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**Review**

# Human papillomavirus and head and neck carcinomas: focus on evidence in the babel of published data

## *Papillomavirus umano e carcinomi del tratto aerodigestivo: il punto sulle evidenze nella babele dei dati scientifici*

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SUMMARY

Human papillomavirus (HPV)-associated squamous cell carcinoma of the oropharynx is a well-defined entity mostly affecting young to middle-aged male non-smokers. It is generally associated with a favourable outcome, and for this reason a less intensive therapeutic approach has been proposed for this subset of patients. The incidence of HPV-associated oropharyngeal cancers is rapidly increasing in most Western countries, but detailed epidemiological data are not available for the Italian population. Furthermore, among other head and neck regions, a smaller proportion of oral high-grade dysplasia and cancers seems to depend on HPV infection, whereas its role in laryngeal cancer is recognised as less relevant. HPV-dependent neoplastic transformation depends on the expression of viral oncogenes in the infected host cell that can only be directly documented through viral oncogene mRNA identification. The consensus on how to classify these patients from clinical and laboratory diagnostic points of view is still limited, with different approaches based on one or more diagnostic techniques including p16 immunostaining, in situ hybridisation and polymerase chain reation (PCR) amplification of viral DNA. The possibility of early diagnosis relying on the identification of HPV infection in oral and oropharyngeal exfoliated cells has so far provided unsatisfactory results, although viral persistence after treatment has been associated with risk of recurrence. Presently, sufficient data are not available to document the natural history and progression from tonsillar HPV infection to oropharyngeal cancer development, and to clearly define the modality of transmission and risk exposure, among which sexual behaviours appear to play a relevant role. The diffusion of HPV vaccination and its administration to both genders will undoubtedly dramatically modify the epidemiology of HPV-related head and neck cancers in the coming years.

KEY WORDS: HPV • Oropharyngeal cancer • Oral cancer • Diagnosis

RIASSUNTO

*I carcinomi squamosi dell'orofaringe associati all'infezione da papillomavirus umano (HPV) costituiscono ormai una entità ben caratterizzata, che interessa prevalentemente maschi, giovani adulti o di mezza età, non fumatori. Essi hanno generalmente una prognosi più favorevole rispetto alla controparte non associate ad infezione, e per questo è stato proposto di dedicare a questo gruppo di pazienti un approccio terapeutico meno aggressivo. L'incidenza dei carcinomi dell'orofaringe associati a HPV è in rapido aumento nella maggior parte dei paesi occidentali, ma per quanto riguarda la popolazione italiana non sono disponibili dati epidemiologici in merito. Per quanto riguarda le altre regioni del distretto testa-collo, una più modesta porzione di lesioni displastiche di alto grado e di neoplasie appare essere correlata all'infezione da HPV, mentre il ruolo del virus nei tumori della laringe è stato parzialmente ridimensionato. HPV determina la trasformazione neoplastica delle cellule infettate tramite l'espressione dei suoi due oncogeni, E6 ed E7, che interagiscono con i meccanismi di apoptosi e regolazione del ciclo cellulare della cellula ospite. L'unica metodica in grado di documentare con certezza l'espressione degli oncogeni virali è attualmente l'amplificazione dell'RNA messaggero trascritto dai due oncogeni. Il consenso riguardo la strategia per l'identificazione dei pazienti affetti da carcinoma dell'orofaringe associato a HPV dal punto di vista clinico e diagnostico è tuttora limitato. Le metodiche diagnostiche più utilizzate, singolarmente o in combinazione, comprendono l'immunocolorazione con anticorpi diretti contro p16, l'ibridazione in situ per genotipi virali ad alto rischio e l'amplificazione del DNA virale mediate PCR. La possibilità di ottenere una diagnosi precoce grazie all'identificazione dell'infezione virale nelle cellule epiteliali esfoliate dal cavo orale o dall'orofaringe non ha finora fornito risultati soddisfacenti, tuttavia la persistenza del virus nel cavo orale in pazienti trattati per carcinoma dell'orofaringe ha dimostrato una significativa associazione con il rischio di recidiva del tumore. Non sono ancora disponibili sufficienti dati che documentino in maniera dettagliata la storia naturale dell'infezione a la sua progressione verso lo sviluppo di una neoplasia, e che definiscano con chiarezza le modalità di trasmissione e i fattori di rischio, comunque è chiaro che i comportamenti sessuali hanno un peso rilevante nel determinare il rischio di sviluppo di neoplasia dell'orofaringe HPV-correlata. La progressive diffusione nelle giovani generazioni del vaccino contro HPV, e soprattutto la sua estensione agli adolescenti di entrambi i generi è sicuramente destinata a modificare in maniera rilevante anche l'epidemiologica dei tumori HPV-correlati nel distretto testa-collo nel prossimo futuro.*

