

The role of human papillomavirus in oral squamous cell carcinoma

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ABSTRACT

Aim: The causative role of human papillomavirus (HPV) has been established into the aetiology of oral squamous cell carcinoma (OSCC). Some authors believe that HPV can determinate the prognosis and module treatment response from this kind of malignancies. **Methods:** Articles published in the last 10 years, focusing on the role of HPV in the development, molecular biology, prognosis and treatment of OSCC were reviewed. **Results:** Thirty-nine articles from 252 were selected, highlighting 4 meta-analysis, 3 prospective and 2 retrospective studies. According to its role in the development of cervical cancer, HPV is classified into a high risk for malignant lesions subtype and a low-grade malignant lesions subtype. Epidemiology and prevalence of HPV varies according to the published data: large studies tend to have lower rates of HPV (< 50%) than smaller ones (0-100%). Interestingly, HPV+ patients are usually diagnosed at a younger age, mainly those with oropharyngeal tumours. There is a predilection for the oropharynx and Waldeyer ring tumours. Regarding prognosis, OSCC HPV+ patients tend to have better outcome and treatment response. **Conclusion:** HPV divides OSCC in two types of tumours with different prognostic and therapeutic implications, with increased survival, better treatment response rates and lower risk of death and recurrences.

Key words:

Papillomavirus infections; carcinoma squamous cell; mouth

INTRODUCTION

Squamous cell carcinoma (SCC) is the most common malignant lesion of the oral cavity and oropharynx. It is characterized by a multifactorial aetiology,^[1-5] where the causative role of papillomavirus (HPV) has been

established.^[6] It is sexually acquired,^[7] usually described in the tonsillar area,^[8-10] affecting younger, non-drinkers and non-smokers patients.^[11-13] DNA from most oncogenic risk HPV is detected in approximately 26% of all oral

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Figure 1: The case of a 70-year-old and ex-smoker female patient with a human papillomavirus+ head and neck squamous cell carcinoma. We can see an exophytic lesion three years of evolution on the left floor of the mouth, surpassing the midline, with a progressive growth. The patient has no dysphagia or dyspnea. We decide to take biopsies, obtaining the diagnosis of squamous cell carcinoma

squamous cell carcinoma (OSCC) throughout the world.^[14]

The appearance of this kind of tumours has changed among the last decades. Some genotypes have been suggested as the most likely causative agents of human papillomavirus, whose carcinogenic effect in oropharynx was first proposed by Syrjänen *et al.*^[15] in 1983 according to common morphological characteristics of HPV and immunohistochemistry. Later, this was confirmed by using new techniques such as "Southern Blot Hybridization".^[16,17] HPV has been proposed as a major risk factor for oropharyngeal squamous cell carcinoma (OPSCC),^[7,18] with a strong association in subjects with or without the established risks of smoking and alcohol.^[7]

The oncogenic potential of certain high-risk HPV genotypes is related to its ability of integrating DNA fragments (E5, E6 and E7) in the host cell, annulling the function of tumour suppressor factors such as p21, p53 and pRb routes.^[19] However, there are many ethno-geographical differences between the examined groups, with detection ranges from 0 to 100%.^[18,20-24] Virus detection is also affected by the sensitivity of the diagnostic test and the location of the lesion, which difficult the clarification of the role of HPV and its carcinogenic potential.^[7,25]

Some authors not only involve the virus in the pathogenesis of OSCC, but also believe that it can determine the prognosis and module treatment response.^[26] The first type of HPV isolated in OSCC was HPV16 in the palatine tonsil, made by Niedobitek *et al.*^[27] in 1990. However, this is not the only subtype identified, varying according to the analysed population sample.^[28] Recently this type of HPV-positive tumours in the oral cavity was described as an entity with different molecular, clinical, etiological, pathological and prognostic characteristics.^[6,20-23,29-32]

METHODS

A review of articles published in the last ten years (since February 29, 2016 until January 1, 2005) in the database of medical literature MEDLINE via PubMed search engine was performed. The following descriptors obtained from "DeCS" were used as keywords: "Papillomavirus Infections", "Carcinoma, Squamous Cell" and "Mouth". All possible associations between them were used.

