

## Advances in testing for human papillomavirus-mediated head and neck cancer

Mehanna, Hisham; Alemany, Laia; von Buchwald, Christian

DOI:

[10.1097/CCO.0000000000001029](https://doi.org/10.1097/CCO.0000000000001029)

License:

Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

*Document Version*

Publisher's PDF, also known as Version of record

*Citation for published version (Harvard):*

Mehanna, H, Alemany, L & von Buchwald, C 2024, 'Advances in testing for human papillomavirus-mediated head and neck cancer', *Current opinion in oncology*, vol. 36, no. 3, pp. 143-146.  
<https://doi.org/10.1097/CCO.0000000000001029>

[Link to publication on Research at Birmingham portal](#)

### General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

### Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.



# Advances in testing for human *papillomavirus*-mediated head and neck cancer

Hisham Mehanna<sup>a</sup>, Laia Alemany<sup>b,c</sup> and Christian von Buchwald<sup>d</sup>

## Purpose of review

New evidence has recently emerged regarding the utility and benefits of dual p16<sup>INKa</sup> (p16) and Human papillomavirus (HPV) status testing when determining the diagnosis and prognosis of patients with oropharyngeal cancer.

## Recent findings

HPV RNA polymerase chain reaction (PCR) is the most accurate diagnostic test. The other assays (HPV DNA PCR, HPV DNA/RNA in-situ hybridization (ISH) and p16) applied to formalin fixed tumour tissue have varying but high sensitivities and specificities. Dual p16 and HPV testing identifies discordant (p16+/HPV– or p16–/HPV+) results in 9.2% of cases, who have significantly poorer prognoses than p16+/HPV+, particularly in smokers. The proportion of discordant cases varies by region, and appears to be highest in regions with lowest attributable (p16+/HPV+) fractions. Dual testing improves prognostication for oropharyngeal cancer cases by identifying discordant cases and improving the prognostic power of the Tumour Node Metastasis (TNM) classification, especially in regions with high discordant rates.

## Summary

Dual testing is essential when considering patients for clinical trials of treatment de-escalation, and may be important when counselling patients on prognosis, especially in regions with high discordant rates and in smokers.

## Keywords

human papillomavirus, oropharyngeal cancer, p16, testing, TNM classification

## INTRODUCTION

Human papillomavirus (HPV) positive oropharyngeal cancer (OPC) is increasing rapidly, especially in high income countries [1–4]. The outcomes of patients with HPV-mediated OPC (HPV+OPC) are considerably better than those with HPV negative disease [5]. As a result, this has led to the concept of de-escalation, with many trials examining this issue having concluded or are in progress (Deescale, RTOG1016, HN006). Central to this concept is the issue of correctly and reliably diagnosing HPV+OPC.

## DIAGNOSIS

There has been a lot of research on the best technique and assay to diagnose HPV+OPC. This has mainly utilized fresh frozen or formalin fixed paraffin embedded (FFPE) tissue from the primary tumour, which is the widely accepted standard. However, there are now newer techniques for the diagnosis of HPV+OPC without the need for tissue –

mainly using cytology from fine needle aspiration (FNA), or oral gargle samples, or circulating tumour DNA in the blood.

<sup>a</sup>Institute of Head and Neck Studies and Education (InHANSE), University of Birmingham, Birmingham, UK, <sup>b</sup>Consortium for Biomedical Research in Epidemiology and Public Health—CIBERESP, Carlos III Institute of Health, Av. De Monforte de Lemos 5, 28029 Madrid, Spain, <sup>c</sup>Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO), Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain and <sup>d</sup>Department of Otolaryngology, Head and Neck Surgery and Audiology, Copenhagen University Hospital – Rigshospitalet, Copenhagen, Denmark

Correspondence to Hisham Mehanna, Director, Institute Head and Neck Studies and Education (InHANSE), University of Birmingham, B15 2TT Birmingham, UK. E-mail: h.mehanna@bham.ac.uk

**Curr Opin Oncol** 2024, 36:143–146

DOI:10.1097/CCO.0000000000001029

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

## KEY POINTS

- Human papillomavirus (HPV) RNA polymerase chain reaction testing is the most accurate diagnostic test, but the other HPV assays and p16 immunohistochemistry also show high sensitivities and specificities.
- Dual p16 and HPV testing identifies 9.2% discordant cases, which show poorer prognosis than p16+ HPV+ cases.
- The rate of discordant cases can vary significantly by geographic region.
- Dual testing provides more accurate results than p16 alone and therefore, has implications for TNM staging, prognostication and clinical trial eligibility.

There are several assays used to ascertain HPV causation from tissue samples. These include detection of HPV DNA by in situ hybridization (ISH) or by polymerase chain reaction (PCR), or E6/E7 HPV mRNA evaluation by ISH or reverse transcriptase-PCR (RT-PCR), or p16INKa (p16) evaluation by immunohistochemistry (IHC), or a combination of these methods. Each of the assays has pros and cons. E6/E7 HPV mRNA evaluation is considered the gold standard for determining HPV positivity, as it identifies transcriptionally active HPV-infection and causation. It is however the most technically difficult and most expensive assay to undertake. On the other hand, p16 is relatively inexpensive and easier to do compared to other techniques, and so is widely used as a surrogate for HPV causation of OPC.