PAROLE CHIAVE: *HPV • Carcinoma dell'orofaringe • Carcinoma orale • Diagnosi*

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## Introduction

Head and neck cancers rank as the sixth most common cancer worldwide and represent a serious challenge for the health community. The typical tumour is a squamous cell carcinoma (SCC) with variable grade of differentiation (from well to undifferentiated); it predominantly affects males in their fifth to sixth decade of life and, in Western countries, it is strongly related to tobacco smoke and alcohol abuse. The estimated annual burden of head and neck squamous cell carcinomas (HNSCC) is about 650,000 cases and the rate of death is approximately 50% [1].

The incidence of head and neck (HN) SCC has remained stable or even declined since the late 1980s because of a gradual decrease in typical risk behaviours. Despite this, SCCs occurring in the oropharyngeal (OP) region (particularly in the tonsils and base of the tongue) have increased from 2-3% to 5.5% of all HNSCCs in the USA and other countries [2 3]. Consistently, this increase has been shown to affect young men (30-40 years of age) with limited or no exposure to the typical risk factors. This epidemiological shift first suggested the involvement of a new driver cause for this type of cancer, which has been supported by epidemiologic and molecular evidence showing a causal role of human papillomavirus (HPV) in the subset of HNSCC originating from the oropharynx. The involvement of HPV in oral and oropharyngeal carcinogenesis was first proposed by Syrjanen in 1983 [4] and subsequently confirmed by several studies and recognised by the international scientific community [5]. At present, there is general agreement that clinical and prognostic implications of HPV-related OPSCC differ from those of conventional, tobacco-related SCC, and for this reason its treatment needs to be adapted accordingly [6-8].

Although HPV is unequivocally recognised as a causative agent for a subset of OPSCC, the abundance of published data regarding the biology and natural history of HPV infection, identification methods and best clinical management of patients with HPV-related cancer can be overwhelming for the general medical professional. In this review, the authors will focus on the main implications of HPV biological, diagnostic and prognostic implications in the wealth of published data on HPV investigation and detection in HN cancer.

## Definition of HPV-associated carcinoma

General consensus has been reached on the definition of HPV-associated tumours, which requires the expression of viral oncogenic proteins E6 and E7, responsible for the neoplastic transformation of infected cells. Less solid evidence, however, supports the belief that HPV-DNA integration into the host cell genome is an essential step for virus oncogene expression in oropharyngeal cancer, as in the case in cervical carcinomas [9]. Since the first observation by Snijders et al. in 1992 [10], several studies have indeed documented the presence of HPV oncogene transcripts in tumours with prevalent episomal viral genomes [9]. Independently of the process leading to oncogene expression, HPV E6/E7 mRNA identification is considered the gold standard for classification of HPV-related tumours in the head and neck, although for patient stratification and epidemiological purposes more accessible strategies based on DNA or protein expression are generally accepted.

## Epidemiological burden of HPV-associated head and neck carcinomas

The prevalence of HPV-associated head and neck carcinomas shows great variation among geographical areas, and is strongly associated with the anatomical site of the tumour. A recent, comprehensive, meta-analysis that considered the methods used to assess HPV tumour status[(11)] provided definite evidence that, among HN compartments, the attributable fraction of E6/E7 mRNA and HPV-DNA positive cases is highest in the oropharynx, close to 40%, whereas it is 16.3% in the oral cavity and less than 10% in the larynx. This result is of great relevance because it provides further confirmation for the current practice of reserving HPV testing to oropharyngeal cancer. Moreover, despite very different prevalences of HPV infection in tumours occurring at subsites such as the larynx [12], no clear prognostic implications have been documented for HPV-positive SCC occurring at these subsites [13].