The main objective was to study the role of HPV in the development, molecular biology, prognosis and treatment of OSCC. We also provided special attention to detection and sampling techniques, risk factors, epidemiology, relationship with other non-malignant lesions and history of the virus.

Inclusion criteria were: (1) studies published between the dates indicated; (2) English language; (3) both observational and experimental studies; (4) reviews and meta-analyses; and (5) items that although published at an earlier date than the cut-off, are cited in the main revised. Exclusion criteria were: (1) publications that do not appear in the set date range and which are not mentioned in any of the included; (2) any type of non-English language; (3) studies lacking internal or external validity; (4) editorials and case reports; (5) studies with a sample size lower than 30, or if it is not mentioned by any of the included; and (6) articles that do not contain information on the main search object.

RESULTS

We preliminarily found 252 articles, of which only 39 were included and reviewed. Among these, 9 publications were highlighted: 4 meta-analysis,^[14,33-35] 3 prospective studies^[32,36,37] and 2 retrospective studies.^[38,39] Main results from these

Table 1: Nine highlighted publications

| Author | Year | Study | Objective | Number | Results |
|---|------|---------------|--|--------|---|
| Miller and Johnstone ^[33] | 2001 | Meta-analysis | To determine the significance of the relationship of HPV in the progressive development of oral cancer | 4,680 | HPV is detected with increased frequency in oral dysplastic and carcinomatous epithelium in comparison with normal oral mucosa |
| Kreimer <i>et al.</i> ^[14] | 2005 | Meta-analysis | To describe the prevalence and type distribution of HPV by anatomic cancer site | 5,046 | Tumor site-specific HPV prevalence was higher among studies from North America compared with Europe and Asia |
| Ragin and Taioli ^[35] | 2007 | Meta-analysis | To study the overall relationship between HPV infection and OS and DFS in HNSCC | 3,151 | The improved OS and DFS for HPV+ HNSCC patients is specific to the oropharynx; these tumours may have a distinct etiology from those tumours in non-oropharyngeal sites |
| Jayaprakash <i>et al.</i> ^[34] | 2011 | Meta-analysis | To provide a prevalence estimate for HPV-16/18 in OPD | 458 | HPV-16/18 were 3 times more common in dysplastic lesions (OR, 3.29; 95% CI, 1.95-5.53%) and invasive cancers (OR, 3.43; 95% CI, 2.07-5.69%), when compared to normal biopsy |
| Rosenquist <i>et al.</i> ^[32] | 2007 | Prospective | To evaluate the influence of different risk factors for recurrence or the appearance of new second primary in the OSCC | 128 | High-risk HPV cases have a higher risk of recurrence/second primary tumours, but lower risk of death in intercurrent disease, compared with HPV- |
| Fakhry <i>et al.</i> ^[36] | 2008 | Prospective | To evaluate the association between tumour HPV status with the therapeutic response and survival | 96 | HPV+ HNSCC respond better to QT and RT-QT, with better overall survival rate at two years and reduced risk of disease progression than HPV- |
| Rischin <i>et al.</i> ^[37] | 2011 | Prospective | To determine the prognostic significance of p16 and HPV in patients with OPC | 185 | HPV+ OPC is a distinct entity with a favorable prognosis (when compared with HPV-). When it is treated with cisplatin-based chemotherapy |
| Ang <i>et al.</i> ^[38] | 2010 | Retrospective | To study the association between tumor HPV status and survival in stage III and IV OPD | 743 | Among patients with OPC, tumor HPV status is a strong and independent prognostic factor for survival |
| Lassen <i>et al.</i> ^[39] | 2014 | Retrospective | To test the hypothesis that the impact of HPV/p16 also extends to non-OP tumours | 1,294 | The prognostic impact of HPV- associated p16-expression may be restricted to OPC only |

Nine highlighted publications are summarized because of their high number of patients, recent date of publication, good study design and highly cited in the literature, including 4 meta-analysis,^[14,33-35] 3 prospective studies,^[32,36,37] and 2 retrospective studies.^[38,39] HPV: human papillomavirus; OPD: oropharyngeal dysplasias; OP: oropharyngeal; OPC: oropharyngeal cancer; HNSCC: head and neck squamous cell carcinoma; OS: overall survival; DFS: disease free survival; QT: chemotherapy; RT: radiotherapy; OSCC: oral squamous cell carcinoma; OR: odds ratio; CI: confidence interval

studies are summarized in Table 1.

