The pathology of HPV-related head and neck cancer: Implications for the diagnostic pathologist

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A subset of head and neck squamous cell carcinomas are caused by the human papillomavirus (HPV). This HPV-related form of head and neck squamous cell carcinoma (HPV-HNSCC) has captured the attention of the oncology community for its rising incidence, its link to non-traditional risk factors, and its divergent clinical behavior. To diagnose this special form of head and neck squamous cell carcinoma is to provide important prognostic information and, in some instances, redirect clinical therapy. The diagnosis of HPV-HNSCC is aided by a strong appreciation for its characteristic microscopic findings and by an awareness of aberrant features that set apart a growing list of HPV-HNSCC morphologic variants. This review will delineate the microscopic appearance of HPV-HNSCC, spotlight ways in which the misinterpretation of these microscopic features can lead to diagnostic confusion, offer recommendations for appropriate terminology when diagnosing HPV-HNSCC, and provide examples of specific diagnostic scenarios where HPV testing can inform the diagnostic process.

HPV as a causative agent in head and neck squamous cell carcinoma

The detection of human papillomavirus (HPV) in head and neck squamous cell carcinoma (HNSCC) dates back to the mid 1980s, but its role in the initiation and maintenance of malignancy was not decisively established until 2000 when evidence began to mount confirming its nature as a causal agent. The biologic plausibility of HPV as a carcinogen of human epithelia is deeply rooted in an extensive experience with cervical cancers. Like cervical cancer, epidemiologic studies have observed a consistent association between HPV exposure risk and HPV-related HNSCC. Studies documenting HPV integration into the host genome, expression of viral mRNA transcripts, translation of viral oncoproteins, and disruption of key tumor suppressor pathways support the biologic activity of HPV and dismiss the notion of "passenger HPV" that is coincidental to tumor development. Indeed, the consistent presence of HPV across all stages of clinical progression underscores its obligatory role in both the initiation and maintenance of the malignant phenotype. Disruption of the p53 and RB pathways by the viral oncoproteins E6 and E7 precludes the need for various genetic alterations induced by chronic cigarette exposure, thus giving HPV-HNSCCs a molecular-genetic profile that is quite distinct from smoking-related cancers. The presence of HPV in squamous cell carcinomas of the oropharynx (HPV-OPSCC) also marks a clinically
distinct form of HNSCC characterized by improved clinical outcomes relative to its HPV-negative counterpart. In effect, HPV is an important causative agent in a subset of HNSCCs, and its recognition amounts to nothing less than the identification of a pathologically, genetically, and clinically distinct tumor entity.

The oropharynx as a preferential site of HPV-related tumorigenesis

Numerous studies have documented a strong predilection of HPV-HNSCC for the oropharynx, specifically the lingual and palatine tonsils. At this site, high-risk HPV, particularly HPV16, is detected in over 80% of squamous cell carcinomas. This preferential targeting likely reflects complex biological interactions between HPV and the specialized lymphoepithelium (i.e., reticulated epithelium) lining the tonsillar crypts including the exploitation of critical immunologic checkpoints. As one important example, PD-1:PD-L1 signaling is selectively activated in the reticulated epithelium (Figs. 1 and 2). Strong expression of PDL1 in the tonsils crypts results in a diminished cytotoxic T-cell response to viral antigens thus creating a potential "immune-privileged" site for viral infection, viral persistence, and viral induced tumorigenesis.

The microscopic and ultrastructural features of the reticulated epithelium lining the tonsillar crypts are distinct from the squamous epithelium that lines the surface of the tonsil and other head and neck sites. As the stratified squamous epithelium covering the surface of the tonsils extends into the recesses of the tonsillar crypts, a dense lymphoid infiltrate obscures the junction between the lymphoid and epithelial components and splinters the epithelial sheath into irregular nests and cords. These nests and cords no longer exhibit the polarization and surface maturation that characterize the stratified squamous epithelium lining the surface of the tonsils. At the cytologic level, the epithelial cells take on a basaloid appearance (i.e., high nuclear to cytoplasmic ratio, absence of cytoplasmic keratinization) with vesicular nuclei and loss of distinct cytoplasmic borders and intercellular bridges. These basaloid cells reside on a non-contiguous basement membrane that is innately disjointed in a way that facilitates the unrestrained migration of immune modulatory cells between the lymphoid stroma and lining epithelium in a manner that blurs the interface of the epithelial and lymphoid stroma (Fig. 2). The unique microscopic, ultrastructural, and immunologic makeup of the tonsillar crypts impacts on fundamental histopathology interpretive tasks such as tumor grading, tumor classification and the recognition of invasive growth (see below).

