this malignancy, highlighted by some very recent publications, there is a lack of consensus on a universal morphological classification of HPV-OPSCC. The advent of immunohistochemistry with p16 $^{ink4a}$ (p16) protein made the diagnosis of HPV-related OPSCC more straightforward; currently patients with OPSCC are stratified in p16-positive and p16-negative. Although p16 is an excellent surrogate of HR HPV infection, it is not the direct demonstration of the presence of virus. At present, there is no univocal 'gold-standard' technique for the detection of oncogenic HPV infection. It is well known that HR HPV-related (OPSCC) bear significantly better survival outcome than HPV-negative cases. Consequently, the eighth edition of the American Joint Committee on Cancer and the Union for International Cancer Control now have separate staging systems for these two distinct malignancies. The present review discusses the salient features of HR HPV-driven OPSCC.

## BRIEF HISTORY

In North American folklore mythology, jackalope is a jackrabbit with antelope horns hence the portmanteau term of jackalope (figure 1). However, mythology reveals a hidden truth because rabbits with simil-horn structures around their head really do exist and have long been known to hunters (figure 2). These protrusions of skin, reminiscent of horns, are true neoplasms (rabbit papillomatosis) caused by a papillomavirus infection (*Sylvilagus floridanus*). In 1932, Richard E. Shope, with a very elegant experiment, purified papilloma virus particles from wild cottontail rabbit (*Sylvilagus*) warts and successfully transmitted them to domestic rabbits (*Oryctolagus*) that consequently developed papillomas. This was the first demonstration of virus that cause cancer.[1] In his very detailed morphological description, Shope depicted the lesions as a sort of 'fibroma' covered by a thick epithelium similar to human 'molluscum contagiosum' that presented dense lymphatic tissue at the base. Currently, without fear of contradiction, we can state that this feature is characteristic of verrucous carcinoma. In 1976, Harald zur Hausen, a German virologist, published his seminal work on the role of human papillomavirus (HPV) as a cause of cancer of the cervix uteri[2] and in early 1980 he and his colleagues identified high risk (HR) HPV in cervical cancer for which zur Hausen received the Nobel Prize in Physiology or Medicine 2008. In 2000 Gillison and his collaborators showed the association between HPV and a subset of head and neck cancers.[3]

## EPIDEMIOLOGY

Oral HPV prevalence shows a bimodal pattern, with peak prevalence at ages 30–34 years (7.3 %) and 60–64 years (11.4%).[4]

Incidence rates of HPV-associated head and neck squamous cell carcinoma (HNSCC) have been increasing despite decreasing rates of alcohol and tobacco-related oral cancer.[5–8] Evidence suggests that HPV-associated oropharyngeal SCC (OPSCC) will be the predominant form of HNSCC by 2030.[7] Published studies report that 4.5% of all cancers worldwide (630 000 new cancer cases per year) are caused by HPV (8.6% in females and 0.8% in males). Almost all SCC and adenocarcinomas of the cervix uteri, except rare subtypes such as gastric type and mesonephric type, are caused by HPV that is also is responsible for a substantial part of other anogenital cancers and oropharyngeal cancers. The relative contributions of HPV16/18 and HPV6/11/16/18/31/33/45/52/58 are 73% and 90%, respectively.

HPV type 16 is the most prevalent genotype found in 87% of HPV-induced OPSCC followed by HPV type 33 and HPV type 18.[9] There are 38 000 cases per year of HPV-associated head and neck cancers, 21 000 of which are located in the oropharynx.[7 10] Patients with HPV-related head and neck carcinoma are treatment sensitive with overall survival figures 5%–33% greater with respect to HPV-negative cancers.[11 12]

HPV-related head and neck cancer shows a threefold higher incidence in males with respect to females[11 13–15] and is apparently more frequent in Caucasians compared with blacks.[16] Patients with HPV-related cancers show less exposure to tobacco and alcohol, tend to be younger with a median age of diagnosis of 54 years,[11 13 17] and have a higher socio-economic status and education.[18]

## RISK FACTORS

### Smoking

Tobacco exposure seems to increase the risk of oropharyngeal cancer progression and death at diagnosis and during therapy and seems to be independent