DISCUSSION

There is much written literature about the relationship of HPV virus and OSCC. Due to the great disparity of published data, it is very difficult to establish rightly the role HPV plays and its etiopathological, clinical and prognostic considerations. This can be related to differences in study populations (genetic, social and cultural factors) and the methodology of study and detection of virus.

There are many HPV genotypes identified, within which, over 130 are related to skin and mucosal lesions.^[40] The first to propose the pathogenic relationship of this type of virus with OSCC was Syrjänen *et al.*^[15] in 1983. And the first type identified in head and neck was HPV16 in palatine tonsil carcinomas.^[27] Since then, there have been many published studies on detection and about its role in OSCC.

HPV molecular biology

HPV belongs to a heterogeneous group corresponding to the "Papillomaviridae" family.^[41] It is characterized as a DNA-double stranded virus. It has a diameter of 50 µm and it is covered by an icosahedral capsid consisting of 72 capsomeres, without casing^[42,43] and presents a particular tropism by keratinocytes, being the synthesis and expression of their genes linked to

the level of their differentiation.^[44]

There are different routes of infection, mainly sexual, vertical and self-inoculation; they all share the need for close contact to occur.^[45,46] Transmission from non-primates to humans is unknown to occur.^[47,48]

To active the infection, the virus must reach the epithelial basal layer, where the specific integrin alpha 6 receptor is present.^[33] Once the infection becomes productive, cytopathic effects can appear, first of all koilocytosis.^[44] To make this happen, the patient's immune response plays an important role. During infection, viral antigen presentation is minimal and thus the infection can persist until years.^[33] In immunocompetent patients lesions usually regress spontaneously, while in immunodeficient the incidence and persistence of them is usually higher.^[49]

Regarding the oncological potential of the virus, there is much controversy about the true role played by the integration of viral DNA into human epithelial cells. Several authors have investigated its pathogenesis in OSCC. According to its role in the development of cervical cancer, HPV is classified into a high risk for malignant lesions subtype (HPV 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, 68, 73 and 82) and low-grade malignant lesions subtype but related to benign lesions (HPV 6, 11, 13, 32, 42, 43, 44).^[33,50]