HPV-related HNSCC in non-oropharyngeal sites

HPV-related carcinomas of the oral cavity, larynx and hypopharynx

Although HPV-related HNSCCs are encountered in non-oropharyngeal sites, the incidence of true HPV-related non-oropharyngeal carcinomas is vastly overstated. There are various factors that contribute to inflated incidence rates (Table 2). Among these, differences in HPV detection methods have a dramatic impact on incidence rates. In particular, highly sensitive assays that are unable to distinguish biologically active from inactive infections tend to overestimate the incidence of HPV-related HNSCCs at non-oropharyngeal sites. In a systematic review of PCR-based HPV incidence studies, Isayeva et al. noted highly variable detection rates with upper limits of HPV positivity reaching 100% in laryngeal carcinomas, 74% in oral cavity carcinomas, and 70% in oral samples from individuals without cancer. The seemingly ubiquitous presence of HPV in these head and neck samples strongly argues for the implementation of detection strategies that couple the presence of virus with evidence of its biologic activity, and that correlate HPV status with meaningful clinical parameters such as disease specific survival.

Other factors may contribute to the erroneous diagnosis of an HPV-related cancer outside of the oropharynx. Occasionally, ectopically displaced tonsillar tissue is encountered in
non-oropharyngeal sites such as the floor of mouth and hypopharynx.\textsuperscript{20,21} Potentially, HPV could target these displaced tonsillar tissues and induce tumors in these non-oropharyngeal locations. In some instances, identification of tumor origin may be elusive when large and bulky tumors involving multiple contiguous anatomic sites. For example, some locally advanced HPV-positive squamous cell carcinomas of presumed supraglottic origin actually represent tumors originating from the base of the tongue. The ability to masquerade as a primary cancer in a non-oropharyngeal site is further enhanced by the propensity of HPV-related oropharyngeal carcinomas to arise from deep within the tonsillar crypts in the absence of surface dysplasia, propagate beneath the surface epithelium in the absence of stromal desmoplasia, and then emerge at some adjacent site along Waldheyer’s ring. In effect, inspection of the pharyngeal membranes often fails to recognize the full extent of tumor spread. Once these factors are properly accounted for, the true incidence of HPV-related HNSCC of the oral cavity, larynx and hypopharynx is only about 5%.\textsuperscript{22–25}

**HPV-related carcinomas of the nasopharynx**

Several studies have detected the presence of biologically active HPV in nasopharyngeal carcinomas.\textsuperscript{26–29} The true incidence of HPV-related nasopharyngeal carcinoma is highly variable and is strongly influenced by the ethnicity of the study population and by the tumor stage. Although Epstein–Barr virus (EBV) is recognized as a major etiologic agent for non-keratinizing carcinomas of the nasopharynx, EBV is not detected in all nasopharyngeal carcinomas. In particular, the constant association between EBV and non-keratinizing nasopharyngeal carcinomas noted in Asian patients is not maintained in Caucasian patients. Those studies of non-keratinizing nasopharyngeal carcinomas that have included a sizeable number of North American Caucasian patients have confirmed a substantial proportion of tumors that are HPV, not EBV, positive.\textsuperscript{26–29}

Tumor stage may also influence the likelihood of detecting HPV in a presumed nasopharyngeal carcinoma. Singhi et al.\textsuperscript{28} observed that HPV was detected only in high-stage nasopharyngeal carcinomas that showed some degree of involvement of the oropharynx. Accordingly, they proposed that most HPV-positive nasopharyngeal carcinomas actually arise in the oropharynx, and then involve the nasopharynx secondarily via tumor migration along Waldheyer’s ring.\textsuperscript{28} Indeed, clonal migration of neoplastic cells along this track of lymphoepithelial tissue has been proposed as a mechanism to account for tumor multifocality in patients with synchronous HPV-related squamous cell carcinomas of Waldheyer’s