Despite being so far limited to OPSCC, HPV oncogenesis in the head and neck region appears to be responsible worldwide for an increasingly relevant burden of new cases that are rapidly changing the traditional landscape of HN oncology. In countries where this phenomenon is more relevant, such as the USA and Northern European countries, the incidence of OPSCC among men younger than 60 years of age has been steadily increasing over the last 3 decades, despite the stability or even reduction of oral cancer incidence [14]. Several studies provided evidence for a role of HPV in the shift of HNSCC epidemiology: in Sweden, Ramqvist et al. reported a substantial increase in tonsillar and tongue base SCC during the period 1960-2006, among which the proportion of HPV-related tumours increased from 23% to more than 90% in the most recent years [15]. Studies in the USA have shown a similar trend and projected that, by 2020, HPV-related OPSCC will become the most common HPV-associated tumour, exceeding cervical cancer [2]. The epidemiology of HPV-associated OPSCC in Italy is less well documented. The Cancer Incidence in Five Continents survey [14] reported a moderate parallel reduction of both oral and oropharyngeal cancer incidence over the last 3 decades, but nationwide data on HPV-related cancer incidence have not been collected. By reviewing the scientific literature produced

in Italy, partial data from the north suggests a lower but nonetheless increasing prevalence: two studies published by the Istituto Tumori of Milan with a 6-year interval from each other reported a prevalence increasing from 17% [16] to 50% [17]. Two recent studies reported, respectively, a 32% and 39.8% prevalence of HPV-associated OPSCC in consecutive series of oropharyngeal cancers collected in two different Roman Institutes between 2009-2011 and 2010-2014 [18 19]. Our group could document a similar raising trend: since the beginning of HPV analysis in 1997 until 2010 less than 30% of OPSCC were HPV-positive [20], while the proportion increased to 48% in the years 2011-2013 [21] and has recently reached 50%.

The clinical relevance of the increasing prevalence of HPV-related SCC is not limited to the number of cases, but needs to take into account the specific characteristics of the affected population, which generally differs from the typical HN tumour patient population in younger age, lack of tobacco and alcohol exposure and higher socioeconomic status [22]. This implies a shift towards a therapeutic approach that takes into account the longer expected life span and lower risk of second exposure-related tumours is needed, as well as the need for increased risk awareness for young non-smoker males who have so far been considered at 'low risk' for their lack of exposure to conventional HNSCC carcinogens.

## Diagnostic classification of HPV-associated carcinoma

Correctly diagnosing HPV-associated HNSCC now represents one of the major challenges faced by otolaryngologists and HN pathologists. When HPV-associated OPSCC was recognised as an independent subgroup of HNSCC [22], more than of a decade of experience had already been accumulated on the diagnosis of HPV-related cervical cancers, and several diagnostic platforms had been implemented and patented for HPV identification, mostly based on DNA identification and/or amplification. Customary cooperative patterns in pathology units led to the widespread translation of the diagnostic protocols for gynaecological cancer into the HN field. This resulted in a wealth of data that can hardly be comparatively analysed, in part because of the heterogeneity of tests employed, and in part because of the inherent difficulty in precisely defining the subsite of tumour origin. Much evidence supports a correlation between HPV oncogenesis and tumours developing from the epithelium of the tonsillar crypts [23]. However, defining the specific subsite of tumour origin in advanced lesions diffusely extending beyond the limits of the original anatomical structures can be difficult, both with clinical evaluation and imaging, and indeed most studies do not specify the topographic criteria of classification. Although histological evidence of non-keratinising, so-called 'basaloid' morphology is suggestive of a deep tonsillar origin of SCC [24], HPV expression in conventional keratinising SCC is also observed [25]. For this reason, studies aimed at correlating HPV status with precise topographical data and histomorphology are required to further clarify the issue of the specific anatomic site of infection and tumour transformation.

