REVIEW

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

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Purpose of review
New evidence has recently emerged regarding the utility and benefits of dual $p16^{INKa}$ (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 (Deescalate, 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.

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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 that 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 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.

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.

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▪▪]. 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.

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Conflicts of interest

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- ▪ of special interest
- ▪▪ of outstanding interest

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