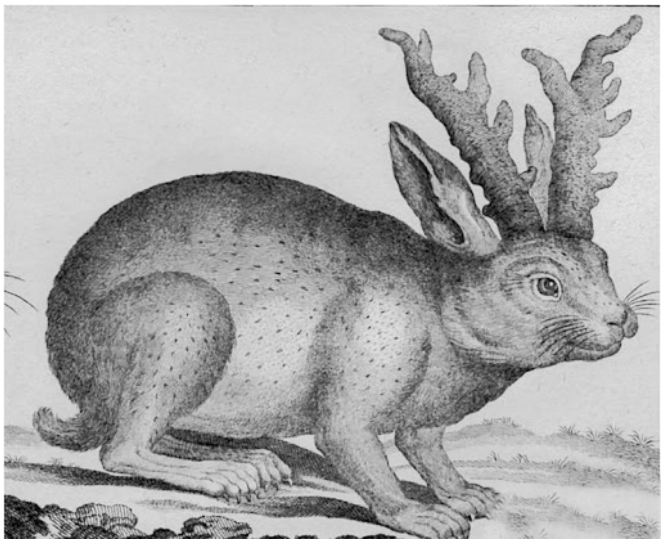

**Figure 1** The mythical animal, the jackalope.

of tumour HPV status.[19–23] Smoking during radiotherapy seems to independently increase the risk of death.[24]

### Sexual behaviour

It is well established that oncogenic HPV is sexually transmitted.[25 26] Compared with patients with HPV-negative oral cancer, those with HPV-driven OPSCC have more than nine lifetime sex partners and more than four oral-genital sex partners and have engaged in oral-genital sex.[27–29]

There is lack of substantial statistically significant information regarding the HPV status of the sexual partners of patients with HPV-related OPSCC.

### Structure and mechanism of action of HPV

HPV is a double-stranded DNA virus. The coding information of HPV is inside three early, late and long-control regions. The early region (E1-8) is responsible for transcription, plasmid replication and transformation. The major (L1) and minor (L2) capsid proteins are found in the late region and the long-control region regulates viral transcription and replication.[30]

The early region contains two viral oncogenes, E6 and E7 that are growth promoting/transforming proteins. E6 binds p53 protein and promotes its degradation while E7 binds and inactivates retinoblastoma (Rb) protein. The inactivation of p53 and Rb causes abnormal cell proliferation, inhibition of apoptosis, cellular immortalisation and genomic instability.[17 31 32]

**Figure 2** The jackrabbit with simil-horn structures around the head caused by papilloma virus.

### Clinical presentation and prognosis

Most HPV-related OPSCC present with early T stage and often cystic and multilevel higher N stage.[33 34] However, HPV-associated OPSCC has improved survival when compared with similarly staged HPV-negative cancers.[11 12] In patients with HPV-associated OPSCC there is an improved response to either chemo-radiotherapy (84% vs 57%) or induction therapy (82% vs 55%).[12] A retrospective clinical trial showed that patients with HPV-related OPSCC have better 3-year rates of overall survival and a 58% reduction in risk of death.[12] The overall survival and progression-free survival rates in HPV-positive patients with OPSCC are 79% and 73%, respectively, compared with 31% and 29% in HPV-negative patients with OPSCC.[35]

The incidence of distant metastases seems to be lower in patients with HPV-related OPSCC compared with HPV-negative tumours.[36 37] Patients with HPV-related OPSCC show improved outcomes compared with patients with HPV-negative cancers even with metastases.[38] Although less than HPV-negative patients, those with HPV-associated tumours may develop recurrence and second primary tumours. However, HPV-positive patients with second primary tumours show improved survival rates compared with HPV-negative patients.[39] In view of the combined weight of clinical evidence the eight edition of the American Joint Committee on Cancer now has a separate staging system for HPV-driven OPSCC.[40]

### Histopathological diagnosis

It is common belief that OPSCC shows 'basaloid' morphology consisting in cells with scanty cytoplasm, ovoid/spindle shaped, relatively monomorphic and hyperchromatic nuclei, which are reminiscent of the basal cell layer of normal squamous epithelium, with frequent mitoses and apoptosis and no keratinisation (figure 3A, B). However, recent published data show that the spectrum of HR-HPV-related OPSCC, although in minor part, comprises classic conventional SCC, with different grades of differentiation and keratinisation and mixed cancers with 'basaloid' morphology and squamous differentiation (figure 4).[41]

