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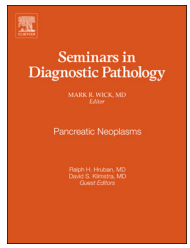

The role of cytology in the era of HPV-related head and neck carcinoma

*Sinchita Roy-Chowdhuri, MD, PhD, Savitri Krishnamurthy, MD**

Department of Pathology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Box 053, Houston, TX 77030-4095

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ABSTRACT

Enlarged neck lymph nodes are very often subject to fine needle aspiration biopsy to detect metastatic disease in patients with suspected or proven squamous cell carcinoma in head and neck region. Cytology specimens of metastatic carcinoma in such patients are routinely evaluated for human papilloma virus (HPV) to identify patients with HPV- related head and neck squamous cell carcinoma. Different types of cytology specimens including smears, cytospins, cell blocks and aspirated material in the rinse can all be used for different types of HPV testing such as immunohistochemistry for p16, HPV-in situ hybridization, and HPV-Polymerase chain reaction. There is currently no consensus regarding the testing of high-risk HPV in cytology specimens. The establishment of standardized HPV testing of cytology specimens is of utmost importance and is eagerly awaited.

Introduction

Human papilloma virus (HPV)-related squamous cell carcinoma is a distinct variant of head and neck squamous cell carcinoma (HNSCC).

Demographics, risk factors and pathogenesis

Human papilloma virus (HPV)-related oropharyngeal carcinoma represents a clinically distinct form of head and neck squamous cell carcinoma (HNSCC) that results from oral HPV infection.[1–6] These tumors are associated with an improved overall survival, progression-free survival, and disease-specific survival in comparison to their HPV-negative counterparts.[7–10] In contrast to HPV-negative HNSCC, which has been gradually decreasing in incidence in the United States due to a decline in tobacco exposure, HPV-related HNSCC has seen a dramatic increase in overall incidence (225% from 1998 to 2004).[1,2,11]

HPV-related HNSCC is typically seen in younger patients (<60 years) whose sexual behavior increases risk factors for HPV infection including early age of sexual contact, high number of lifetime sexual partners, oral–genital and oral–anal sex, and lack of barrier protection during sexual contact.[12–15] HPV-related HNSCC has a strong association with oral high-risk HPV (HR-HPV) infection, most frequently HPV type 16 (85–90% of cases).[16,17] These tumors are more frequently encountered in men than in women who are often non-smokers and without a history of alcohol abuse.[3,17] They most commonly involve the oropharynx, with particular predilection for the tonsil and base of the tongue.[3,16–20] HPV-related HNSCC is driven by the production of viral oncoproteins E6 and E7, which interfere with the p53 and retinoblastoma (Rb) tumor suppressor pathways.[21,22] Inactivation of Rb by E7 leads to upregulation and overexpression of p16.[23] Immunohistochemical (IHC) detection of p16 in tumor cells is thus often used as a surrogate for HR-HPV infection.[24,25]

HPV-related HNSCC forms a distinct clinicopathologic entity primarily because of its much improved prognosis

* *Corresponding author.*
E-mail address: skrishna@mdanderson.org (S. Krishnamurthy).

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and clinical outcomes when compared with non-HPV associated HNSCC.[10] The option of treating these patients with less intense chemo/radiotherapy than conventional treatment for non-HPV HNSCC is currently being addressed in clinical trials.[26,27]

Histopathology of HPV-related HNSCC

The most common histomorphology seen in HPV-related HNSCC is a non-keratinizing *basaloid* form of squamous cell carcinoma (SCC). This tumor typically infiltrates in sheets, cords, and lobules with associated central necrosis often leading to cystic degeneration.[3,28–30] Strong stromal desmoplastic response is uncommon, but hyalinization is usual, and the tumor is often surrounded by lymphoid tissue which may infiltrate as tumor-infiltrating lymphocytes (TILs) thereby imparting a lymphoepithelial-like appearance.[31] Cytologically, malignant cells display high nuclear–cytoplasmic ratios giving a classic basaloid appearance. Cytoplasmic keratinization and intercellular bridges are absent (Fig. 1A). The distinction of HPV-related HNSCC with basaloid cytomorphology from clinically aggressive non-HPV-related basaloid variant of HNSCC is important.[32,33] Other morphologic variants of HPV-related HNSCCs reported in the literature include papillary,[34] adenosquamous,[35] and rare reports of small cell transformation which is associated with a more aggressive behavior and poor prognosis.[36,37]

