# Current status and future perspectives of oral HPV testing in the diagnosis and monitoring of oropharyngeal cancer. A review

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HPV16 status in oropharyngeal cancer (OPC) is an important prognostic factor. Its determination, based on immunistochemical analysis of p16 oncoprotein requires an invasive biopsy. Thus, alternative methods are being sought. Determining oral HPV16 status appears to be a promising alternative. However, it is not used routinely. This prompted us to perform a systematic literature review enabling us to evaluate the diagnostic and predictive ability of this approach. Thirty-four relevant studies were finally selected. For determination of HPV status in OPC, the calculated average sensitivity and specificity for oral sampling was 74% and 91%, respectively, with p16 tumour tissue marker being the gold standard. The method appears to be valuable in monitoring treatment response as well as the biological activity of the tumour, enabling early detection of persistent or relapsing carcinoma sufficiently long before its clinical and/or radiological manifestation. It can also contribute to identification of the primary tumour in cases of metastases of unknown origin. Last but not least, the screening HPV oral testing would help to identify individuals with persistent HPV oral infection who are at increased risk of development of OPC.

**Key words:** head neck cancer, human papilloma virus, oropharyngeal cancer, oral HPV infection, oncologic marker

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## INTRODUCTION

Squamous cell carcinoma of the head and neck is the sixth most common human malignancy worldwide with oropharyngeal cancer (OPC) accounting for 3–5% of all malignant tumours. In general, the incidence of head and neck cancer has dropped in the past three decades, probably due to decreased tabacco and alcohol comsumption. On the other hand, significantly growing incidence of HPV-associated OPC (HPVOPC) has been evident over the last 30 years[1-3].

Data show that HPVOPC is a distinct entity characterized by better treatment sensitivity and prognosis compared to "traditional" OPC (ref.[1,4]). Consequently, the TNM classification has recently accepted HPV status as up to now the only non-clinical prognostic marker in OPC, whose positivity is expected to de-escalate treatment protocols[5-9].

Testing of HPV status is based on analysis of p16 oncoprotein in a biopsy specimen. However, this may fail in cases with clinically inapparent primary or recurrent tumours and is useless in monitoring tumour progression and therapy response. These flaws are not intrinsic to detection of HPV DNA/RNA and relevant antibodies in blood or gargle oral samples. This systematic review summarizes so far published papers on methods of determining oral HPV status and its relation to the presence and biological activity of HPV associated OPC. The results are expected to highlight recent advantages of these methods in the diagnostics and follow up of this tumour.

## AIMS AND METHODOLOGY

For identification of relevant articles, a PubMed assisted literature search was performed using the following key words: (HPV OR human papillomavirus) AND (oral OR oropharyngeal OR pharyngeal) AND (cancer OR carcinoma OR tumour OR neoplasm) AND (saliva OR rinses OR gargle OR swab). Using this search, 170 studies published in the past 30 years (1990–2020) were identified, and 3 more studies were found from citations. A total of 173 full-text articles were reviewed.

Of these 139 articles were excluded for the following reasons:

a/ 18 studies on a topic different from HPV
b/ 19 studies on other than HPV marker in HNC
c/ 67 studies on healthy or non-oncologic subjects
d/ 8 studies solely on techniques of HPV detection, without relevant clinical data
e/ 16 studies on theoretical aspects of HPV associated carcinogenesis or reviewed results of the previously published ones, included in our study
f/ 1 study lacked sufficient relevant clinical data
g/ 6 studies elaborated data included as a subset of previous larger studies
h/ 4 studies lacked correlation of the HPV oral status with that of tumour tissue

Finally, 34 studies were analysed. (Fig. 1)

**Data extraction**

The following information was extracted from each of the 34 articles: cancer type and location, number and types of controls (healthy or non-oncologic subjects), testing method for HPV in tumour tissue and oral swabs and/or gargle samples, p16 in situ hybridization (ISH), HPV DNA or mRNA polymerase chain reaction (PCR), detection of specific anti HPV antibodies, changes in DNA methylation, HPV type, and results of statistical comparison of oral vs. tumoural HPV status (sensitivity, specificity, accuracy, positive and negative predictive values).