### Immunohistochemical and molecular diagnosis

The most popular and cost-effective method to detect HR-HPV infection is immunohistochemistry with p16 protein. The p16 $^{ink4a}$ (p16) protein is a tumour suppressor protein, belonging to the family of INK4 cyclin-dependent-kinase inhibitors. p16 inhibits the cyclin-dependent kinases 4 and 6 (CDK4/6), which phosphorylate the Rb protein with consequent accumulation of

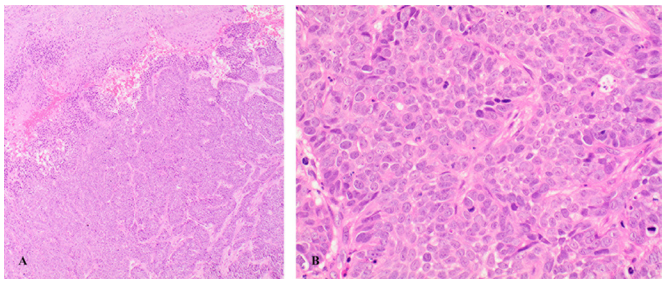


**Figure 3** (A, B) 'Basaloid' squamous cell carcinoma composed of cells with scanty cytoplasm, high mitotic rate and no keratinisation.

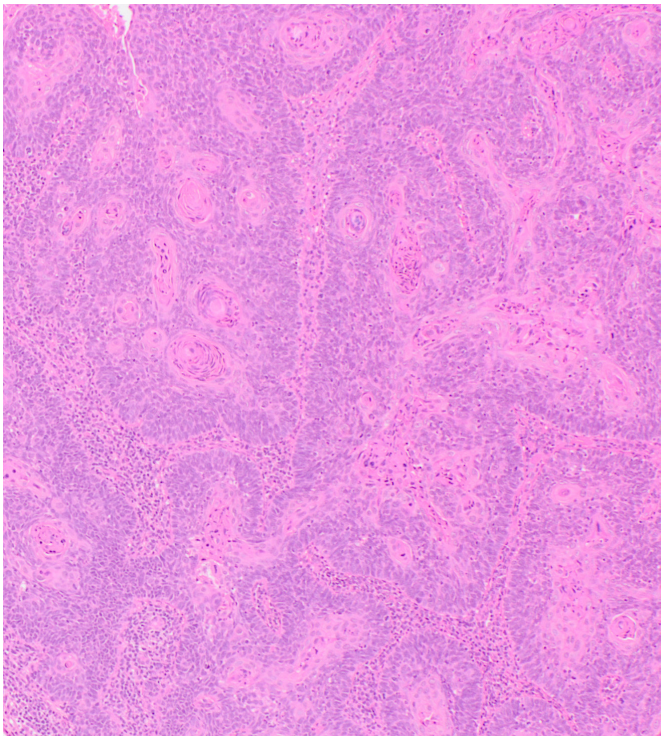

**Figure 4** 'Basaloid' cell carcinoma with squamous differentiation and keratosis.

hypophosphorylated Rb, which in turn signals cell cycle arrest. Overexpression of p16 in the lesional squamous cells of HPV-driven OPSCC is due to the inactivation of Rb by HR-HPV E7 oncoprotein. In normal cells, p16 is epigenetically silenced by polycomb repressive complexes. It is well established that the overexpression of p16 is a surrogate marker for transcriptionally active HR-HPV infection.[31 42–45] The new version (eighth edition) of the TNM classification includes p16-status as a single marker in the staging of OPSCC. However, p16 overexpression alone has proven as an insufficient marker for HPV-positivity, as well as for predicting prognosis.[46–48]

With regard to the immunohistochemical expression of p16, various points should be kept in mind as follows:

- Approximately 10%–15% of all OPSCC are p16 positive but do not show presence of HR-HPV DNA with molecular investigation. This subset of patients present a significantly worse survival rate with respect to p16/HR-HPV DNA positive patients.[47 49]
- In unknown primary neck SCC metastasis, positive p16 immunoreactivity should be interpreted with extreme caution because approximately 20% of metastatic skin and lung SCC, with no associated HR-HPV, show more than 70% of nuclear and cytoplasmic positive staining with p16.[50]
- p16 is only a surrogate marker for HR-HPV as, in the cervix uteri, 37% of non-HPV-related adenocarcinomas express diffuse p16.[51]

