Assessment of Fine Needle Aspiration Specimen Adequacy for High-Risk HPV Detection and Genotyping in Oropharyngeal Squamous Cell Carcinoma

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Key Words
Fine needle aspiration · HPV · Genotyping · Squamous cell carcinoma

Abstract
Objective: The aim of this study was to determine the adequacy of archived and fresh fine needle aspiration (FNA) specimens from metastatic head and neck squamous cell carcinoma (SCC) for the molecular detection and genotyping of high-risk (HR) HPV. Study Design: Thirty-seven specimens from 26 patients diagnosed by FNA with metastatic SCC were included as retrospective specimens [19 slides stained with Papanicolaou (Pap) and 18 with Diff-Quik® (DQ)]. Twenty fresh FNA specimens from 18 patients were included as prospective specimens. These specimens were analyzed using the standard protocol for ThinPrep® cervical specimens, with a Cervista HR HPV detection kit. The positive specimens were tested for the HPV 16 and 18 genotypes. Results: Forty-four of 57 specimens (77%) had sufficient cells to yield a valid HPV result. The adequacy rate for Pap-stained slides was 15/19 (79%), for DQ-stained slides it was 13/18 (72%), and for fresh needle aspirates it was 16/20 (80%). HR HPV was detected in 23/44 (52%) specimens. Among the 23 HPV-positive specimens, 19 were genotyped as HPV 16 and 1 as HPV 18. Conclusions: HR HPV detection and genotyping can be performed on FNA specimens of head and neck SCC prospectively collected in PreservCyt as well as on archival slides with either Pap or DQ stain.

Objective
Head and neck squamous cell carcinoma (HNSCC) is a heterogeneous tumor group. One recently recognized subtype is the HPV-positive oropharyngeal carcinoma (OPC).

The HPV-positive OPC shows increasing incidence and usually affects younger patients, predominantly white males, non-cigarette smokers, non-alcohol drinkers, and individuals with a high number of sexual partners and a history of oral-genital or oral-anal sex [1].

These tumors usually arise from the crypts of the lingual and palatine tonsils, have no association with dysplasia of the surface squamous epithelium, show a lobular growth pattern, and demonstrate basaloid morphology, lacking significant keratinization.

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HPV-positive HNSCC express the viral oncoproteins E6 and E7, they overexpress P16 gene product, and they infrequently harbor p53 gene mutations and are associated with an improved prognosis [2]. Tumor HPV status is a strong and independent prognostic factor for survival among individuals with OPC in nonsmoking patients [3]. HPV 16 has been detected in up to 70% of OPC. The aim of this study was to determine the adequacy of archival and fresh FNA specimens for molecular detection and genotyping of HPV using Cervista HR HPV detection assay.

### Study Design

After obtaining approval from the institutional review board, a retrospective review of our pathology files was undertaken to identify those patients with a diagnosis of metastatic SCC in fine needle aspirations (FNA) of the head and neck area in the past 5 years. A total of 37 adequate specimens from 26 patients were available in the cytology laboratory of Thomas Jefferson University Hospital and were included as retrospective specimens. Among these specimens, 19 had slides stained with Papanicolaou (Pap) and 18 had slides stained with Diff-Quik® (DQ) stain. Twenty fresh FNA specimens from 18 patients with metastatic SCC diagnosed in the last 6 months were included as prospective specimens.

**HPV Analysis**

The archival Pap- and DQ-stained slides were soaked in xylene overnight to remove the coverslips. Scraped cells from the slides were placed in tubes and washed with ethanol, and dry pellets were resuspended in 2 ml of PreservCyt solution. For the prospective samples, fresh residual needle material from FNA specimens was directly rinsed in 2 ml of PreservCyt solution. All specimens were then analyzed using the standard protocol for ThinPrep® cervical specimens with a Cervista HR HPV detection kit. Positive specimens were then tested for HPV 16 and 18 genotypes.

### Results

Overall 44 of 57 specimens (77%) had a sufficient number of cells to yield a valid HPV result. The adequacy rate for Pap-stained slides was 15/19 (79%), for DQ-stained slides it was 13/18 (72%), and for fresh needle aspirates it was 16/20 (80%) (table 1). High-risk (HR) HPV was detected in 23/44 (52%) specimens. Identical HR HPV results were obtained from parallel DQ- and Pap-stained specimens in 6 patients. Among the 23 HPV-positive specimens, 19 (83%) were genotyped as HPV 16 and 1 was HPV 18 (tables 2, 3).

### Discussion

HPV genotyping has been used in gynecologic cytopathology in the past few years [4, 5] but only recently is emerging as an important tool in the management of patients with OPC. Most publications in the literature are based on HPV 16 detection in surgical pathology speci-
mens and use HPV detection methods such as PCR and ISH [6–10].

Cervista HPV HR testing has analytical sensitivity comparable to that of Hybrid Capture 2 (HC2). The assay uses a housekeeping gene as an internal control to ensure sufficient cells are tested to avoid false-negative results.

Our adequacy results (77%) are comparable with those of one study based on HPV testing in FNA specimens performed in metastatic lesions of SCC, prior to treatment or after tumor recurrence. The authors reported the Hologic method as reliable, with adequate DNA for molecular analysis in 70% of aspirates. No genotyping data was reported [11].

The aim of our study was to determine adequacy rates for archival and fresh FNA specimens for the molecular detection and genotyping of HPV. The inclusion criteria for this study was a diagnosis of metastatic SCC in the head and neck area, not targeting only lingual and palatine tonsil SCC. This could account for the detection rates and genotyping results.

**Conclusion**

HR HPV detection and genotyping can be performed on FNA specimens of HNSCC prospectively collected in PreservCyt as well as on archival slides with either Pap or DQ stain.

**References**