With the above-mentioned limitations, the proportion of HPV-DNA-positive cases in different oropharyngeal subsites ranges from 53.9% (CI 46.4-61.3) to 47.8 (CI 43.1-61.8), respectively, in the tonsil and tongue base [11], However, at variance with cervical tumours, the presence of HPV-DNA in OPSCC cells does not equal HPV-driven oncogenesis [26], being possibly explained by passenger infections facilitated by lowered immune resistance (tumour, therapy, previous smoking), although other explanations, including false-negative mRNA amplification and contaminations, should be taken into account. As previously mentioned, HPV-mediated oncogenesis depends on the expression of the two viral oncogene proteins E6 and E7 that interact with cellular pathways of apoptosis and cell cycle control, and which are also the unequivocal markers of HPV-associated SCC [27]. Although the availability of standardised laboratory methods for mRNA extraction and amplification is increasing, it is not yet easily available for routine diagnosis in general pathology labs, mostly because fresh samples are still required for reliable results. Therefore, we are relying on different diagnostic strategies, whose diagnostic accuracy has to be assessed against the gold standard of HPV-mRNA amplification. Currently used tests that can be applied on routine formalin-fixed, paraffin-embedded samples include DNA amplification, DNA in situ hybridisation (ISH) and immunohistochemical identification of the cell cycle regulator $p16^{ink4a}$. As far as *PCR* amplification is concerned, the vast range of primer sets, most of them patented, targeting either DNA sequences shared by more high-risk (HR) and low-risk (LR) HPV genotypes [28] or specific sequences for a single genotype (esp. HPV16), and the parallel heterogeneity of methods used to analyse and interpret PCR results, make it very difficult to assess overall PCR accuracy with respect to the gold standard of HPV oncogene mRNA expression. The reported sensitivity of various PCR tests is generally high (60-99%), whereas the specificity is low (33-76%) [29-36]. Recently, real-time quantitative (q-) PCR has been applied to the study of HPV-associated OPSCC. Although the use of different experimental approaches (primer sets, target gene, technologies) also impairs comparisons in this setting, this approach can increase test accuracy given that HPV oncogene mRNA expression is strictly correlated with high viral load [32 35]. Commercial qPCR platforms for HPV screening and typing of cervical samples have been released on the market by several companies, but their use in OPSCC is still limited. A recent study analysed the performance of the Roche Cobas™ HPV test on cytological samples of HNSCC, documenting 100% sensitivity and

86% specificity compared with p16 ICH and ISH, but further studies are required to establish the test accuracy with respect to mRNA expression [37].

ISH has acquired a relevant role in HPV identification in OPSCC because of the development of standardised automated protocols using either genotype-specific (HPV16) probes or probes that target several HR genotypes, and the inherent morphological correlations on tissue slides (Fig. 1a). Although the reported sensitivity is ideally 1-2 viral copies per cell 23, ISH sensitivity with respect to mRNA gold standard is generally lower than that of PCR, although its specificity is good (88-100%) [29 30 32 38-40].

p16 immunostaining is the only method showing, albeit indirectly, evidence of HPV transcriptional activity, and contemporarily allowing morphological correlations (Fig. 1b). p16 is a cyclin-dependent kinase inhibitor whose expression is linked via a negative feed-back loop with pRB expression; pRB inactivation by HPV E7 leads to p16 overexpression that can be demonstrated immunohistochemically with specific monoclonal antibodies, while normal expression levels rest below the detection threshold. The prognostic role of p16 expression is sufficiently well documented to support its use in oropharyngeal cancer patient classification even independently of HPV status [41 42], to the point that it was the only selection criterion in multicentre trials aimed at assessing the efficacy of deintensified therapy protocols in HPV-positive patients [43 44]. It is necessary to remark however, that while p16 sensitivity is very high compared to the gold standard of mRNA expression, consistent evidence shows that its specificity is lower (72-80%) [29 30 32 45]. Poor specificity is due to the presence of other regulatory pathways of p16 expression apart from HPV oncogenes [46]. Recent studies have suggested that p16 overexpression in HPV-negative tumour cells may be associated with mechanisms of cell senescence [47]. A further issue that challenges the use of p16 as a surrogate marker of HPV oncogenic infection is its common expression in normal tonsil reticulated cells from which HPV-positive SCC are believed to originate [47]. p16-negative, HPV-mRNA-positive cases have also been reported by some authors [48]. Finally, the optimal cut-off of expression for positivity has not yet been univocally established, although 70% nuclear and cytoplasmic positivity appears to better predict the presence of HPV [49]. Despite these limitations, p16 immunostaining is still considered an acceptable and accessible surrogate marker for the classification of HPV-related OPSCC [31 50 51], provided that the interpretation of staining results follows the reported guidelines and is not translated to non-oropharyngeal sites [52 53].

*Diagnostic algorithms*

The suboptimal diagnostic accuracy of the above-summarised diagnostic methods can be partially overcome by the use of diagnostic algorithms that pair, either in parallel or sequentially, more than one test. Given a sensitivity approaching 100% and lower cost, there is general agreement on the use of p16 immunostaining as the first diagnostic step, followed by either HPV-DNA amplification or ISH [29 54]. More recently, RNAscope™ (Advanced Cell Diagnostics, Hayward, CA) has been validated as a new ISH method to directly document the presence of HPV mRNA in histological tissue sections [39 55] (Fig. 1c, d). De-