Evidence-based recommendations for the various techniques and applications in HR-HPV testing in HNSCC have been developed by a panel of experts in head and neck and molecular pathology convened by the the College of American Pathologists.[52]

The highlights of the recommendations are as follows:

- All newly diagnosed OPSCC (primary or regional lymph node metastasis) should be tested for HR-HPV.
- HR-HPV testing by surrogate marker p16 immunohistochemistry should be carried out. Discretionally, additional HPV-specific testing may also be performed.
- Routinely HR-HPV testing on non-squamous carcinomas of the oropharynx and non-oropharyngeal primary cancers of the head and neck should not be performed.
- HR-HPV testing on patients with metastatic SCC of unknown primary in a cervical upper or mid jugular chain lymph node should be carried out.
- HR-HPV testing should be done on head and neck fine needle aspiration SCC samples from all patients with known OPSCC not previously tested for HR-HPV, with suspected OPSCC, or with metastatic SCC of unknown primary.
- p16 immunohistochemical positivity, as a surrogate for HR-HPV, should be reported when there is at least 70% nuclear and cytoplasmic expression with at least moderate to strong intensity.
- Locoregional recurrent cancer should not be tested. If initial HR-HPV status was never assessed or results are unknown, then testing is recommended.
- Distant metastases should not be tested if primary tumour HR-HPV status has been established.
- Tumour grade status for HPV-positive/p16-positive OPSCCs should not be provided.
- Additional HR-HPV testing on p16-positive cases should be performed for tumours located outside level II or III (non-routine testing) in the neck and/or for tumours with keratinising morphology.

Although the guideline from the College of American Pathologists is an excellent 'road map' for diagnosis and in developing recommendations for OPSCC, similar to any consensus document, presents some weaknesses such as:

- 'Pathologists should not routinely perform HR-HPV testing on patients with non-oropharyngeal primary tumours of the head and neck': it is well known that some non-squamous type head and neck carcinomas are HPV-related and, therefore, need HR-HPV testing.[53–55]
- 'p16 immunohistochemical positivity, as a surrogate for HR-HPV, should be reported when there is at least 70% nuclear and cytoplasmic expression with at least moderate to strong intensity': the cut-off value of 'at least' 70% and 'moderate/intense' positive staining is purely subjective and may potentially create borderline cases.
- 'Tumour grade status for HPV-positive/p16-positive OPSCCs should not be provided': it has been shown that, although the majority, but not all OPSCCs, are 'basaloid' type and pure, conventional squamous type or mixed type ('basaloid'/squamous type) can also exist. Defining the differentiation of the squamous component in these subtypes would be a good practice.[41 56]

Currently, there is no univocal standard approach for HPV testing of clinical samples. There are different targets to be detected and determining the appropriate tool is based on accuracy, feasibility and cost-effectiveness. The targets include p16 protein, HPV DNA/RNA, viral oncoproteins and HPV-specific serum antibodies. RNA in situ hybridisation probes complementary to E6/E7 mRNA enable direct visualisation of viral transcripts in routinely processed tissues (figure 5) and show high sensitivity and specificity of 97% and 93%, respectively. This technique has been proposed as the clinical standard for assigning a diagnosis of HPV-related OPSCC.[57–60]

However, the method has limited application in day-to-day routine diagnostic work due to the high demand for expertise and complex tissue processing. In the era of innovation and