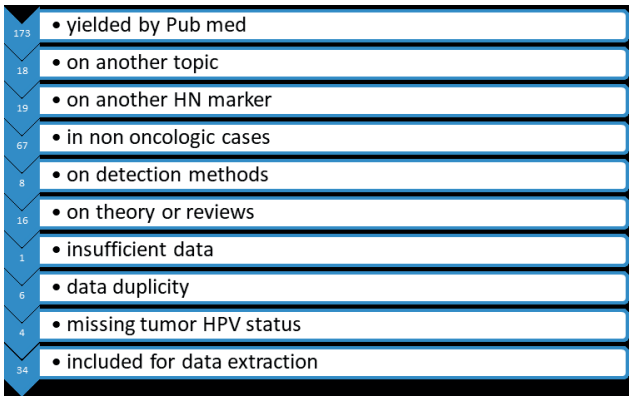


**Fig. 1.** The flowchart of the process of selection of the studies included in the review.

**Table 1.** Studies included in the review.

| Author | Method of HPV detection | HPV type | Tumour location | Study design | Conclusion | Ref. |
|---|---|---|---|---|---|---|
| Agrawal, 2009 | PCR | 16 | HN | diagnostic, monitoring | posttreatment monitoring and early detection of recurrence | 10 |
| Ahn, 2014 | PCR | 16 | OP | diagnostic, monitoring, prognostic | posttreatment monitoring and early detection of recurrence | 11 |
| Asvadi, 2012 | PCR | 16; 18 | HN | screening | significant difference in case and control groups | 12 |
| Auguste, 2017 | PCR | 16 | HN | screening | significant difference in case and control groups | 13 |
| Bhosale, 2016 | PCR | 16 | HN | screening | does not necessarily reflect transcriptionally active virus in tumours | 14 |
| Cohen, 2017 | PCR; Ab | 16 | OP | diagnostic, monitoring | prediction HPV tumour status | 15 |
| D'Souza, 2014 | PCR | 16 | OP | screening | oral HPV16 infection is commonly detected among patients with HPV-OPC at diagnosis, but not among their partners | 16 |
| Dang, 2015 | PCR | 16; 18 | OP | screening, diagnostic | significant difference in case and control groups | 17 |
| Fakhy, 2019 | PCR | 16; HR | OP + OC | monitoring | posttreatment monitoring and early detection of recurrence | 3 |
| Giuliano, 2019 | DNA methylation | 16 | OP | screening, diagnostic | DNA methylation as HPV-tumour biomarkers | 18 |
| Grewal, 2018 | PCR; Ab | 16; 18 | OP | diagnostic | prediction HPV tumour status | 19 |
| Hama, 2014 | PCR | 16 | OP | diagnostic | prediction HPV tumour status | 20 |
| Hama, 2017 | Ab | 16 | OP | monitoring, prognostic | posttreatment monitoring and early detection of recurrence | 21 |
| Hanna, 2019 | PCR | 16 | OP | monitoring, prognostic | posttreatment monitoring and early detection of recurrence | 22 |
| Hettman, 2018 | PCR | 16; 13 | HN | screening, diagnostic | predicts HPV tumour status | 23 |
| Chai, 2016 | PCR | 16 | HN | diagnostic | prediction HPV tumour status | 24 |
| Chuang, 2008 | PCR | 16 | HN | monitoring | posttreatment monitoring and early detection of recurrence | 25 |
| Imai, 2016 | PCR | 16 | OP | diagnostic | prediction HPV tumour status limited in very small tumours | 26 |
| Isaak, 2017 | PCR | 16 | OP + OC | screening, diagnostic | prediction HPV tumour status | 27 |
| Khyani, 2015 | PCR | 16; 18 | OC | screening, diagnostic | limited due to small sample size | 28 |