### Table 2 – Factors that contribute to the detection of HPV in head and neck carcinomas from non-oropharyngeal sites.

<table>
<thead>
<tr>
<th>Factor</th>
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<tr>
<td>Presence of biologically inactive HPV (passenger virus, viral contaminant) detected by highly sensitive assays (e.g., PCR-based assays)</td>
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<tr>
<td>Large bulky tumor involving multiple contiguous anatomic subsites with no clear epicenter</td>
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<tr>
<td>Tumor migration along Waldheyer’s ring</td>
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<tr>
<td>Ectopically displaced tonsillar tissue</td>
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<tr>
<td>Presence of biologically active HPV (i.e., true HPV-positive non-oropharyngeal carcinomas)</td>
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ring including dual involvement of the oropharynx and nasopharynx.30,31

**HPV-related carcinomas of the sinonasal tract**

The sinonasal tract is not a common site of HNSCC, but up to 20% of carcinomas that arise from this anatomic subsite harbor transcriptionally active HPV.32 The likelihood of detecting HPV in sinonasal carcinomas is influenced by certain phenotypic features. Like the oropharynx, HPV positivity closely tracks with the non-keratinizing morphology. In non-keratinizing squamous cell carcinomas of the sinonasal tract, HPV detection rates have ranged from 34% to 50%.33,34 The likelihood of detecting HPV is also increased in variant forms of squamous cell carcinoma such as those that exhibit papillary (i.e., papillary squamous cell carcinoma) or glandular (i.e., adenosquamous cell carcinoma) features. The prognostic importance of detecting HPV is still unclear due to small study populations. One of the larger studies to date demonstrated a strong trend toward improved overall survival in the HPV-positive patients, but a trend that was not statistically significant.33,34

Bishop et al.35,36 have described an unusual form of HPV-related carcinoma that appears to be restricted to the sinonasal tract. These tumors are set apart by their distinctive adenoid cystic-like appearance and thus have been termed “HPV-related adenoid cystic-like carcinomas of the sinonasal tract” (Fig. 3). Unlike adenoid cystic carcinomas of other head and neck sites, these tumors harbor transcriptionally active HPV, are often associated with surface squamous dysplasia, and are not associated with the MYB gene rearrangement that characterizes most adenoid cystic carcinomas of the head and neck.35 In the sentinel report of 8 patients, there was no documented regional or distant metastasis; however, the number of study cases was small and clinical follow-up was limited.35

Although some carcinomas of the sinonasal tract harbor biologically active HPV, clear cut differences between HPV-positive tumors of the sinonasal tract and oropharynx should discourage any conjecture that they represent the same disease entity. It is not known how HPV is transmitted to the sinonasal tract. Unlike HPV-OPSCC, sexual transmission has not been established for those HPV-positive tumors arising in the sinonasal tract. The rate of OPSCC has been increasing, whereas the rate of sinonasal SCC has been slowly decreasing. Finally, the clinical implication of detecting HPV in sinonasal carcinomas, in contrast to OPSCCs, is unclear. Given the relatively low incidence of sinonasal carcinomas, multi-institutional studies will be required to address many unanswered questions relating to the epidemiology, pathology, and clinical significance of HPV-positive sinonasal carcinomas.

**The morphology of HPV-related oropharyngeal carcinoma**

The histologic features of the reticulated epithelium are retained, to varying degrees, in HPV-OPSCC. HPV-OPSSCs consistently arise from tonsillar crypts. Involvement of the tonsillar surface, when it occurs, is generally a secondary phenomenon reflecting colonization of the surface epithelium as the carcinomas spill over from the tonsillar crypts (Fig. 4). This transition between HPV-HNSCCs and the adjacent surface epithelium tends to be abrupt without transitional zones of epithelial precursor lesions. Indeed, the histologic progression through the sequential stages of dysplasia culminating in carcinoma in situ and invasive growth that characterize non-HPV-HNSCCs is not generally evident for HPV-OPSCCs. The inability to histologically characterize the early stages of HPV-induced tumorigenesis continues to deter efforts to assess cancer risk and diagnose early cancers.

