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Perspective

P16 and HPV Discordance in Oropharyngeal Squamous Cell Carcinoma: What are the Clinical Implications?

Osborn HA* and Lin D

Department of Otolaryngology, Massachusetts Eye and Ear Infirmary, USA

***Corresponding author:** HA Osborn, Department of Otolaryngology, Head and Neck Surgery, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston MA, 02114, USA

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Perspective

One of the great advances in the field of head and neck oncology over the past several decades has been the identification of human papilloma virus (HPV)-related oropharyngeal squamous cell carcinoma (OPSCC) and its relatively favorable prognosis[1-3]. Clinical trials are underway investigating de-escalation of therapy in this patient population due to its improved treatment-sensitivity and overall survival [4]. This improved prognosis and shift in therapeutic approach makes accurate identification of HPV-positivity in oropharyngeal carcinoma critically important [5,6].

Methods of HPV detection include in-situ hybridization for high-risk HPV subtypes (HPV ISH), polymerase chain reaction for HPV DNA (DNA PCR) or viral E6 mRNA (RNA PCR), or the use of surrogate biomarker p16, assessed using immunohistochemistry (IHC) [6,7]. P16 is a cyclin-dependent kinase inhibitor which activates cyclin D1 CKD4 and 6 complex, preventing phosphorylation of retinoblastoma protein (pRb) and leading to cell cycle arrest [8-10]. HPV-infected cells express oncoprotein E7, which binds to pRB, inactivating it and resulting in over-expression of p16 [8,10,11]. Immunostaining for p16 is based on a high correlation between transcriptionally active HPV and p16 over expression in tumor cells, with sensitivity approaching 100% [1,12-14]. Based on this, p16 has been used as a surrogate marker in place of more laborious HPV detection methods in both research and clinical settings [1]. The sensitivities and specificities of HPV detection methods are summarized in table 1.

HPV is an ubiquitous infection in our society, and the presence of HPV alone does not signify that the virus is the driving force of carcinogensis. Boscolo-Rizzo and colleagues attempted to clarify the difference between HPV-positive and HPV-driven tumors [15]. According to these authors, the high sensitivity of PCR-based assays for HPV DNA presents a problem. HPV DNA may be detected, not only when HPV is the driver of carcinogensis, but also when a non-transforming infection is present in the tumor or in the surrounding tissue. A positive result on DNA PCR may represent a past infection that has not resulted in carcinogenesis, or a recent oral HPV exposure. In response to the spurious positive results stemming from the very high sensitivity of DNA PCR, some authors have advocated the use of ISH using HPV-specific probes as an alternative. The benefit of this modality is that it allows direct visualization of HPV in tissue [15]. Theoretically, this allows discrimination between oncologically relevant and non-relevant infections. However, multiple studies have suggested that ISH alone is inadequately sensitive [15,16]. Overall, neither of these tools adequately discriminate between a transient infection and a causative infection which is driving carcinogenesis.

Since HPV as a driver of carcinogenesis requires active transcription of E6 and E7, Boscolo-Rizzo, et al. note that E6 and E7 should be consistently detectable in all HPV-driven tumors, making PCR detection of HPV E6 mRNA the most reliable technique. This is supported by other authors who argue for the establishment of RNA PCR as the gold standard [17,18]. However, RNA analysis is complex and labor-intensive, and requires frozen as opposed to formalin-fixed specimens, creating logistical problems. In light of these concerns, a proposed alternative is p16 immunostaining, following by high-risk HPV DNA PCR in only positive specimens. This has been shown to have comparable sensitivity and specificity (96-97% and 94-98%) relative to RNA analysis [7,15,19,20]. Several institutions have developed testing protocols in which p16 IHC is followed by PCR [1,6,7]. However, this has not yet been established as the standard of care, and in an analysis of 14 trials examining treatment de-escalation and therapeutic vaccination in HPV-positive OPSCC, the authors found that a majority of these trials use only p16 IHC to identify HPV-positive lesions and only three trials reported the use of both p16 IHC and HPV DNA [4].

Multiple landmark studies have examined treatment outcomes in patients with p16 positive OPSCC compared to patients with p16 negative disease. As a group, it is clear that p16 positivity correlates with an improved prognosis. The TROG 02.02 trial found improved two-year overall survival and failure-free survival in p16 positive patients treated with concurrent chemoradiation. They also noted a lower T-stage, but a higher N-stage compared to p16-negative patients [21]. The Danish Head and Neck Cancer Group similarly obtained improved 5-year disease specific survival and overall survival in p16 positive patients [22]. These studies have helped to establish p16 over expression as an independent prognostic marker in patients with OPSCC.