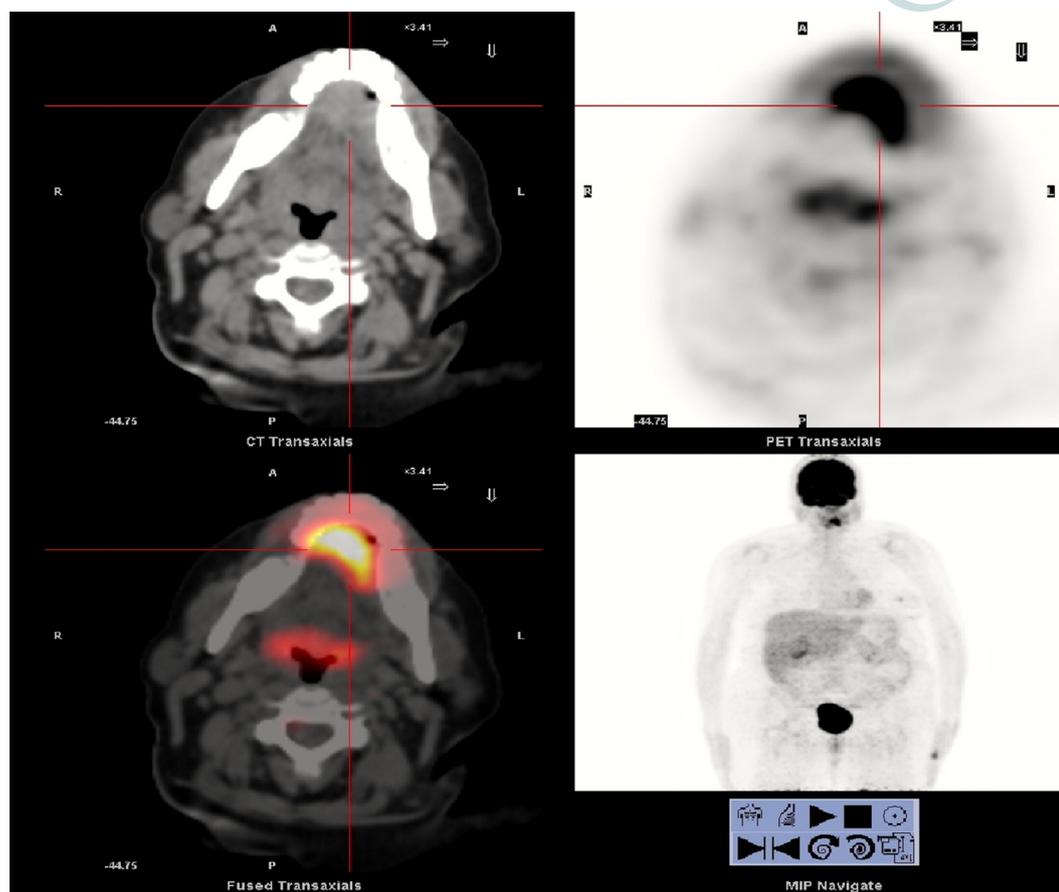


Figure 2: Positron emission tomography-computed tomography shows a mass in the left region of the anterior mouth floor, with marked increase glucose metabolism, about 3 cm in diameter and high probability of malignancy. The other cervical structures have normal glucose metabolism, showing no other hypermetabolic neoplastic involvement

The HPV genome is divided into about eight open reading frames (ORFs) divided into three regions:^[2,33] (1) early region (E): it is required for replication, cell transformation and control of viral transcription; (2) late region (L): it encodes structural proteins; and (3) long control region (LCR): it is required for replication and transcription of viral DNA.

In E, three proteins are encoded, which are often described as involved in the carcinogenesis related to the virus: pE7, pE6 and pE5.^[2,33,44] PE5 stimulates proliferation and inhibits apoptosis, while pE7 and pE6 act as oncogenes.^[2,33,47,48,51,52]

The final result is an induced and unregulated cell proliferation, with consequent immortality of the keratinocyte^[19] due to the integration and expression of the viral genome into the host cell. Chromosome aberrations and excessive production of viral DNA^[53,54] all occur due to inhibition of tumor suppressor factors (p21, p53 and pRb roads).^[19]

However, although the involvement of inhibition of tumor suppressor gene p53 in the carcinogenic effect of HPV seems to be clear, there are some publications that question the relationship of p53 polymorphism with the risk of oral cancer,^[55] suggesting that HPV does not play an important role oral lesions due to low detection in their analysed.^[56-59] This could be justified by population differences, sample size, detection techniques and tumor location. Some studies suggest that in HPV+ OSCC, p53 mutation is conditioned by tumor localization and expression of E6 and E7 viral genes, appearing a mutated p53 when the tumor is HPV+ but it does

not express these genes (mainly oropharynx), or when the tumor is HPV-.^[60,61]

Epidemiology and prevalence

Epidemiology and prevalence of HPV infection associated with OSCC varies according to the published data. Large studies tend to have lower rates of HPV (< 50%) than smaller studies (0-100%).^[25,62] Miller and Johnstone^[33] in a meta-analysis about 4,680 patients with OSCC from 94 reports reported that HPV was present in 46.5% of the cases (95% CI, 37.6-55.5%). However, the oral cavity was not the most often location, being surpassed by the oropharynx.^[63,64]

Kreimer *et al.*^[14] in a meta-analysis from 60 publications in 2005 (5,046 patients) reported that the overall prevalence of HPV in OSCC was 25.9% to 34.5%.^[14,25] The prevalence of OSCC ranges from < 2% to 100%^[10,25,57,65] and it may be because some studies do not differentiate between Paraffine Embedded and Fresh Frozen biopsies or different classification criteria, including incorrectly OPSCC within the OSCC, making an overestimation.^[25]

There is an association between the presence of HPV and age; patients older than 60 years have a lower HPV+ prevalence (29.4%) compared to patients under that age (77.8%).^[66] Within the OPSCC HPV+, HPV16 is higher in patients younger than fifty years.^[67,68] In relationship to sexual behaviour, the risk of oral cancer increases in male patients with decreasing age of first intercourse, with increasing numbers of partners and history of genital warts.^[69]