As these carcinomas infiltrate, they tend to invade as sheets, lobules, or ribbons of cells (Fig. 5). Central necrosis within expanding tumor lobules, sometimes giving rise to cystic degeneration, is a frequent finding. Invasive growth often does not elicit a strong desmoplastic stromal reaction. Instead, the tumor nests are often surrounded by a zone of...
lymphoid cells that penetrate the tumor nests as tumor-infiltrating lymphocytes. The tumor cells often display a high nuclear to cytoplasmic ratio, syncytial cytoplasm without intercellular bridges, and lack significant cytoplasmic keratinization. These cellular features can impart a distinct basaloïd appearance. When these features are present and well developed in an oropharyngeal carcinoma, the likelihood that the tumor is HPV related is very high. Although the finding of keratinization certainly does not exclude the possibility of an HPV-OPSCC, its presence in association with surface dysplasia and a prominent desmoplastic stroma strongly points away from an HPV etiology.

In lymph node metastases, the presence of cystic degeneration is a common finding. Its presence should warrant strong consideration of an HPV-related metastasis from the oropharynx. In cytologic preparations from fine-needle aspirates of lymph node metastases, the tumor cells form cohesive sheets and clusters and exhibit hyperchromatic, pleomorphic, and overlapping nuclei (Fig. 6). Their high nuclear to cytoplasmic ratio is in contrast to conventional keratinizing HNSCC where the keratinized cells exhibit abundant orangeophilic cytoplasm.

**Histologic grading of HPV-OPSCC**

Tumor grade is a semi-quantitative measurement of differentiation expressed as the degree to which a tumor resembles the normal tissue from which it arises. As a rule of thumb, the more poorly differentiated a tumor, the more aggressive its behavior. Numerous studies have uniformly reported a strong and direct correlation between HPV positivity and a high histologic grade. Accordingly, HPV-OPSCCs are widely perceived as poorly or undifferentiated carcinomas. This perception is largely based on the immature appearance of a tumor cell that widely diverges from the

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**Fig. 4** – HPV-related squamous cell carcinoma of the oropharynx (OPSCC) consistently arises from the tonsillar crypts and not the surface epithelium (A). A p16 immunohistochemical stain highlights the distribution of the carcinoma in the tonsil (B).

**Fig. 5** – HPV-related OPSCC typically invade the lymphoid stroma as expanding lobules that are permeated by tumor-infiltrating lymphocytes. The absence of a desmoplastic stromal reaction is not unusual.

**Fig. 6** – Fine-needle aspiration of HPV-related squamous cell carcinoma yields cellular material in cohesive sheets. The tumor cells lack overt keratinization, and exhibit a high nuclear to cytoplasmic ratio with nuclear hyperchromasia and nuclear overlapping.
stratified squamous epithelium that lines the surface of the tonsil. Using the surface epithelium as a point of reference for phenotypic divergence, however, may not be appropriate, given the common origin of HPV-OPSCCs from the tonsillar crypts. HPV-OPSCCs often retain the appearance of the reticulated epithelium from which they arise (i.e., permeating lymphocytes and basaloid cells), and thus might best be regarded as highly differentiated tumors based on this more suitable comparison (Fig. 7). Recognition of HPV-OPSCC as well differentiated, not poorly or undifferentiated, is appropriate as it more accurately reflects histogenic derivation, more fittingly associates tumor grade with expected clinical behavior, and seems to explain the perplexing epidemiologic trends toward improving patient survival in the face of worsening tumor grade for patients with OPSCC.39

Few studies have tried to identify histopathologic parameters that might help identify the subgroup of HPV-OPSCC associated with unfavorable clinical outcomes. Lewis et al.40 made a preliminary observation that the presence of anaplasia and tumor cell multinucleation are predictive of poorer clinical outcomes in patients with non-keratinizing p16-positive oropharyngeal carcinomas, but this initial observation has yet to be confirmed by others.