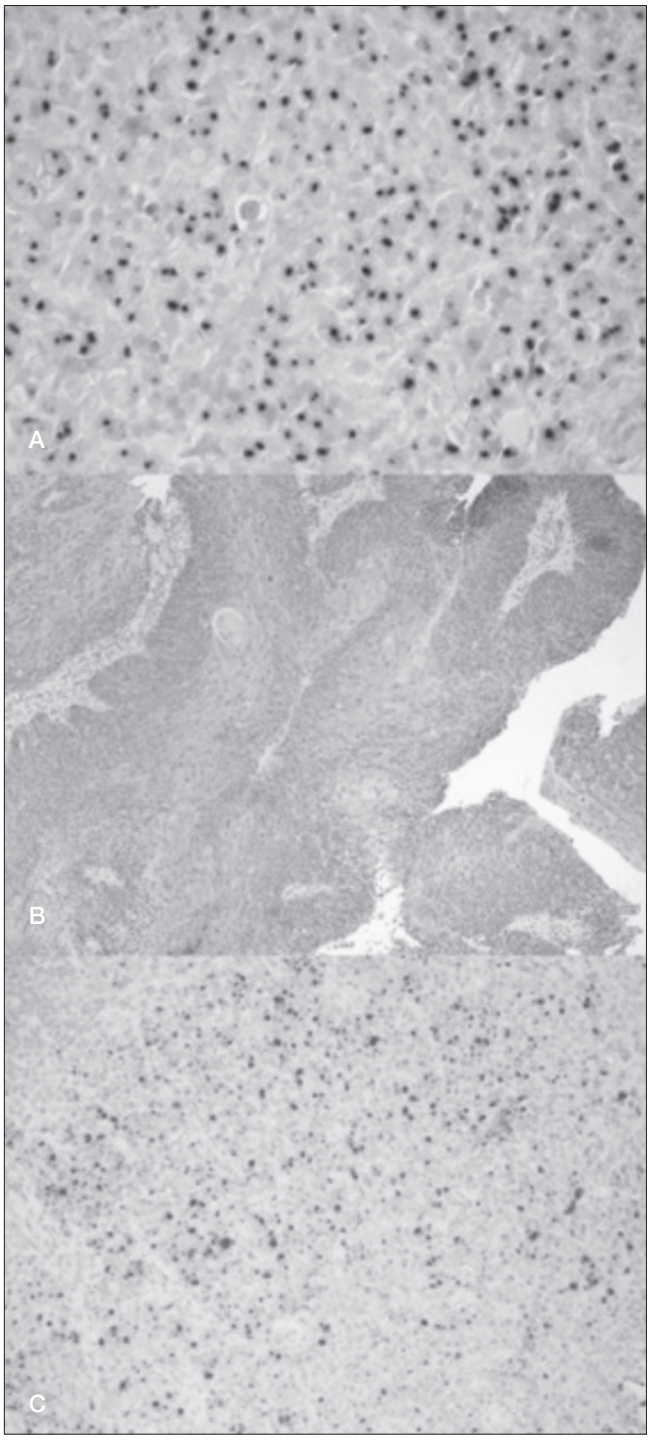


**Fig. 1**. Light micrographs showing a positive reaction in ISH (a), p16 positive immunostaining (b), and mRNA ISH positive reaction (c) in HPV-positive OPSCC.

spite its excellent diagnostic accuracy [39 56], application of RNAscope in routine diagnosis is probably limited by its high cost and complex protocols. We have demonstrated that a stepwise diagnostic algorithm that includes both ISH and HR-HPV-DNA amplification (Fig. 2) can correctly classify all mRNA ISH-positive cases [21]; however, RNAscope is the only tool for the direct recognition of HPV transcriptional activity in paraffin-embedded samples.

A further issue is whether only HPV16 or all HR genotypes should be investigated in OPSCC, and which viral gene should be amplified. In our experience, we adopted a very sensitive broad-spectrum primer set (SPF10) paired with reverse-line blot genotyping [LiPA, Genotyping Assay Version Extra (Fujirebio Europe, Ghent, Belgium)] that is widely used in genital and head and neck pathology in paraffin samples [2 57] and found non-HPV16 HR infections in 5-10% of cases. When amplifying viral sequences comprised in the L1-L2 region in oropharyngeal SCC, false negative and discordant results can be observed because of possible L1 deletion upon viral integration [20 58 59]. The amplification of the HPV16 E6 gene in a separate reaction can help in resolving ambiguous results (i.e. p16+/ISH+/PCR-).