HPV-related HNSCCs frequently undergo necrosis and cystic degeneration, especially when they metastasize to cervical lymph nodes resulting in cystic degeneration.[38] The cystic change in an enlarged cervical node with a metastatic deposit is so characteristic of HPV-related HNSCC that in the absence of a known primary, an occult oropharyngeal HPV-related HNSCC should always be suspected.[39–41] Oropharyngeal HNSCCs frequently present with small, often occult tumors, and low tumor (T) stage disease but with higher nodal (N) stage and enlarged metastatic cervical lymph nodes.[42–44]

The role of cytology in the diagnosis of HPV-related HNSCC

The role of cytology for the diagnosis of HPV-related HNSCC at the primary site is very limited. The small size of the tumors, together with the most prevalent sites of occurrence in the base of tongue and tonsillar crypts, makes cytological sampling techniques such as oral brushing/rinsing relatively ineffective for detection of these tumors.[45] Therefore, an oral Papanicolaou (Pap) screening test similar to the hugely successful cervical cytology screening has not been considered for early diagnosis of HPV-related HNSCC.[45–47]

In contrast to the limited role of traditional cytology for the diagnosis of HPV-related HNSCC in the oropharynx, fine-needle aspiration (FNA) biopsy of enlarged metastatic neck lymph nodes very often plays a significant role in the initial diagnosis of HPV-related HNSCC.[38–41] The role of neck lymph node FNA in the recognition of occult HPV-related HNSCC in patients who present with metastatic carcinoma of unknown primary is now well accepted clinically. Therefore, evaluating HPV status in any patient with a newly diagnosed metastatic SCC to a neck node without a known primary is highly recommended in determining a primary site of origin. Having a positive HPV result is a strong indicator of oropharyngeal origin and facilitates clinical management. FNA biopsy results in these patients lead to targeted endoscopic examination of the oropharynx, particularly the tongue base and tonsils, to identify the primary tumor site.[39–41] In the rare event of an unidentified primary site despite exhaustive clinical/radiological evaluation,[48] positive HPV status can be used to direct localized radiation therapy to the oropharynx instead of irradiating the entire upper respiratory mucosa, thereby sparing the patient significant morbidity and associated complications. The possibility of metastatic HPV-related squamous cell carcinoma (SCC) from distant sites such as lung and cervix needs to be excluded in such patients because of the significant morphologic and immunophenotypic overlap in SCCs arising from different sites.[49,50]

Cytomorphology of HPV-related HNSCC

The morphologic features of FNA smears of metastatic HPV-related HNSCC have been described in prior studies.[51] As previously mentioned, any metastatic SCC in the head and neck needs to be tested for HPV; however, certain characteristic features (e.g., basaloid morphology and lack of keratinization) should raise suspicion for HPV-related HNSCC. The following morphologic patterns have been described.

The cystic pattern

Cystic metastatic SCC in cervical lymph nodes is highly suspicious of an oropharyngeal primary.[38–41] Cytologically, the aspirate may be composed of predominantly bland cells in a cystic background of macrophages, inflammatory cells, anucleated/nucleated squamous cells, and keratin debris. The findings are non-specific and overlap with other benign conditions including branchial cleft cyst or lymphoepithelial cystic lesions in the parotid gland. A high index of suspicion together with a thorough screening of the aspirate for atypical squamous cells is needed to make a diagnosis of SCC. HPV testing of the aspirate can be useful in such a situation, although one needs to use caution in interpreting HPV status using p16 immunostaining of cellblock material, since rare branchial cleft cysts have been reported to show p16 positivity.[52,53]

The basaloid pattern

This pattern is one of the most frequently encountered in HPV-related HNSCC. FNA smears in this pattern are generally very cellular displaying both sheets and loose clusters of poorly differentiated “small” cells with high nuclear–cytoplasmic ratios. Nuclei are hyperchromatic without enlarged nucleoli.[54] Presence of intercellular bridges and keratinization may be clues to diagnosis of SCC; however, these features, if present, are usually focal and not readily identified. The lack of nucleoli, presence of mitosis, necrosis, and even nuclear molding raise the differential diagnosis of small cell neuroendocrine carcinoma (SCNEC).[55,56] The use of CK5/6, p63, and p40 in identifying squamous differentiation and neuroendocrine markers such as CD56, synaptophysin, and chromogranin are useful for distinguishing basaloid SCC from SCNEC.[57,58] Basaloid salivary gland tumors, particularly the solid variant of adenoid cystic carcinoma (AdCC), enter into the differential diagnosis of the basaloid HPV-related