Diagnosing invasion in HPV-OPSCC

For many of the HPV-OPSCCs, the difficulty in distinguishing carcinoma in situ from invasive carcinoma is heightened by (1) origin from the tonsillar crypts beneath the surface epithelium; (2) the oft absent tell-tale sign of invasion—stromal desmoplasia; (3) the well recognized propensity of small, sometimes occult, tonsillar carcinomas to metastasize to regional nodes in the absence of clear cut stromal invasion; (4) the blurred junction of the reticulated epithelium and the underlying lymphoid stroma; and (5) the porous nature of this epithelial/lymphoid junction where the basal cell layer is incomplete and its supporting basement membrane is disrupted and non-contiguous (Fig. 2).18,41 In effect, the time honored microscopic approach to the recognition of tumor invasion may not be valid for those HPV-OPSCCs arising from the tonsillar crypts. Until the histologic progression of HPV-related neoplasia of the tonsils is better characterized and the critical transition marking infiltrative growth is more clearly demarcated, an aggressive approach that regards all HPV-related neoplasia of the tonsils as potentially malignant, even in the absence of those histologic features that have been traditionally used to diagnosis invasion, may be warranted.

Diagnostic terminology in the reporting of HPV-OPSCC

The same morphologic features that characterize HPV-OPSCCs also can cause considerable confusion when it comes to reporting these tumors. Given the distinctiveness of HPV-OPSCC as a biological and clinical subtype of HNSCC, the reporting of these cancers must make careful use of diagnostic terminology that asserts their relationship with HPV, and yet does not confound the treating clinician. As noted, the current and widespread practice of assigning these tumors with an advanced histologic grade (e.g., poorly differentiated or undifferentiated) is misleading for the way it inappropriately infers an aggressive clinical course. Similarly, the common use of the term “basaloid” as a diagnostic descriptor, though morphologically correct, invites an erroneous connection with the basaloid variant of squamous cell carcinoma—the subtype of HNSCC notorious for aggressive clinical behavior.41,45 When reporting HPV-OPSCCs, it is our current practice to suspend the use of inapt descriptors such as “basaloid,” do away with the conventional grading scheme for HNSCC, and routinely report on the HPV status of all HNSCCs arising in the oropharynx.

For OPSCCs that are found to be HPV positive, we prefer the term “HPV-related squamous cell carcinoma” for its simplicity, clarity, and directness. At the same time, blunt use of the term “HPV-related” can have a powerful psychological impact on patients, patient partners, and family members, given considerable uncertainty regarding transmission, latency, and communicability of oral HPV. Use of the term should only be used in those HNSCCs where the presence of biologically active HPV has been confirmed by reliable HPV detection assays, and only for those HPV-related carcinomas arising in the oropharynx. Clearly, there is a growing need for a consensus panel to put forward a classification scheme that, like the nasopharynx, is customized for carcinomas of the oropharynx.

Morphologic variants of HPV-related OPSCC

On occasion, HPV-OPSCC can deviate from its usual morphologic profile. Recognized morphologic variants include papillary squamous cell carcinoma,43,44 adenosquamous carcinoma,45 basaloid squamous cell carcinoma,42 lymphoepithelial-like carcinoma,46 sarcomatoid carcinoma,47
and small cell carcinoma.\textsuperscript{48,49} With the notable exception of HPV-related small cell carcinoma, morphologic variance does not seem to influence clinical behavior. An awareness of these variant forms is important so that HPV-OPSCC is not overlooked and mistaken as some more aggressive type of carcinoma. Accordingly, a few of these variants warrant special consideration.

**HPV-HNSCC with papillary features**

Papillary squamous cell carcinoma (PSCC) is an uncommon variant of HNSCC that is characterized by exophytic papillary growth. The papillary fronds may be lined by keratinized squamous cells or non-keratinized basaloid cells. The presence of biologically active HPV is most commonly noted in those PSCCs of the non-keratinized variety.\textsuperscript{43,44} Most HPV-positive PSCCs arise in the oropharynx, but they also occur in the sinonasal tract and, less commonly, the larynx.\textsuperscript{43,44} Patients with PSCCs are generally believed to have a better prognosis than patients with conventional SCCs.\textsuperscript{50} In large part this improved prognosis reflects its limited invasiveness. It is not clear whether the presence of HPV adds any survival benefit.