A recent systematic review synthesized the data regarding the accuracy of the different techniques [4]. There were nine studies evaluating HPV detection in FFPE – which is the most used and easiest tissue type to obtain clinically. Sensitivities were high for all assay types ranging from 74% [95% confidence interval (CI) 64–82] to 99% (95% CI 89–100). Specificities on the other hand showed a wide range from 55% to 100%. The pooled sensitivity and specificities for each of the assays is shown

**Table 1.** Sensitivities and specificities of different HPV assays

Assay	Sensitivity (95% CI)	Specificity (95% CI)
RNA ISH	93.1 (87.4–96.4)	91.9 (78.8–97.2)
DNA ISH	81.1 (71.9–87.8)	94.9 (79.1–98.9)
DNA PCR	90.4 (81.4–95.3)	81.1 (71.9–87.8)
p16 IHC	83.3 (69.0–91.8)	93.5 (88.4–96.5)

Adapted from [6].

95% CI, 95% confidence intervals; IHC, immunohistochemistry; ISH, in situ hybridization; PCR, polymerase chain reaction.

in Table 1 below, with the highest sensitivity being RNA ISH and the lowest being DNA ISH. The highest specificity was for DNA ISH and the lowest for DNA PCR.

Regarding other less invasive sample types, five studies assessed the diagnostic accuracy of HPV detection in FNA with small patient numbers. The studies used a variety of assays, sometimes in combination. All studies reported sensitivities above 94% and four studies reported a specificity of 100%. Nine studies assessed HPV detection in the blood, mainly by circulating HPV DNA. The pooled overall sensitivity was 81.4% (95% CI 62.9–91.9), and the pooled overall specificity was 94.8% (95% CI 91.4–96.9).

When assessing the use of oral samples for the diagnosis of HPV+OPC, pooled sensitivity and specificity were 77.0% (95% CI 68.8–83.6) and 74.0% (95% CI), respectively. However, there was high variability detected between studies, ranging from 60.9% (95% CI 40.8–77.8) [47] to 86.1% (95% CI 80.4–90.3) for sensitivity, and between 50% (95% CI 27–73) [46] to 91% (95% CI 62–98) for specificity.

## PROGNOSIS OF DISCORDANT CASES

p16 is strongly prognostic for outcomes, and because of its relative ease of application and low cost, it has been incorporated into the most recent AJCC TNM version 8 classification for OPC. However, there appears to be a proportion of patients who have tumours that are p16 positive, but are negative on testing for HPV [5,7]. Some studies reported that the outcome of these patients is significantly worse than the outcomes of patients who are p16 positive and HPV positive [7–9]. This would have important implications for the use of p16 alone to determine selection of patients for de-escalation of treatment, as p16 positive, HPV negative patients who may be at high risk of recurrence could potentially undergo treatment de-escalation.

One of the main reasons for the continuation of this controversy was the fact that most previous cohorts had amassed relatively few HPV negative/p16 positive cases. We therefore undertook a large collaborative international study [8] to clearly define the prognosis of patients with OPC with discordant p16 and HPV test results. In a cohort of 7654 patients from nine countries, we identified discordant results in 9.2% of patients. 10.9% of p16 positive patients were HPV–. The discordance rate differed significantly by region ( $P < 0.001$ ), and increased as the attributable fraction (proportion of p16+/HPV+ cases) decreased ( $r = -0.744$ ,  $P = 0.0035$ ). It should be noted however that the regions included were only in Europe and North America.

Patients with discordant results showed overall and disease-free survival outcomes that were significantly worse than p16+/HPV+ cases, but were significantly better than p16-/HPV- cases. Five-year overall survival was 81.1% (95% CI 79.5–82.7) for p16+/HPV+, 40.4% (38.6–42.4) for p16-/HPV-, 53.2% (46.6–60.8) for p16-/HPV+, and 54.7% (49.2–60.9) for p16+/HPV-. The prognosis of discordant p16+/HPV- tumours was further stratified by smoking status: Compared to p16+/HPV+ tumours, p16+/HPV- smokers demonstrated significantly worse prognosis (adjusted hazards ratio for 5 year overall survival aHR 2.94; 95% CI 2.37, 3.64,  $P < 0.001$ ), and comparable to the p16-/HPV+ group (aHR 3.13; 95% CI 2.44, 4.02), and slightly better than p16-/HPV- group (aHR 4.06, 95% CI 3.56, 4.64). Disease free survival (DFS) outcomes showed similar patterns. Conversely, nonsmokers had outcomes (aHR 1.53; 95% CI 0.82, 2.87); that were similar to p16+/HPV+ disease.

This study therefore demonstrated that, along with routine p16 IHC testing, HPV testing for all patients is strongly recommended where HPV status determines eligibility for clinical trials and is also recommended where it affects patient counselling, and in future, where treatment de-escalation or intensification are being considered, especially for smokers and in areas with low HPV attributable fractions.