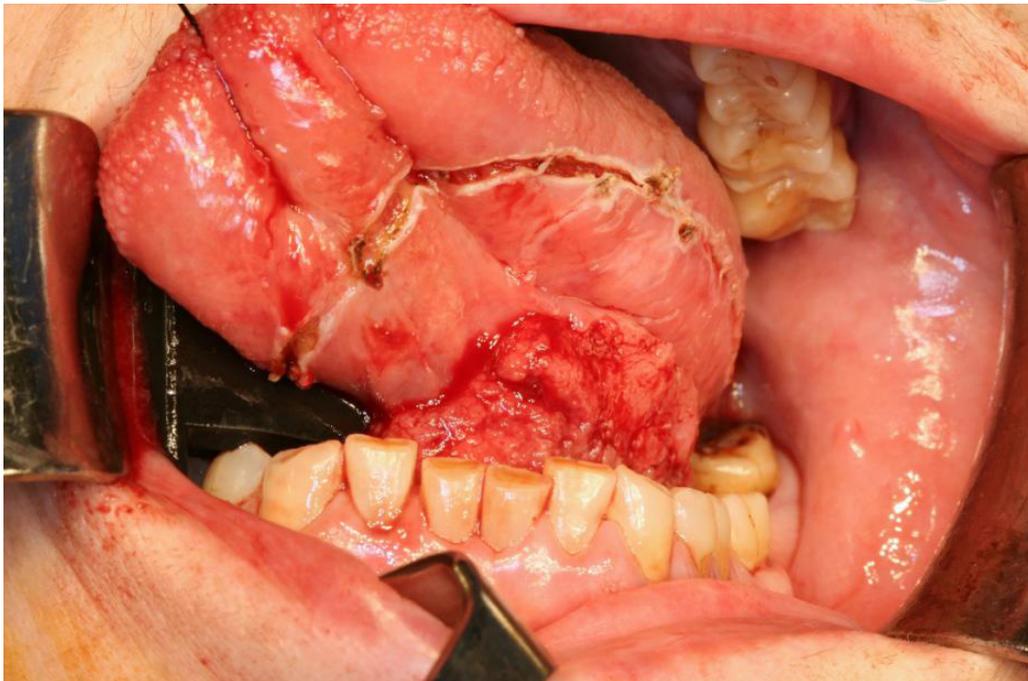


Figure 3: Intraoperative image. After performing tracheotomy, double functional bilateral neck dissection, excision of the lesion with macroscopic margins above the centimeter and subsequent reconstruction with radial forearm flap was performed. Histological results reveals a pT3N0M0 human papillomavirus 16+ squamous cell carcinoma, with close resection margins, 6 mm thickness, with no vascular or perineural infiltration. After surgery, the patient received adjuvant radiotherapy. She is currently without signs of loco-regional recurrence

Regarding to ethnogeographical differences, some authors suggest that Japanese studies tend to have the highest rate of HPV,^[70,71] while Africans tend to have the lowest rate.^[59] Kreimer *et al.*^[14] established that HPV+ prevalence was higher among studies from North America compared with those from Europe and Asia. In 2016, Mehanna *et al.*^[72] conducted a prospective study of 801 patients with head and neck cancers. They established the geographic variability (differences between continents) as an independent risk factor for HPV+ prevalence of OPSCC. It is most prevalent in Western Europe, when compared to Eastern Europe (37%, 155 of 422 vs. 6%, 8 of 144; $P < 0.0001$) and Asia (37% vs. 2%, 4 of 217; $P < 0.0001$).

Regarding the genotype, the most prevalent is HPV16 (68.2-90%)^[14,33,66] [Figures 1-3] followed by HPV18 (34.1%).^[14,73] But this varies depending on the series analysed and the techniques used, and that proportion may be reversed, being higher HPV18.^[28,59] Although the association between HPV and OSCC is described,^[32,62,68,74-77] it is important to note that high-risk genotypes HPV16 have been detected in normal oropharyngeal mucosa,^[78,79,62] questioning this causal relationship. In 2001, Mork *et al.*^[80] defined HPV infection as a risk factor for OSCC, whose exposure may precede the occurrence of OPSCC in 10 years and older.