**HPC-HNSCC with basaloid features**

The lobular arrangement of compact tumor cells with a high nuclear to cytoplasmic ratio in the absence of keratinization conveys a distinctly “basaloid” appearance to HPV-HNSCCs (Fig. 8). When the basaloid morphology is highly developed, HPV-related SCC may be histologically indistinguishable from the basaloid squamous variant of SCC—a variant of HNSCC that is set apart as a distinct subtype based on its striking basaloid morphology and its aggressive clinical behavior.\textsuperscript{51} At the microscopic level, the distinction between HPV-HNSCC and the basaloid variant of HNSCC can be difficult as both tumors are characterized by the lobular growth of basaloid cells. A subset of basaloid squamous cell carcinomas harbors biologically active HPV. These HPV-positive tumors usually arise in the oropharynx, while the HPV-negative tumors are more likely to be encountered in the supraglottic larynx and hypopharynx. Morphologic similarities aside, HPV-OPSCCs do not share the same aggressive clinical behavior that characterizes the basaloid variant of squamous cell carcinoma.\textsuperscript{42,52,53} In their evaluation of basaloid HNSCCs, Begum et al.\textsuperscript{42} found that the presence of HPV was significantly associated with improved overall survival even though patients with HPV-HNSCCs were more likely to present with lymph nodes metastases. In effect, the detection of HPV essentially downgrades what would otherwise be regarded as a high-grade HNSCC.

**HPC-OPSCC with lymphoepithelial features**

Some HPV-related OPSCCs demonstrate lymphoepithelial features including syncytial cytoplasm, vesicular nuclei, prominent nucleoli, and a dense lymphoplasmacytic infiltrate (Fig. 9). The cells are dispersed in a lymphoplasmacytic background as cords, clusters, or single cells.\textsuperscript{46,54} When these lymphoepithelial features are highly developed, an HPV-HNSCC may be mistaken for an Epstein–Barr virus (EBV)-induced undifferentiated carcinoma of the nasopharynx. Based on this morphologic overlap, one cannot assume an EBV-driven process by phenotype alone. Despite its “undifferentiated” histologic appearance, the clinical outcome associated with the lymphoepithelial variant seems to be favorable and comparable to that associated with conventional HPV-related OPSCC.\textsuperscript{46,54}

**HPV-HNSCC with small cell features**

Recent reports have drawn attention to a variant form of HPV-HNSCC characterized by a small cell phenotype that is indistinguishable from small cell carcinoma of the lung and other sites.\textsuperscript{48,49} We have also detected HPV in high-grade neuroendocrine carcinomas of the oropharynx that are best classified as large cell neuroendocrine carcinomas (unpublished observations). The small cell variant is comprised of small anaplastic cells with hyperchromatic nuclei, scant
cytoplasm, a high mitotic rate and tumor necrosis. In 4 of the 9 tumors reported by Bishop and Westra, the small cell component arose in close association with a more conventional HPV-HNSCC component. In these cases, HPV was detected in both the small cell and conventional components. For patients with HPV-related cancer of the oropharynx, recognition of the small cell variant and its distinction from HPV-related squamous cell carcinoma is important but not necessarily straightforward. Both tumor types share certain morphologic features including small hyperchromatic cells with scant cytoplasm and tumor necrosis. In these instances, immunohistochemistry may play a role in confirming the presence of a small cell component. By immunohistochemistry, the small cell component consistently demonstrates neuroendocrine differentiation (e.g., positive for synaptophysin and chromogranin) and loss of squamous differentiation (e.g., negative for p63 and CK5/6).

Importantly, HPV does not convey a favorable prognosis when its presence is detected in this small cell variant. HPV-related small cell carcinoma of the oropharynx appears to share the same aggressive clinical features of its counterpart in the uterine cervix and lung where the small cell phenotype is associated with early distant spread and poor overall survival. Consequently, the small cell phenotype should be regarded as an undifferentiated form of HPV-related oropharyngeal carcinoma where tumor morphology supersedes HPV positivity as a prognostic indicator. In turn, the presence of a small cell component should disqualify any patient with an HPV-OPSCC from consideration as a candidate for less intensive multimodality therapy (i.e., de-escalation therapy) solely on the grounds of HPV positivity.