*Topographic correlations*. Evidence is accumulating that supports a specific correlation between HPV infection and neoplastic transformation of the reticulated epithelium lining the tonsillar crypts [23], which can in turn explain the typical non-keratinising or 'basaloid' morphology of HPV-associated OPSCC (Fig. 3) [24]. The biological peculiarities of tonsillar structures have been claimed to be responsible for this selective tropism. The deep mucosal crypts may trap HPV viral particles and prolong the contact time between the virus and the mucosa; furthermore, tonsillar reticulated epithelia have intercellular 'gaps' that mimic the microlesions known to allow viral access to the basal cell layers in the cervical epithelium [60]. Another possibility is that the local immune environment of the tonsil is directly involved in malignant transformation. Although HPV-related tumours can by no means arise from superficial tonsillar keratinising epithelium [25], within the oropharyngeal region HPV-related oncogenesis appears to be strongly related to tonsillar structures, whereas other oropharyngeal subsites are rarely involved. Importantly, a clear distinction of the site of origin of the tumour is often not straightforward from a clinical point of view, especially with larger tumours. Therefore, new cases of OPSCC should not be excluded from HPV testing on a purely topographical basis.

## Non-oropharyngeal HPV-associated SCC

We have previously mentioned the low impact of HPV infection in non-oropharyngeal oncogenesis. One possible exception can be the oral cavity, where HPV infection has been demonstrated in the periodontal pockets [61], and HPV replication appears to be favoured by the epithelial cell proliferation induced by chronic periodontal inflammation [62]. Recent studies documented HPV association in a morphologically characteristic subset of oral high-grade dysplasia and SCC, mostly originating from the floor of the mouth and mobile tongue [63 64], which accounts for