In the oropharynx there is no hard evidence linking HPV with alcohol or tobacco use, and the absence of synergism is the most accepted hypothesis,^[81] suggesting two ways for the development of OPSCC, one derived from smoking with or without alcohol and another derived from the HPV induced genomic instability.^[31]

Most frequent location

HPV has a predilection for the oropharynx and the Waldeyer ring.^[14,24,59] It is estimated that the most frequent location

for detecting papillomavirus DNA is the palatine tonsil and the base of the tongue, with a strong causal association,^[14,82] independently of the influence of smoking or alcohol. Oropharyngeal HPV+ tumours appears in up to six times more often than in other tumours of the head and neck.^[6] Snijders *et al.*^[83] were the first to suggest the amygdala is linked with the HPV, in 1992.

Detection, diagnosis and typing techniques

Molecular assays are the gold standard for HPV identification,^[84] mainly polymerase chain reaction (PCR),^[85] specifically the reverse transcription PCR (RT-PCR) to measure viral mRNA E6 and E7 in fresh tissue.^[86] It has a high sensitivity.^[80,85-88] It is even able to detect latent infections. Other tests that have been used for detection of HPV are “Southern Blot” (less sensitivity than PCR)^[89] and *in situ* hybridization (ISH) (less sensitive and less expensive than PCR). Some authors have proposed the combination of PCR with ISH, combining the advantages of the two tests: the high sensitivity of PCR and the ability of ISH to identify and localize genomic sequences linked to HPV in this kind of tumours.^[90,91]

P16 is a protein used by some authors as a biomarker for HPV infection, which can be expressed when viral DNA is integrated into the host cell. It reflects the functional effects derived from the inactivation of pRb, induced by E7. It is detected by immunohistochemistry staining and it can be used as a predictor of HPV infection in OPSCC, even being proposed by some authors the detection of p16INK4A as an initial test, followed by the detection of HPV in which are positive for this.^[92-94]

Regarding to the sample being sent for testing, the most commonly accepted it is taking biopsies or tumour specimen analysis [Figure 1]. This allows not only molecular analysis

but also morphological analysis of the piece, including all cell layers where the virus may be latently.^[95] As a method of screening for epidemiological studies, Lawton *et al.*^[96] reported that mouthwash is the technique of choice, although higher performance by combining different sampling techniques is obtained.

Virus relationship with other oral lesions

Since the early 1980s some authors have reported the presence of HPV not only in cancerous lesions of the oral cavity, but also in premalignant lesions.^[15,97,98] Recently, the presence of HPV has been identified as an independent prognostic factor for survival in patients with OPSCC.^[38] Miller and Johnstone^[33] indicate that HPV (low and high risk serotypes) are 2-3 times more detected in precancerous mucosa and almost 5 times more detected in carcinoma than in non-neoplastic mucosa: (1) 22.2% in benign leukoplakia; (2) 26.2% in intraepithelial neoplasias; and (3) 46.5% in OSCC, with a detection probability of high-risk ones 2.8 times higher than low risk.

Jayaprakash *et al.*^[34] published in 2011 a meta-analysis about 458 oropharyngeal dysplasias, estimating that the prevalence of HPV16/18 is 24.5%. They reported that HPV16/18 were 3 times more common in dysplastic lesions (OR, 3.29; 95% CI, 1.95-5.53%) and invasive cancers (OR, 3.43; 95% CI, 2.07-5.69%), when compared to normal biopsies. In addition, they found these two genotypes are at least 2.5 times more common in men than in women. Within oral leukoplakia, proliferative verrucous leukoplakia is believed to have a stronger relationship with HPV^[44] mainly 16, with a range of onset between 10% and 85%,^[99,100] and higher rate of malignant transformation.^[101] Some authors have also reported a relationship between lichen planus and HPV, ranging from 0 to 100%,^[102] which indicates the existing controversy about this association.

Many publications are studying virus connection with benign lesions or even in normal mucosa, varying its prevalence depending on the technique used, many times no PCR techniques are used, which may underestimate measurements. As a summary:

Appearance in normal mucosa: varies between 0 and 81%.^[78,103] It may appear subclinical or latent,^[104] being detected by the extreme sensitivity of the PCR and may be or not related to the emergence of a future lesion.