**HPV-HNSCC with spindle cell features (HPV-related sarcomatoid carcinoma)**

Sarcomatoid carcinoma (SA) of the upper respiratory tract is a distinct variant of squamous cell carcinoma trademarked by a prominent or even exclusive spindle cell component. Although the spindle cells may take on a highly mesenchymal appearance, they are in fact derived from the surface squamous epithelium. Recently, HPV has been detected in a subset of sarcomatoid carcinomas arising in the oropharynx. Importantly, HPV is present in both the conventional and spindle cell components (Fig. 10). The strict epithelial

![Fig. 10 – HPV-related sarcomatoid carcinoma. This example of sarcomatoid carcinoma (A) exhibited pancytokeratin staining only in the epithelial component (B), but both the conventional and spindle cell components were p16 positive by immunohistochemistry (C) and HPV16 positive by in situ hybridization (D).](image-url)
tropism of HPV infection confirms the epithelial nature of the spindle cell component and serves as an important diagnostic tool in those difficult cases where, at the one extreme, the spindle cell proliferation may closely resemble a reactive fibroblastic proliferation, and at the other extreme, where it may resemble a true sarcoma.

**Methods of HPV detection**

Given the clinical implications of HPV status, even to the point of dictating the type and intensity of cancer therapy, the need for routine and accurate HPV testing of oropharyngeal carcinomas is compelling and urgent. Indeed, the College of American Pathologists has recommended routine HPV testing as part of the standard pathologic evaluation of resected oropharyngeal squamous cell carcinomas (http://www.cap.org/apps/docs/committees/cancer/cancer_protocols/2013/Pharynx_13protocol_3300.pdf), and Cancer Care Ontario has published evidence based guidelines for routine testing of HNSCCs (https://www.cancercare.on.ca/common/pages/User File.aspx?id=279836). Despite the expectation for routine HPV testing, there is currently no standard approach. Instead, methods of HPV testing across laboratories vary considerably, reflecting the biases and tendencies of individual investigators and the cost to benefit ratio of each technique.

Immunostaining for p16 protein has recently been regarded as a practical alternative or complimentary procedure for HPV testing of oropharyngeal cancers based on a high correlation between the HPV detection and p16 overexpression in recent studies. The simplicity, low cost, and high sensitivity of p16 immunohistochemistry have prompted consideration of replacing more intensive PCR-based methods as a standalone HPV test. To be truly useful as a surrogate marker of HPV infection, the interpretation of p16 immunohistochemistry must be informed by various histological, anatomical, and clinical considerations. First, p16 IHC may substitute for HPV testing when strong staining is present in the nucleus and cytoplasm of the tumor cells throughout all or most (>70%) of the tumor. Focal or weak staining should be supported by other forms of HPV testing. Second, while the sensitivity and specificity of p16 staining as a marker of HPV infection is sufficiently high to serve as a reliable test for squamous cell carcinomas of oropharyngeal origin, these values are either unknown or acceptably low for HNSCCs arising in non-oropharyngeal sites. Third, interpretation of p16 staining must be informed by the morphologic features of the tumor as outlined above. P16 IHC staining may substitute for HPV detection in those oropharyngeal carcinomas that demonstrate the typical morphology of HPV-related HNSCC. Additional HPV testing should be performed in p16-negative oropharyngeal carcinomas that exhibit classic HPV-related histomorphology, and in p16-positive oropharyngeal carcinomas that do not exhibit classic HPV morphology. Fourth, p16 IHC is currently used primarily as a prognostic indicator for patients with oropharyngeal carcinoma, and any expanded clinical role for HPV detection (e.g., selection for HPV therapeutic vaccine or therapeutic de-escalation) may necessitate more stringent detection methods.

**Diagnostic applications of HPV testing**

Although HPV testing is generally performed for prognostic purposes, there are specific diagnostic scenarios where knowledge of HPV status can directly inform the diagnostic process. As a result, HPV testing is finding increasing use by the pathologist as a very helpful diagnostic adjunct (Table 3).