**Table 1.** *(Continued)*

| | | | | | | |
|---|---|---|---|---|---|---|
| Koslabova, 2013 | Ab | 16 | OP | monitoring, prognostic | prediction HPV tumour status, monitoring treatment responce and early detection of recurrence | 29 |
| Lim, 2016 | DNA methylation | 16 | HN | screening, diagnostic | DNA methylation as HPV-tumour biomarkers | 30 |
| Martin, 2019 | PCR | 16 | OP | diagnostic | prediction HPV tumour status, significantly lower among younger cases and earlier disease | 31 |
| Nordfors, 2014 | PCR | 16; 18; HR | OP | screening, diagnostic | prediction HPV tumour status | 32 |
| Qureishi, 2018 | PCR | 16 | OP | diagnostic | prediction HPV tumour status | 33 |
| Rettig, 2015 | PCR | 16 | OP | monitoring, prognostic, predictive | posttreatment monitoring and early detection of recurrence | 34 |
| Rosentahl, 2017 | PCR | HR | OP + OC | screening, diagnostic | prediction HPV tumour status; a potentially useful screening test | 35 |
| Smith, 2004 | PCR | 16; 18; HR | OP + OC | diagnostic | prediction HPV tumour status | 36 |
| Tang, 2019 | PCR | 16 | OP | diagnostic | prediction HPV tumour status; a potentially useful screening test | 37 |
| Tsao, 2016 | PCR | HR | OP | screening, diagnostic | Partners of OPC patients may have a higher prevalence | 38 |
| Wang, 2014 | PCR | 16 | HN | diagnostic, monitoring | prediction HPV tumour status | 39 |
| Wasserman, 2017 | PCR | HR | HN | diagnostic | prediction HPV tumour status | 40 |
| Yoshida, 2017 | PCR | 16 | OP | monitoring | posttreatment monitoring and early detection of recurrence | 41 |
| Zhao, 2005 | PCR | 16 | HN | screening, diagnostic | prediction HPV tumour status; limited for population sccreening | 42 |

## RESULTS

Of the 34 articles reviewed, only four[10,26,37,43] were published before 2010 (Table 1).

Eleven studies reported SCC of various head and neck locations[10,12-14,24-26,31,40,41,43], four were restricted to carcinoma of the oropharynx and oral cavity (OCC) (ref.[18,28,36,37]). Seventeen and one paper dealt exclusively with OPC (ref.[11,15-17,19,20-23,27,30,32-35,38,39,42]) and OCC (ref.[28]), respectively. The oral HPV status was analysed separately for OPC in all but the one study[12]. Generally, better correlation between the HPV status of oral gargles and tumour tissue was evident in OPC than in other carcinomas.

PCR analysis of oral samples was performed in 32 studies, 29 (ref.[3,10-13,14,16-18,20-24,26,27,29,30,32-42]) and 3 (ref.[14,25,28]) of which used HPV16 DNA and HPV16 E6/E7 mRNA, respectively. In 4 of them[15,20,22,30], HPV antibodies were also used. DNA methylation was applied in 2 studies[19,31].

Twenty-three studies tested solely the HPV16 (ref.[10,11,13-16,19,21-23,25-28,30-32,34,35,38,40,42,43]), eight others analysed also other high-risk HPV types[12,17,18,20,24,29,33,37] and three, the latter exclusively[36,39,41]. In all the above cited studies, there was better correlation between oral and tumour HPV status for HPV16 than for high-risk HPV types.

HPV status of tumour tissue samples was assessed solely by p16 protein expression in 11 studies[10,14,15,22,25,28,31,36,41-43], by both the p16 IHC and PCR HPV DNA in 4 studies[27,32,34,39], while only PCR was performed in 19 studies[11-13,16-21,23,24,26,29,30,33,35,37,38,40].

The type of control group varied with the study design. Non-oncologic and healthy subjects were enrolled in 16 (ref.[12-14,16-19,24,28,29,31-33,36,39,43]) and 12 studies[13,17-19,24,28,29,31,33,36,43], respectively, one study included both control groups[29]. Five studies tested patients with premalignant mucosal lesions[14,16,29,32,36].

The number of control subjects in particular studies ranged from 20 to 604 cases, that of patients with carcinoma from 14 to 218 cases (Fig. 2).

Twelve studies tested the HPV oral status as a predictor of biological activity of carcinomas[10,11,15,16,18,22,23,26,30,35,40,42]. Sixteen papers compared that status in carcinoma vs. control subjects[12-14,16-19,24,28,29,31-33,36,39,43] and 33 others compared the HPV oral status in HPV positive to negative tumours[3,10-21,23-42].