Squamous papilloma: clinically often indistinguishable from common warts. HPV genotypes 6 and 11 are most frequently associated, detected by ISH.^[105]

Condyloma accuminatum: it is a sexually transmitted infection and it is usually related to HPV 6 and 11 infection, varying its positivity between 75% and 85% in oral lesions.^[106,107] Furthermore it is also related to the HPV 16.^[108,109] It is usually present in HIV+ patients.^[110]

Common wart (verruca vulgaris): oral lesions usually result from autoinoculation from the fingers. It usually occurs in children. The HPV 2 is described as the most frequently related, followed by HPV 57,^[106,111] detected most of the time

with no PCR techniques between 80% and 90%. Other authors with more recent publications detected more frequently HPV2 and 4.^[109]

Focal epithelial hyperplasia (Heck's disease): they usually occur in children and young adults. There is usually genetic predisposition.^[112] HPV13 (20%) and HPV32 (60%) are related to those lesions.^[113-115]

Prognosis and treatment

There is much controversy about the role that infection by the HPV plays in the prognosis and treatment of patients with OSCC. Most of the published studies are retrospective. But they do generally conclude that the presence of HPV divides these tumours in two different entities with different prognostic and therapeutic implications. The most commonly accepted is that patients with OPSCC HPV+ have a better prognosis due to increased survival, showing better treatment response rates.^[36,38,63,116-118]

The most cited paper in the literature is the one published by Fakhry *et al.*^[36] in 2008. They conducted a prospective phase 2 study of 96 patients with oral, oropharyngeal and laryngeal SCC. All patients received two cycles of induction chemotherapy with paclitaxel and carboplatin followed by concurrent weekly paclitaxel and radiotherapy. They detected HPV (types 16, 33 and 35) with PCR and ISH in 40% of all patients. They compared their response to treatment with HPV-: OSCC HPV+ have better respond to chemotherapy (82% vs. 55%, difference = 27%, 95% CI, 9.3-44.7%; $P = 0.01$) and chemo-radiotherapy (84% vs. 57%, difference = 27%, 95% CI, 9.7- 44.3%; $P = 0.007$).

Patients with OSCC HPV + have a better overall survival rate at two years [95% (95% CI, 87-100%) vs. 62% (95% CI, 49-74%), (difference = 33%, 95% CI, 18.6- 47.4%; $P = 0.005$, log-rank test)] and a lower risk of disease progression than HPV- [Hazard Ratio (HR), 0.27; 95% CI, 0.10-0.75%].

In 2007, Ragin and Taioli^[35] performed a meta-analysis of 37 studies, which conclude that patients with OSCC HPV+ had a lower risk of death (HR = 0.85 target; 95% CI, 0.7-1.0) and lower risk of recurrence (HR = 0.62% target; 95% CI, 0.5-0.8) than in HPV-. Regarding OPSCC they conclude that HPV+ had a reduced risk of death of 28% (Target HR 0.72; 95% CI, 0.5-1.0) compared with HPV- with a similar result for disease-free survival (Meta HR, 0.51; 95% CI, 0.4-0.7).

In the same year, Rosenquist *et al.*^[32] conducted a prospective study of cases and controls over 128 Swedish patients with OPSCC to evaluate the influence of different risk factors for recurrence or appearance of new second primaries in the first 3 years after the diagnosis. They found, unlike other published studies that high-risk HPV+ cases had a higher risk of recurrence/second primary tumour, but lower risk of death in intercurrent disease, compared with HPV- ones.

In 2008, Worden *et al.*^[119] conducted a study about the response to treatment of 66 patients with OPSCC. They found that the presence of HPV was significantly associated with response to chemotherapy ($P = 0.001$), chemo-radiotherapy (P

= 0.005), with better overall survival ($P = 0.007$) and disease-free survival ($P = 0.008$). They conclude that chemotherapy followed by chemo-radiation therapy is an effective treatment especially in patients with HPV + OPSCC.

In 2010, Ang *et al.*^[38] conducted a retrospective study of the association between tumor HPV status and survival among 743 patients with stage III or IV OPSCC who were enrolled in a randomized trial comparing treatment with accelerated-fractionation RT+ cisplatin vs. standard-fractionation RT+ cisplatin. Among 323 OPSCC, 63.8% were HPV+, which presented better 3-year rates of overall survival (82.4% vs. 57.1% among patients with HPV- negative tumours; $P < 0.001$ by the log-rank test) and they also had a 58% reduction in the risk of death (HR, 0.42; 95% CI, 0.27 to 0.66). They concluded that among patients with OPSCC, tumor HPV status is a strong and independent prognostic factor for survival.