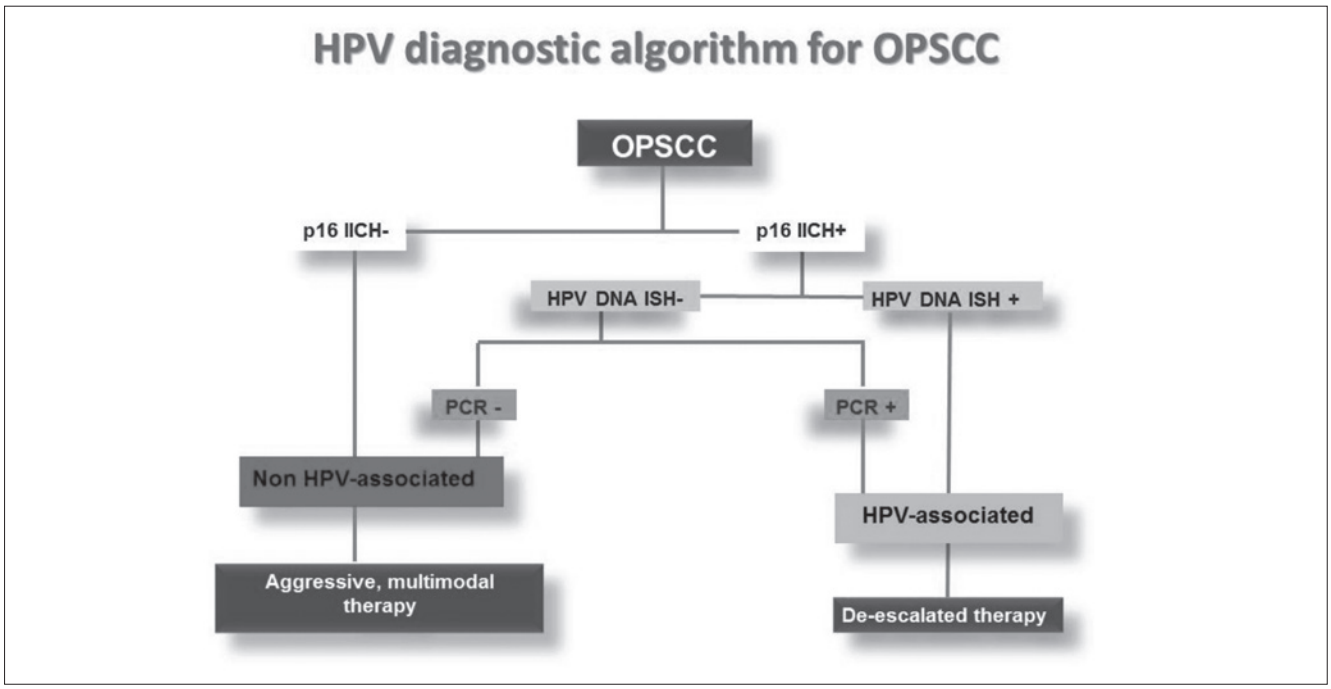


**Fig. 2**.The diagnostic algorithm currently in use at our centre for the assessment of HPV-status in OPSCC.

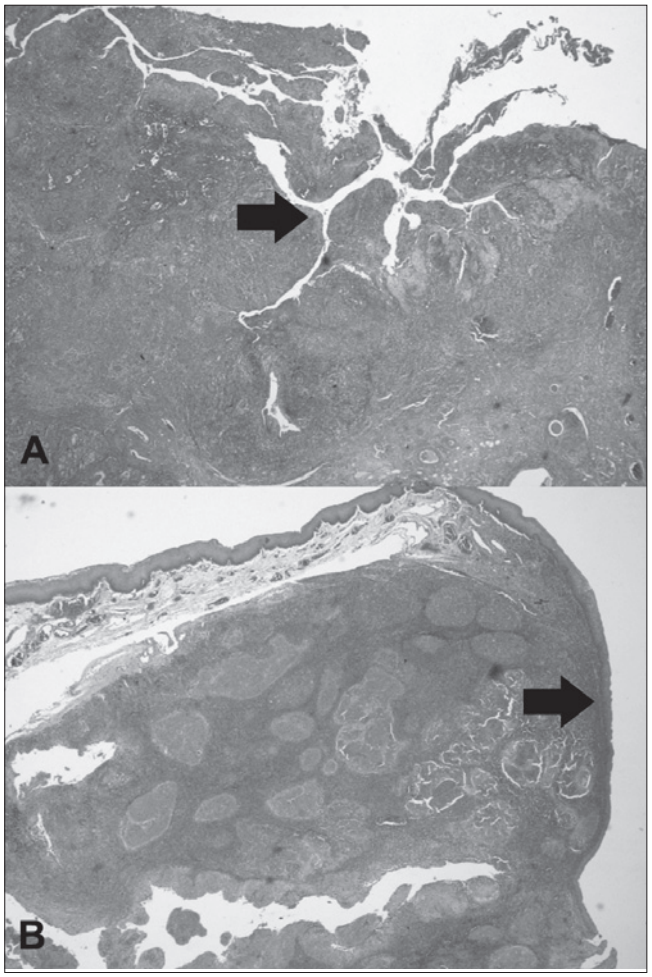


**Fig. 3**. Different morphological features of HPV-independent (a) and HPV-related OPSCC (b). OPSCC not associated with HPV generally arise from the surface tonsillar epithelium (arrow), have keratinising features and show both superficial and invasive growth. HPV-related OPSCC originate from the tonsillar crypts, do not produce keratin, grow deeply in the tonsil and are covered by intact squamous epithelium (arrow) (a,b, H&E stain).

5.9% of all oral cases [65]. The clinical relevance of these observations must be fully assessed before extending routine HPV characterisation to oral preneoplastic lesions.

## Early diagnosis and prevention of HPV-related oropharyngeal SCC

Early identification by Papanicolau smear of HPV-related preneoplastic lesions in the uterine cervix has dramatically changed the epidemiology of female genital tract tumours. Attempts at reproducing the PAP-smear approach in the oral cavity for early diagnosis of OPSCC, however, have not been successful [66]. The failure of this approach can be explained with the anatomical peculiarities of HPV-related tumours: because they most frequently arise from HPV infection and neoplastic transformation of the deep tonsillar crypts, as previously described, the sampling of superficial exfoliated cells, even when specifically targeting the tonsillar surface, will hardly provide transformed cells from the crypts, which are located under the surface [67].

Oropharyngeal cytology and HPV-DNA analysis, on the contrary, represent a valid diagnostic possibility for HPV-status characterisation in patients with clinically-evident oropharyngeal abnormalities. In a recent study, abnormal brush cytology was significantly associated with the risk of HNSCC, whereas HPV positivity in cytobrush samples was strongly associated with a diagnosis of OPSCC [68] and could be used as an alternative to invasive sampling. Furthermore, oral HPV persistence after successful tumour treatment has been shown to be associated with disease recurrence and poor prognosis [69].