## DISCUSSION

### Epidemiology

HPV oral infection affects mostly young populations and is present in about 11% of newborns. The virus is usually eliminated within one year. However, it persists for more years in about 4% of infected subjects. In general, the average prevalence of oral HPV16 infection is

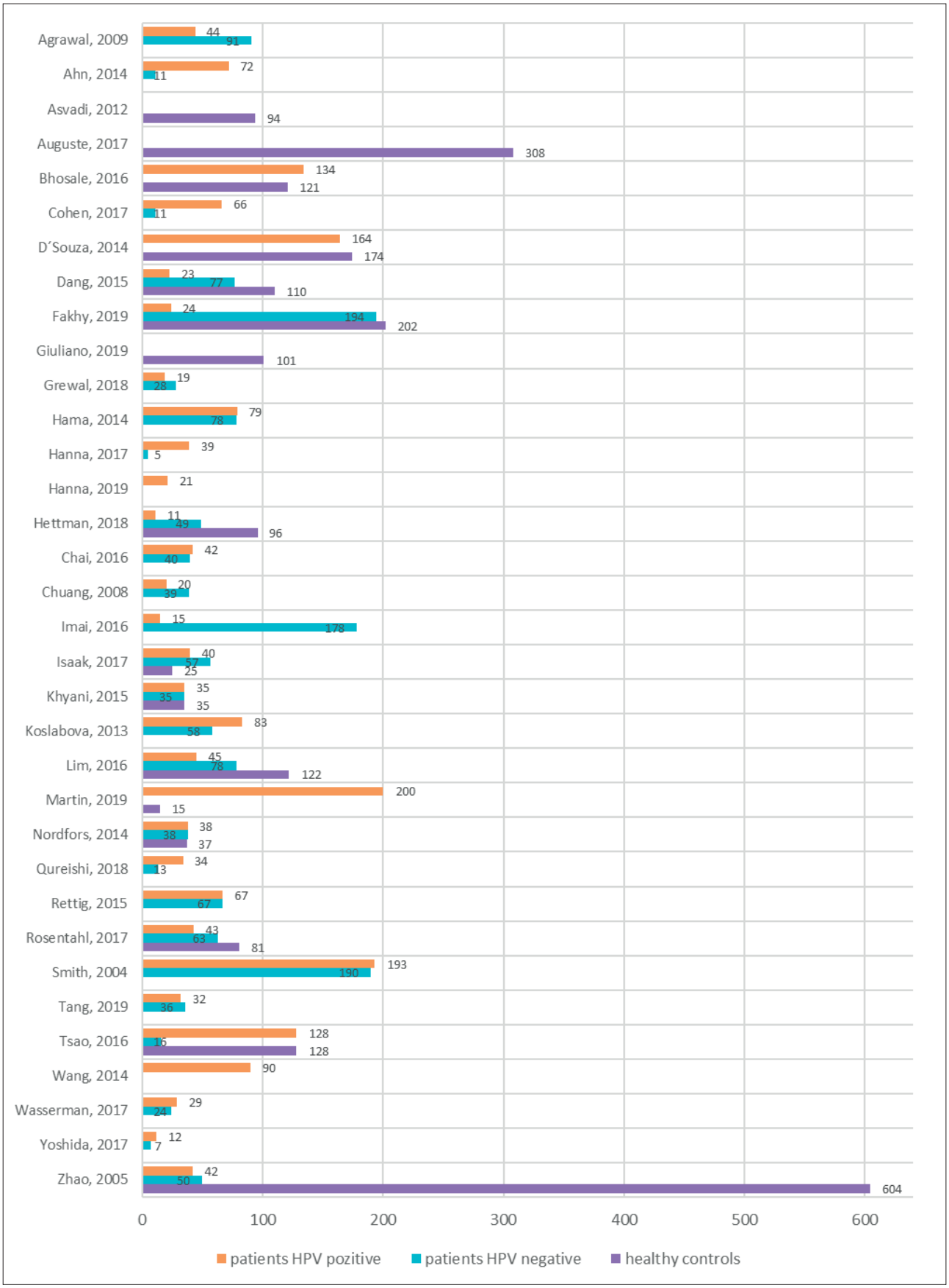


**Fig. 2.** Number of patients and controls in sets of included studies.

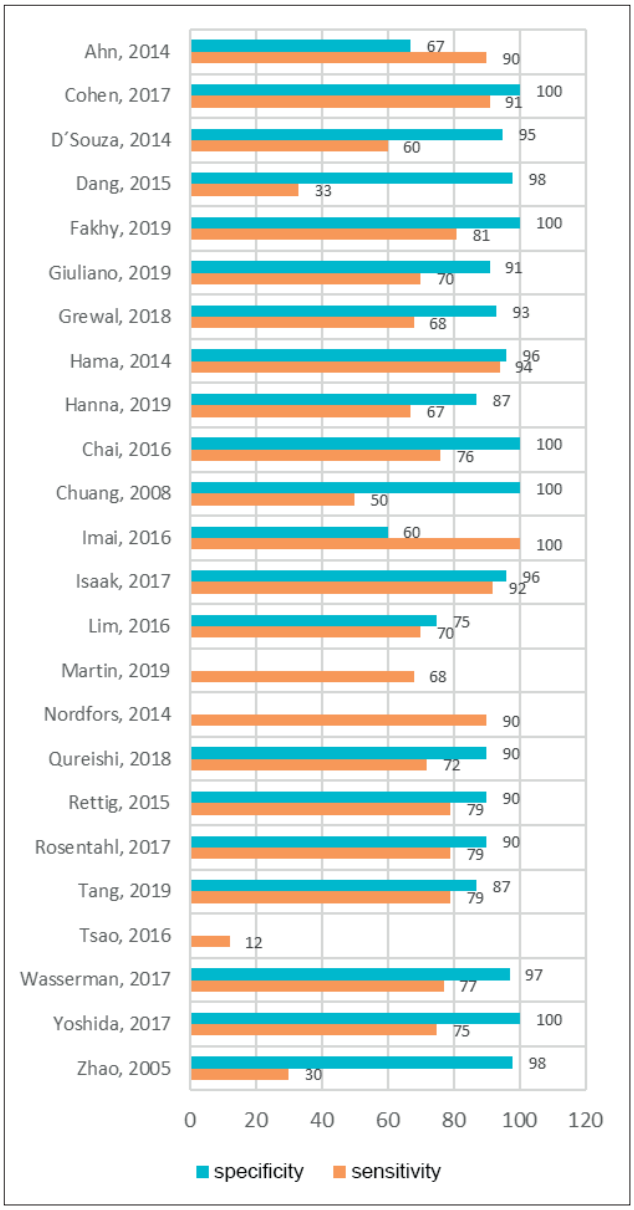


**Fig. 3.** Sensitivity and specificity of oral HPV16 test for detection of OPC of reviewed studies.

about 1%, seropositivity is found in 0.5–5% of healthy individuals, with two peak ages of 25–30 and 55–60 years, respectively[3,43-45].

## Oral HPV infection and its role in cancerogenesis

The presence of oral HPV DNA in salivary rinses or swabs reflects the inability of the organism to eliminate infected cells. The viral particle can spread throughout the body by vesicular transport, ultrafiltration, passive diffusion, active transport or directly released from disintegrated tumour cells. Therefore, positive HPV DNA finding in oral samples is considered to be a marker of either latent infection or the presence of an HPV associated carcinoma[43].

Persistent HPV infection may give rise to a carcinoma with a latency ranging from 10 to 30 years[45].Viral DNA integrates into the host DNA eliciting up-regulated expression of E6 or E7 oncoproteins. They interfere with the activity of tumour suppressors p53 and Rb by promoting their degradation with subsequent upregulation of the host protein p16. This pathway leads to anti-apoptosis, genetic instability and promotion of carcinogenesis[3].

## Diagnostic methods

### Liquid biopsy

HPV can be discovered at different stages of its biological activity by identifying viral mRNA, DNA or incorporation of the latter into the host DNA or downstream viral (E6, E7) or host (p16) proteins.

HPV is detectable in various types of biologic samples, e.g. tissues, cytologic aspirates or swab, liquids (blood, saliva, urine).

Analytic methods based on blood sampling, called liquid biopsies, have gained popularity in recent years. Compared to tissue testing, these methods are much less invasive, minimizing the risk of inconclusive fine-needle aspiration or tissue biopsy, primarily in a case of a small tumour arising in a post-radiation-altered area.