## EFFECT ON PROGNOSTICATION BY THE TNM CLASSIFICATION

Such a conclusion naturally led to an evaluation of whether combined p16/HPV testing resulted in significantly better prognostic power than p16 alone when applying the TNM 8 staging system to the same multicentre cohort [10<sup>11</sup>]. On multivariable analysis, in addition to p16 status, HPV status was a significant independent prognostic indicator of overall survival (OS) and DFS, regardless of stage of disease. For p16+ cases, HPV detection and stage significantly affected survival probability ( $P < 0.001$ ) with HPV+/stage I–II patients having the best survival probability and HPV-/stage III–IVb patients having a worse overall survival (hazard ratio (HR): 3.30 [95% CI 2.26–4.83]). For p16- cases, HPV-/stage III–IVb patients also had worse survival (HR: 2.40 [95% CI 1.94–2.97]) with a significantly lower survival probability than HPV+/stage III–IVb patients (HR: 1.61 [95% CI 1.17–2.21]). Survival probability was similar for both HPV+ and HPV- stage I–II patients. HPV status also conferred additional information beyond p16 status for discordant cases.

When evaluating the cohort as a whole, p16+/HPV+ stage I/II had the best survival, and p16-/

HPV- stage III/IVb had the worst survival. The other combinations had intermediate prognoses. The findings of this study strongly suggest that HPV status should be integrated into the TNM staging system, due to the improved prognostication it confers. This would be especially marked in regions with lower attributable fractions.

## CONCLUSION

p16 has become the standard test for diagnosis and prognostication for OPC due to its ease of clinical application, relatively low cost and strong prognostic power. However, recent studies show that there is a proportion of patients with discordant p16 and HPV results, and their prognosis, especially when they are smokers, is significantly worse than p16+/HPV+ cases. These cases would not therefore be suitable for de-intensification studies. Furthermore, this discordance affects the prognostic power of the TNM classification. As a result, especially in regions where there are lower attributable fractions, combined p16 and HPV testing would be recommended.

## Acknowledgements

*We would like to thank Kathrine Kronberg Jakobsen for her help in preparing the manuscript.*

*Professor Mehanna is a National Institute for Health Research (NIHR) Senior Investigator. The views expressed in this article are those of the author(s) and not necessarily those of the NIHR, or the Department of Health and Social Care*

## Financial support and sponsorship

NIHR Senior Investigator Award.

## Conflicts of interest

*H.M. reports personal fees from Warwickshire Head Neck Clinic Ltd, other from Warwickshire Head Neck Clinic Ltd, personal fees from Astra Zeneca, MSD, Sanofi Pasteur, Merck, Nanobiotix, Eisdsai Inc, Seagen, grants from GSK Biologicals, Sanofi Pasteur, GSK PLC, Astra-Zeneca, Cancer Research UK nonfinancial support from Sanofi Pasteur, MSD, Merck. CB reports no conflicts of interest. LA reports grants to institution from Merc & Co, Roche, GSK, Vitro, Hologic and Seagene. Consulting fees to institution from Merck & Co.*

## REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. *J Clin Oncol* 2013; 31:4550–4559.

2. Mehanna H, Beech T, Nicholson T, *et al.* Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer—systematic review and meta-analysis of trends by time and region. *Head Neck* 2013; 35:747–755.
3. Louie KS, Mehanna H, Sasieni P. Trends in head and neck cancers in England from 1995 to 2011 and projections up to 2025. *Oral Oncol* 2015; 51:341–348.
4. Carlander AF, Jakobsen KK, Bendtsen SK, *et al.* A contemporary systematic review on repartition of HPV-positivity in oropharyngeal cancer worldwide. *Viruses* 2021; 13:1326.
5. Ang KK, Harris J, Wheeler R, *et al.* Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* 2010; 363:24–35.
6. Jakobsen KK, Carlander ALF, Bendtsen SK, *et al.* Diagnostic accuracy of HPV detection in patients with oropharyngeal squamous cell carcinomas: A systematic review and meta-analysis. *Viruses MDPI* 2021; 13.
7. Quabius ES, Haag J, Kuhnel A, *et al.* Geographical and anatomical influences on human papillomavirus prevalence diversity in head and neck squamous cell carcinoma in Germany. *Int J Oncol* 2015; 46:414–422.
8. Mehanna H, Taberna M, von Buchwald C, *et al.* Prognostic implications of p16 and HPV discordance in oropharyngeal cancer (HNCIG-EPIC-OPC): a multi-centre, multinational, individual patient data analysis. *Lancet Oncol* 2023; 24:239–251.
9. Taberna M, Mena M, Tous S, *et al.* HPV-relatedness definitions for classifying HPV-related oropharyngeal cancer patient do impact on TNM classification and patients' survival. *PLoS One* 2018; 13:e0194107.
10. von Buchwald C, Jakobsen, KK, Carlander, A, *et al.* TNM 8 staging ■ system beyond p16: double HPV/p16 status better predicts outcome in oropharyngeal squamous cell carcinoma than p16 alone. submitted, 2024.

Identifies the benefit of dual testing on prognostication by the TNM8 classification.