**Determination of site of tumor origin**

In HPV-related OPSCCs, the presence of HPV persists across all stages of clinical progression such that it is just as readily detected in metastatic implants as in the corresponding primary cancers. Consequently, a lymph node metastasis is quite suitable as a substrate for HPV testing, obviating the need for additional tissue acquisition in those patients with small or even occult primary cancers. For those patients who present with neck metastases in the absence of an obvious primary tumor, HPV testing of a lymph node metastasis is an effective strategy for localizing the site of origin. In these patients, the detection of HPV in a lymph node metastasis is a reliable predictor of oropharyngeal origin. Similarly, for the squamous cell carcinoma in the lung of a patient with a prior HPV-related oropharyngeal carcinoma, HPV detection provides a direct link between the two tumors and provides compelling evidence that the tumor in the lung represents a metastasis rather than a new primary lung primary. This link to an oropharyngeal primary is particularly useful given the observation that HPV-related OPSCCs can sometimes metastasize to distant sites long after treatment of their primary cancers, even well beyond the 2- to 5-year interval that is usually set as the threshold for distinguishing a primary lung cancer from a metastatic HNSCC.

**Distinction from non-HPV types of HNSCC**

As noted above, lymphoepithelial features may be present and fully developed in a subset of HPV-related OPSCCs. Failure to recognize that the lymphoepithelial phenotype is not entirely restricted to EBV-driven undifferentiated nasopharyngeal carcinomas may be problematic when it is...
encountered in cervical lymph node metastases. Assumptions about nasopharyngeal origin based solely on the morphologic findings run the risk of inappropriately diverting treatment away from the oropharynx and toward the nasopharynx. Accordingly, testing for both HPV and EBV is advisable when a lymphoepithelial phenotype is encountered in lymph node metastases in the absence of a known primary tumor site.

Alternatively, HPV-related OPSCCs may be easily confused with the basaloid variant of squamous cell carcinoma based on similar morphologic features, yet the distinction is important as these tumors set apart by very different clinical behavior. When dealing with basaloid tumors of the oropharynx, HPV testing is advisable to separate the clinically favorable HPV-positive carcinoma with basaloid morphology from the highly aggressive HPV-negative basaloid variant of squamous cell carcinoma.42

Squamous-lined cysts of the lateral neck

Patients with squamous cell carcinomas of the head and neck often present with a neck mass as the initial and only manifestation of their disease. These cervical metastases frequently undergo cystic degeneration causing clinical confusion with benign cystic squamous lesions. Even fine-needle aspiration cytopathology is not always helpful in making a clear cut distinction between benign and malignant cystic squamous lesions of the neck, especially when it comes to separating benign lymphoepithelial cysts with reactive squamous atypia from well-differentiated squamous cell carcinomas.68 This diagnostic difficulty has prompted a pursuit of additional diagnostic techniques including the immunohistochemical detection of differentially expressed markers. When struggling with this diagnostic dilemma, detection of HPV in the cyst wall provides very compelling evidence for a cystic metastasis from the oropharynx. Of note, p16 is overexpressed in almost one-half of benign branchial cleft cysts, rendering p16 immunostaining of little if any value in this diagnostic scenario.69 Accordingly, some other method of HPV detection such as in situ hybridization is necessary to confirm the malignant nature of a squamous-lined cyst in the neck.

Recognition of HPV-related cancer and its distinction from non-neoplastic tissues

The histologic recognition of an HPV-related OPSCC is not always straightforward and may benefit from HPV detection assays such as p16 immunohistochemical staining that highlights the presence of HPV infected tumor cells. Several factors contribute to diagnostic difficulty. First, HPV-related OPSCCs are often very well differentiated, retaining a close resemblance to the reticulated epithelium lining the tonsillar crypts. Second, the tumors are often masked by an obscuring lymphoid background. Third, the tumors may be inconspicuously small, even in patients who present with advanced metastatic spread to regional lymph nodes. Indeed, in patients with HPV-positive lymph node metastases from tumors of an unknown primary site, p16 staining of the tonsillectomy specimens sometimes discloses small clusters of HPV carcinoma that were not apparent by routine histology alone.64

In summary, the emergence of a distinct subset of head and neck cancer that is caused by high-risk HPV is re-shaping the oncology landscape and is impacting on current diagnostic practices. The morphology of these HPV-related are characteristic, but these same histologic features are challenging traditional diagnostic paradigms relating to fundamental concerns such as tumor grading, tumor classification and the histologic recognition of tumor invasion. Confirmation of HPV status is not only important for estimating clinical outcomes and directing therapy, but can directly inform the diagnostic process in specific diagnostic scenarios.

References