The proof of cancer presence with liquid biopsy is based on detection of selected tumour-specific cell-free DNA (cfDNA) mutations. This method is not only of diagnostic benefit, but has prognostic and predictive value too.

Some of these biomarkers can be measured in blood as well as other body fluids, including saliva[46,47]. The liquid biopsy method is rarely used in monitoring biological activity in EBV-associated nasopharyngeal carcinoma[48,49]. Similarily, tumour specific cfDNA as well as non-tumoural HPV DNA can be detected in blood and saliva[47,50].

### HPV detection

Viral DNA is usually detected with two methods, namely conventional polymerase chain reaction (PCR) and in situ hybridization (ISH). PCR-based methods are very sensitive and can be performed on frozen or formalin-fixed paraffin embedded tissue as well as cytological specimens taken with fine-needle aspiration cytology or oral swabs[51]. Detection of viral HPV E6/E7 mRNA by reverse transcriptase PCR (RT-PCR) or by ISH has become popular and is regarded as the "new gold standard" for detecting a transcriptionally active HPV infection[16,52].

Cost and time-effective immunohistochemical detection of p16 protein showing at least 90% sensitivity and specificity[53], has been accepted as a proof of HPV tumour etiology. Expression of the surrogate oncoprotein is scored as positive when the cut-off value is reached at least by 70% of tumour cells revealing diffuse and strong nuclear and cytoplasmic reactivity[51,52].

In the reviewed studies, tissue HPV analysis based on DNA or mRNA detection predominated, in a large number of studies[11-13,16-21,23,24,26,29,30,33,35,37,38,40] these methods were combined with p16 testing, in 12 others[10,14,15,22,25,27,28,31,36,41-43] but only the latter was applied.

Commercially available certified smear HPV tests used primarily in cervical cancer screening are based on PCR detection of amplified specific HPV DNA sequences E6/E7, mRNA or DNA (ref.[11,16,21,22,39]). These PCR meth-

ods providing not only qualitative[36] but also quantitative[42] analysis of viral load within the tumour are very sensitive, able to detect as little as 0.001 copy per HPV-16 DNA genome[3,11]. Quantitative analysis would be useful in monitoring the disease activity as HPV levels are related to tumour burden in both plasma and saliva[21,22,36,37].

Unfortunately, the techniques of sampling and analysis have not been fully standardized and reliable cut-off value of number of HPV-16 DNA copies has not been set so far[21,22,37].

A previously published review showed that as fluid markers of the presence of HPV infection in human body cells, PCR detected HPV DNA or mRNA are mostly used, with sensitivity and specificity approaching 100% (ref.[51]).

Other methods, such as detection of E6, E7 and L1 antibodies, miRNA, cell-free viral DNA or episomal DNA methylation are not routinely performed[18,21,29,30,47,51,52]. For oral rinses, the sensitivity and specificity of the antibody tests range from 91–96% and 96–98%, respectively[16,54]. Comparable efficacy (sensitivity and specificity range of 70–72% and 90–95%, respectively) was recorded for oral swabs[7,39,55,56].

**Correlation of HPV tests of oral and tissue samples**

The sensitivity and specificity of all oral HPV tests in detection of HPV positive status of pharyngeal SCCs was determined in 24 studies[11,15-21,23,25,26-28,31-36,38,39,41-43]. The average sensitivity and specificity of rinse and swab HPV tests were 74% (range 30–100%) and specificity of 91% (ranging 50 to 100%), respectively[11,15-21,23,25,26-28,31-36,38,39,41-43] (Fig. 3). These results are very similar to those published by Gibson[56] who in his early review found an average sensitivity of 72%, and specificity 94%.

Our review demonstrated that the accuracy of all oral HPV tests was higher for HPV-16 than for other high-risk HPV types[17,18,20,24,29,33,37,57]. Many studies revealed better results for OPC than for cancers of other locatio ns[10,13,14,24-26,31,40,41,43].