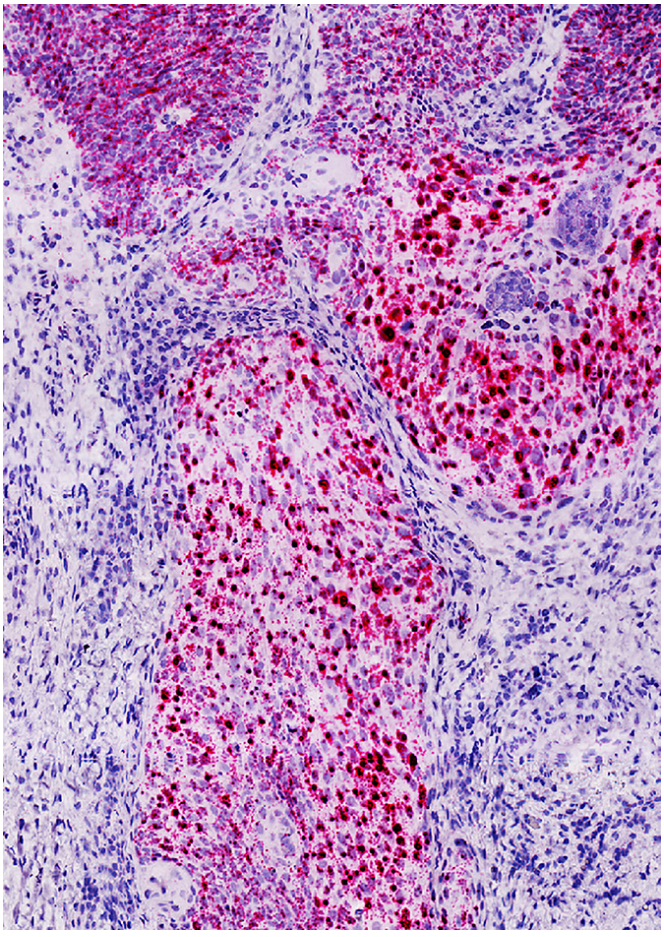

**Figure 5** RNA scope ISH showing E6/E7 mRNA HR-HPV. HPV, human papilloma virus; HR, high risk; ISH, in situ hybridisation.

digitalisation, direct searching of HPV genome and/or related proteins on tissue will no longer be the 'gold-standard' method. Detecting HR-HPV DNA/RNA or circulating neoplastic cells in blood or other biological fluids will replace the current techniques of HPV detection on tissues. With regard to the non-invasive methods, analyses of digitalised radiological and histological images seem to show excellent performance in terms of prediction of survival of patients with HPV-associated OPSCC almost identical to immunohistochemical analysis of p16 protein on tissues.[61 62]

## Follow-up

Detection of HPV DNA in biological fluids (eg, blood, saliva, etc), when tested along with clinical and radiological assessment, may help to detect residual disease in a very early stage. This is of paramount importance for the clinical decision regarding salvage in patients after first line treatment. Currently, second-line systemic treatment, immunotherapy, stereotactic re-radiation and surgical salvage are available and give patients a further chance when early diagnosis is made. Furthermore, regular assessment of HR-HPV DNA in biological fluids during follow-up may help detect locoregional or distant metastasis prior to onset of clinical symptoms enabling more prompt and effective salvage therapy. The correlation between HR-HPV DNA in biological fluids and radiotherapy or chemoradiotherapy findings is particularly important because of the increasing number of patients with HPV-associated OPSCC and their longer survival despite recurrence. Some studies demonstrated that circulating HPV16 DNA could be detected in the plasma of most patients with HPV-associated OPSCC and that the amount of DNA reflects response to treatment.[63–67]

## Vaccination

Despite the fact that universal access to vaccination is the key to avoiding most cases of HPV-related cancers, the efficacy of vaccine shown in genital cancers cannot be directly translatable to HPV-associated OPSCC. This is due to the differences in the epidemiology of OPSCC and genital HPV-positive cancers such as differences in age and gender distribution and the lack of oropharyngeal premalignant lesions analogous to cervical intraepithelial neoplasia in cervical cancer.[68]

## Take home messages

- The rate of human papilloma virus (HPV)-driven squamous cell carcinoma is increasing and in the next future this malignancy will be the predominant form of head and neck cancer.
- HPV-related cancers show better prognosis compared with their HPV-negative counterpart.
- The last edition of American Joint Committee on Cancer dedicated a separate staging system for HPV-positive cancers.
- Although immunohistochemistry with p16 is the most popular technique to detect HPV infection, molecular techniques for detection of HPV oncoproteins are more reliable.
- Recent advances in artificial intelligence based examination of standard digitised radiographic and histopathological images might replace tissue-based search of HPV.
- The challenging problem is to determine a standard univocal test on blood or other biological fluids to follow up treated patients for HPV-related cancers.
- Such a test, along with clinical and radiological assessment, may help to detect residual disease in the very early stage that may facilitate salvage management in patients after first-line treatment.

**Handling editor** Runjan Chetty.

**Contributors** SR designed and wrote the manuscript.

**Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests** None declared.

**Patient consent for publication** Not required.

**Provenance and peer review** Commissioned; externally peer reviewed.

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