However, other pathways may lead to p16 over expression, and a significant proportion of patients exhibit p16 positivity while testing negative for HPV using ISH or PCR. P16+/HPV- discordance rates have been reported ranging from 11-20% [1,16,23]. The clinical implications of such discordance have not been fully elucidated and there is a lack of clarity as to how this population, once detected, should be treated.

Three retrospective reviews have attempted to tackle this problem, with differing results. Lewis and colleagues examined 26 discordant

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Table 1: Methods of HPV detection [7,15,20].

Method	Sensitivity	Specificity
HPV ISH	83-88%	88-100%
DNA PCR	97-100%	87-89%
RNA PCR	100% (gold standard)	100% (gold standard)
P16 IHC	94-100%	79-82%
P16 + DNA PCR	96-97%	94-98%
P16 + HPV ISH	88%	90%

tumors which were p16 positive but HPV negative for high risk HPV subtypes using both ISH and DNR PCR. They found that these patients had improved survival relative to a p16-negative cohort, and maintained a prognosis that was not significantly different from a p16-positive and HPV positive group [24]. The significant survival difference was retained in multivariate analysis, accounting for differences in T-stage, N-stage, race and treatment regimens. Overall, they argued that p16 functions as an independent prognostic indicator, regardless of HPV detection, and that HPV-specific testing adds little to the prognostic and clinical information already provided by p16 status.

In contrast, Rietbergen, et al. retrospectively analyzed 723 patients with oropharyngeal carcinoma, of which 26 patients were p16-positive but HPV negative for 14 high risk subtypes by PCR. These patients showed significantly decreased 5-year overall survival (46.2% *vs.* 73.5%) and 5-year progression-free survival (45.8% *vs.* 70.0%) compared to an HPV-positive group. These authors argued that tumors which are p16 positive but HPV negative have a prognosis equivalent to truly HPV-negative patients, and therefore should be considered negative for clinical purposes [25].

Liu, et al. examined 185 patients with OPSCC, 15 of which exhibited p16 positivity while obtaining a negative result for HPV 16 DNA via PCR. Further testing revealed that 6 of these cases were positive for other high risk HPV subtypes (33, 35, 51, 58 and 59), leaving 9 p16+/HPV– discordant patients. This small group exhibited an intermediate prognosis relative to the p16+/HPV+ and p16-/HPV- cohorts. Overall survival in the discordant group was 62.41 months, compared to 105.43 months in the p16+/HPV+ cohort and 14.11 months in the p16-/HPV- group [26].

Although other reviews have examined discordant tumors, these have included multiple head and neck sites [27], or have excluded other high-risk HPV strains from their HPV analysis [28], limiting clinical applicability. Overall, at this time, there is limited clinical evidence providing guidance as to the management of p16+/HPV- discordant tumors in the oropharynnx. Rooper, et al. suggested that cases of discordance could be resolved by the use of E6/E7 mRNA in situ hybridization to detect HPV [29]. While this is the most reliable clinical test and the gold standard for HPV testing in head and neck cancer, it largely remains a research tool and is unavailable for clinical purposes at most institutions.

When a clinician is required to manage the p16+/HPV- discordant patient, without the benefit of mRNA ISH, several factors must be considered. Of primary importance is the relative hazards of over- and under-treatment. Conventional chemotherapy and radiation offer excellent response rates in the HPV-positive population. The downside is that this may represent over-treatment, resulting in unnecessary long-term morbidity, particularly important in the more youthful HPV-positive population. At this point in time, HPV testing is used largely for prognostication and for consideration of participation in clinical trials. Conflicting evidence has been presented regarding the prognosis of discordant patients, and affected patients should be aware of this uncertainty. Vigilant, long-term surveillance after treatment is essential. As noted above, clinical trials have used varying criteria for HPV positivity, including p16 alone, and a combination of p16 and HPV DNA. The results of these trials may offer further insights into the relative prognosis of p16+/HPV- discordant patients. If de-escalation is considered outside the setting of a clinical trial, our recommendation is to exercise caution, as the long-term effects of de-escalation are still being clarified in patients with HPV-positivity, and in patients in whom HPV status is uncertain, the relative risk may be significantly higher.

Emerging results from ongoing clinical trials will better elucidate the significance of p16/HPV discordance and the applicability of deintensification protocols to this patient population. Moreover, tools are being developed to improve clinical access to E6/E7 mRNA ISH, the gold standard test for the presence of clinically relevant HPV infection, which may eliminate the problem of identifying HPV-driven tumors. As HPV status currently informs both prognostication and treatment decisions, the hazards of misidentification of HPV status are clear, and careful attention should be paid to the prognostic and therapeutic uncertainty when treating this patient population.

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