Wang[39] detected the presence of HPV-16 in saliva in 47% to 70% of patients diagnosed with HPV positive carcinomas. The HPV-16 positivity prevalence also depended on the tumour location. By combining saliva and blood tests, the sensitivity rises to 100% in oral, laryngeal and hypopharyngeal carcinomas and 91% of those originating in the oropharynx. The benefit of that combination has been confirmed also by Ahn in OPC, reporting sensitivity increase from 53% for oral sampling only to 76 % and 100 % specificity and positive predictive value, respectively[11]. Similarly, Hanna confirmed 100% sensitivity and specificity in detection of recurrent or persisting HPV+ OPCs (ref.[21]).

Thus, in OPC patients, detection of HPV-DNA in oral samples (saliva, gargles, swab) may provide a rapid non-invasive way to determine the HPV status of carcinomas, and, eventually replace the p16 tissue biopsy test. Moreover, the liquid oral HPV PCR testing represents a promising diagnostic tool in patients with cervical lymph nodes metatastasis from unknown primary. In one study, 100% sensitivity and 92% specificity of HPV-DNA detection was reported, leading to a targeted biopsy from relevant areas, i.e. primarily palatine tonsil or base of the tongue, where small carcinomas may go undetected[15].

**Screening of HPVOPC**

HPV-PCR testing of oral samples is expected to become very valuable in the screening, diagnostics and follow-up of HPV-associated ENT carcinomas. Therefore, this topic was addressed by many population studies focused on identification of the most effective PCR methods.

Unfortunately, the screening capability of the HPV tests of oral samples has the following limitations: about 8% of healthy subjects test as false positive[3,16,43], which may put them under unnecessary psychological stress[16,35,41]. Detection of a persistent HPV infection could select the population at risk of HPV positive carcinomas, though, but the sensitivity of relevant tests does not exceed 75%, leaving at least some HPV infected tumour candidates undetected[56].

The reported specificity of oral HPV tests appears to be sufficient for population screening, but only some of the positively tested patients develop OPC. This makes the tests inapplicable for one step definitive detection of OPC by a population based screening test. However, HPV tests can be useful in subjects with immunosuppresion, poor oral hygiene, risky sexual practices and smokers which have higher risk of OPC development[16,56,57]. In these cases, blood samples should also be taken, increasing diagnostic accuracy of the screening (vide supra) (ref.[11,16,22,40,54,57]). For the monitoring of potential tumour initiation, only the proof of a persistent HPV oral infection if performed by consecutive positive tests, is significant.

However, there is a lack of concensus on the approprate time interval both for repeating the tests in the at-risk population after the first negative test or for follow-up testing after the first positive detection of HPV DNA to confirm the persistence of the infection[16,58].

**Oral HPV tests in the surveillance of recurrent or persistent HPVOPC**

Locoregional posttreatment surveillance of recurrent or persisting head and and neck carcinomas may be difficult due to tissue and anatomical alterations developed secondary to preceding surgery and/or radio(chemo) therapy which reduces the accuracy of imaging methods and complicates the biopsy sampling, which in addition must be frequently performed repeatedly and under general anestesia[59,60]. For these purposes, fusion of positron emission tomography and computed tomography (PET/CT) reaching 94% sensitivity and 82% specificity is recommended. Diagnostic potential of this method is limited mainly due to incapability to detect small or superficially growing tumours. Moreover, the relatively low (75%) positive predictive value may result into an inadequate therapeutic scenario in false-positively tested patients[46].

Liquid biopsy based on detection of either viral or tumour cell-free circulating nucleic acids proved to be a prospective, diagnostic, predictive and prognostic method for EBV associated nasopharyngeal and other head and neck non-viral carcinomas[46]. Similarly, in HPV positive

tumours, analysis of blood and/or saliva HPV16 DNA emerges as a promising tool in monitoring the presence and biologic activity of both primary and recurrent tumours. This would be mainly helpful in discovering of microscopic tumours undetectable with conventional biopsy or clinical and imaging methods as well as in prediction of the disease relapse and in selected high-risk patients suitable for high-intensity treatment or closer monitoring.

In twelve studies monitoring saliva HPV16 DNA/RNA levels, the presence of a recurrent or persistent tumour was predicted with sensitivity and specificity exceeding 74% and 92%, respectively[10,11,15,16,18,22,23,26,30,35,40,42]. Similar results, with positive and negative predictive value of 100% and 89%, respectively, were reported by Chuang using exclusively the saliva HPV16 DNA test[25]. It is of considerable clinical significance, that the method is reliable in detection of small locoregional recurrences as early as 19 months before their clinical manifestations[11,25,34].