More importantly, several studies have been undertaken to define the prevalence and natural history of HPV infection in the oral cavity of healthy subjects to find a possible connection with oropharyngeal tumour development. In the USA, Gillison et al. [70] have shown that the overall incidence of oral HPV infection is 7%, lower than in the genital tract, and is more common in men (10.1% *vs.* 3.6%). Considering HR genotypes, HPV16 has been found in 1% of subjects of both sexes, corresponding to an estimated 2.13 million infected individuals in the USA. Tobacco use appears to be strongly associated with HPV oral infection in healthy subjects in most studies, together with male sex [71]. However, one-time measurement of incident HPV infection does not provide evidence for risk of developing HPV-related cancer, as we know from cervical pathology. The HIM study confirmed that most newly acquired oral infections are cleared within 1 year, similarly to what occurs at genital sites. The median duration of infection was shown to be 6.9 months for any HPV, and 7.3 months for HPV16 [72]. Given that in the female genital tract HPV persistence in the cervical mucosa is the strongest risk factor for high-grade intraepithelial and invasive SCC [73 74], we expect a similar mechanism to take place in tonsillar cancerogenesis. Although persistent oral HR-HPV infection can be found in a proportion of high-risk (HIV-positive) subjects and appears to be associated with tonsillar HPV infection [66], there is as yet no prospective evidence (cytological or epidemiological) that correlates oral or tonsillar HPV infection with risk of OPSC in the general population. Only one study documented a temporal relationship between HPV infection and oropharyngeal cancer development, by showing that seropositivity for anti-HPV16 E6 antibodies in healthy subjects followed longitudinally predated cancer, and was associated with the risk of head and neck SCC and of HPV-associated oropharyngeal SCC [75]. Anti-E6 antibodies were also correlated with risk of tumour recurrence in patients treated for HPV-associated oropharyngeal SCC [76].

The low prevalence of persistent oral HPV infection suggests that the cost-benefit ratio of a large-scale oral

screening programme to identify subjects at risk of oropharyngeal SCC would be extremely low, especially in our population where HPV-associated tumours account for a relatively limited proportion of cases. An alternative strategy could be that of targeting subjects at increased risk of oral HPV infection for screening. In addition to HIV-positive immunosuppressed patients [66], it has been demonstrated that the risk of acquiring oral HPV infection is related to sexual behaviour, both hetero- and homosexual [77 78]. Preliminary evidence suggests that, besides individual sexual habits, the partners of patients with HPV-related squamous epithelial lesions of the genital area are at increased risk for oral HPV infection and oropharyngeal SCC [79-81], so they could represent a potential target for oral HPV infection and oropharyngeal cancer screening protocols.

## Future perspectives

Quadrivalent (HPV 6/11/16/18) and bivalent (HPV 16/18) anti-HPV vaccines have been available since 2006 and 2007, respectively. Vaccination policies vary worldwide concerning age of administration, population coverage and gender. Only a few countries have so far introduced gender-neutral vaccination for pre-adolescents, and its introduction was generally delayed by a few years with respect to female vaccinations [82]. Despite this variability, in countries where coverage has been high a dramatic reduction in cervical high-grade squamous intraepithelial lesions as well as warts [83 84] has been observed. It is relevant to note that a significant reduction in cervical but also oral HPV prevalence was demonstrated in the Swedish female population a few years after the introduction of the vaccination [85]. Although the interval between tonsillar HPV infection and SCC diagnosis is unknown, we expect that vaccination benefits in HN cancer epidemiology would be delayed by several years. Notably, a small retrospective study has suggested that previous resection of the palatine tonsils significantly reduces the risk of tonsillar carcinoma, even though it does not affect the risk of HPV-related cancers arising in other oropharyngeal subsites, and opens new interesting opportunities for primary prevention [86].

## Conclusions

Five-year survival rates are significantly different for patients with HPV-related OPSCC compared with HPV-negative patients (75-80% *vs.* 45-50%) [6 16 87 88]. Based on the higher survival rates registered among patients with HPV-positive OPSCC, the application of de-intensified protocols has been proposed in this patient group, regardless of the specific treatment strategy (surgery, radiation therapy, concurrent chemoradiotherapy or induction chemotherapy plus concurrent chemoradiation). In addition, the reduced risk of second malignancies in patients with HPV-related OPSCC [89] is also expected to modify the natural history of OPSCC patients, and further supports the need for treatments that do not persistently affect patients' quality of life. Future clinical research will provide further insights, but the combination of tumour HPV status, pack/year amount of tobacco exposure, and cancer stage should already be used routinely to classify patients as having low, intermediate or high risk as proposed by reputed authors [6].

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