Some authors have studied the prognostic influence of some biomarkers related to HPV infection in OSCC. One of the most studied biomarkers is p16, being observed that p16+ and HPV+ patients have a better overall survival compared with HPV- or HPV+ but p16-.^[93] This was corroborated in the prospective phase III study of concomitant chemotherapy published in 2011 by Rischin *et al.*^[37] In a sample of 465 patients with OPSCC stage III or IV, 172 were analysed with evaluable HPV and p^{16INK4A} status, and 185 with eligible p16 status. They randomized RT+ cisplatin with or without tirapazamine, concomitantly. They found that p16+ tumours compared to p16- presented: (1) higher rates of overall survival at 2 years (91% vs. 74%; HR, 0.36; 95% CI, 0.17-0.74; $P = 0.004$); (2) higher rates of relapse-free survival (87% vs. 72%; HR, 0.39; 95% CI, 0.20-0.74; $P = 0.003$); and (3) lower loco-regional recurrence and death rates from other causes. They also observed a trend in favour of tirapazamine group in terms of improved loco-regional control disease in p16- patients (HR, 0.33; 95% CI, 0.09-1.24; $P = 0.13$). They concluded that OPSCC HPV+ have a favourable prognosis when treated with cisplatin-based chemotherapy, compared to HPV-.

In 2014, Lassen *et al.*^[39] published a retrospective study among 1,294 Danish patients with advanced stage OPSCC. They observed that p16 positivity was significantly higher in oropharyngeal than non-oropharyngeal SCC ($P < 0.0001$). OPSCC p16+ presented a statistically significant improvement in loco-regional disease control with primary RT [HR (95% CI), 0.38 (0.29-0.49)], free survival events [HR (95% CI), 0.44 (0.35-0.56)] and overall survival [HR (95% CI), 0.38 (0.29-0.49)], unlike in non-OP.

Future therapeutic lines

HPV+ OSCC response to RT, chemotherapy and the combination of both are topics widely approached in the literature and specialized forums. However, little or nothing is known about immunotherapy techniques and their effectiveness. In 2015, Rosenthal *et al.*^[120] published a retrospective assessment of the IMCL-9815 study, trying to find if there were any differences in treatment patients with RT alone vs. RT+ cetuximab, in a series of 182 OSCC patients, in relation to the presence or absence of HPV and p16. They concluded that the addition of cetuximab to RT improved

clinical outcomes regardless of p16 or HPV positivity. They also indicated that p16 does not predicted response to cetuximab.

Unlike cervical cancer, regarding OSCC there is not much literature on the use of HPV vaccines to treat these tumours. The effectiveness of the HPV vaccine against OSCC is not yet proven.

In conclusion, there is much controversy about the carcinogenic potential of HPV. Its mechanism usually involves the pE7 and pE6 proteins, which can delete p53, p21 and pRb routes.

HPV+ patients are usually diagnosed at a younger age, mainly those with oropharyngeal tumours, presenting positivity first of all for HPV16 > HPV18, although it varies depending on the population and the test used to detect the infection.

For more diagnostic performance, the most advisable is to use the combination of several techniques. P16 positivity needs to be mentioned in special attention as a predictor of HPV infection in the OPSCC for their prognostic and therapeutic considerations.

HPV can appear in normal mucosa, benign and precancerous lesions.

The most commonly accepted is that the presence of HPV divides OSCC, mainly oropharyngeal, in two types of tumours with different prognostic and therapeutic implications.

Despite the great controversy in prognosis, most studies tend to indicate that HPV+ OSCC have an increased survival, better treatment response rates, lower risk of death and lower risk of recurrence [Figure 3].

The oropharyngeal region should be analysed separately. OPSCC HPV+ tend to respond better to radio-chemotherapy treatments, considering the HPV positivity as a strong and independent survival prognostic factor. In addition, if p16+, these tumours tend to have better survival and loco-regional disease-control.

Future research should evaluate the possibility of new treatments.

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Conflicts of interest

There are no conflicts of interest.

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