Retting[34] assessed the capability of the posttreatment saliva HPV16-DNA test in prediction of a tumour recurrence. Persistent oral HPV16-DNA was associated with a higher risk of recurrence (hazard ratio 29.7) and risk of death (hazard ratio 23.5). In all patients with persistent oral HPV16-DNA but only in 8% of those without persistent oral HPV16-DNA a recurrence has developed. The oral HPV16-DNA was detected 4–11 months before the recurrence was diagnosed.

Hanna[21] found 20-times higher salivary HPV16-DNA in patients with a solely local tumour than in those with distant matastases only. In the latter cases however, the plasma HPV16-DNA levels were significantly higher than the salivary ones[22]. In a previous study, the author analysing salivary HPV antibodies found out that their persisting levels indicated treatment failure with sensitivity 87%, specificity 67%. A combination of salivary and plasmatic HPV antibody levels increased sensitivity to 100% (ref.[22]).

Chuang[25] reported 50% sensitivity and specificity of positive saliva HPV16 DNA test in prediction of a local tumour recurrence. For detection of distant metastases, 60% specificity of positive blood HPV DNA test was recorded by Capone[42].

Ahn[11] in his multivariate analysis found out that positive posttreatment saliva HPV16-DNA status identified patients with significantly higher risk of a recurrence and disease associated death (hazard ratio 10.7 and 25.9, respectively), and the 3-year specific disease-free survival was predicted with 19% sensitivity and 97% specificity. When combined with plasma posttreatment HPV-16 DNA status, the two mentioned test parameters reached 70% and 91%, respectively. These values are even better than those reported for the PET/CT scans[61].

## CONCLUSIONS

In this systematic review of published literature, we summarized 34 studies focused on oral HPV16 sampling and its applicability in clinical aspects of HPV16 associated OPC. For determination of the HPV status of OPC, the calculated average sensitivity and specificity was 74% and 91%. This method appears to be valuable in the monitoring of treatment response as well as biological activity of the tumour, suggesting early detection of persistent or relapsing tumours sufficiently long before their clinical and/or radiological manifestation.

Based on the review performed, we would believe that oral sample testing would be most useful for clinical practice during the post-treatment period. First, it would help in the evaluation of treatment results, i.e. the identification of residual tumor. In addition, it could provide prognostic information, i.e. select patients who are at increased risk of recurrence and will benefit from closer follow-up. And above all, this inexpensive and minimally burdensome method for the patient can be repeated at regular intervals, optimally together with blood sample testing. If the dynamics of oral HPV DNA values were correlated with the activity of the disease, it could allow an early diagnosis of a recurrence. The tangible benefit of this marker is mostly important in diagnoses in which regular clinical examinations and imaging methods are of limited significance.

That sampling should be employed in a diagnostic procedure of identification of the primary in cases of metastases of unknown origin. Last but not least, the screening HPV oral testing could help to identify individuals with persistent HPV oral infection who are at increased risk of developing OPC.

We would suggest to include HPV testing of oral samples as a non-invasive, easy-to-perform and sufficiently accurate method that could be part of regular post-treatment follow-up of patients with HPVOPC.

This method could be very useful not only in clinical practice but also as a form of self-examination for regular preventive testing of individuals at risk of developing OPC.

### Search strategy and selection criteria

Our research strategy was aimed at evaluating studies on the role of the oral detection of HPV infection to determine if they can be used as marker of oropharyngeal squamous cell carcinoma. Scientific articles from 1990 to 2020 were searched using the PubMed database. All searches were up to date as of December 2020. The search terms used included: “HPV OR human papillomavirus” AND “oral OR oropharyngeal OR pharyngeal” AND “cancer OR carcinoma OR tumour OR neoplasm” AND “saliva OR rinses OR gargle OR swab”. Only English language papers were reviewed.

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**Author contributions:** ZH, IS: study design, manuscript writing; ZH: performed the database search and data extraction; RS: supervised the project; All authors discussed the results and contributed to the final manuscript.

**Conflict of interest statement:** None declared.

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