Cytopathology in focus: p16 immunostaining in cytology specimens—a diagnostic pitfall

Efrain A. Ribeiro, MD, PhD

January 2022—Cytopathologists are often the first pathologists to diagnose HPV-related head and neck squamous cell carcinomas (HNSCC). These head and neck cancers can present as superficial masses amenable to fine-needle aspiration, where p16 immunostaining is used as a surrogate marker for HPV in situ hybridization in a subset of squamous cell carcinomas. However, there remains uncertainty within the practicing community regarding the interpretation of p16 staining as it relates to HPV status in cytology specimens, particularly in specimens outside of the head and neck. In this article is a review of a recent study that aims to address the diagnostic utility of p16 staining in cytology specimens (Ribeiro EA, Maleki Z. *J Am Soc Cytopathol.* 2021;10[4]:414–422).

There are several challenges related to the interpretation of p16 staining in cytology specimens, which are often sparsely cellular and may represent only a subset of neoplastic cells from the target lesion on a cell block preparation. With respect to head and neck cytology specimens, there is still no consensus on the minimum staining required to consider a p16 stain as unequivocally positive. Recent studies suggest that a lower threshold may be warranted in head and neck cytology specimens compared with the 70 percent cutoff used in surgical pathology. Ultimately, these challenges are compounded by a lack of data on the concordance of p16 and HPV in situ hybridization outside of the head and neck in cytology specimens. The recommended reading article aims to address this gap in knowledge.

In the study by Ribeiro and Maleki, 372 cases in which p16 staining was performed were reviewed and grouped by body site. Retrospective analysis confirmed that the vast majority of cases with diffuse p16 staining in the head and neck, particularly in men, were HPV-positive. However, there was a subset of p16-positive cases in the head and neck that were HPV-negative and were not ultimately diagnosed as an HPV-related cancer. Approximately one quarter of the cases reviewed were obtained from body sites outside of the head and neck and included specimens from the thoracic cavity, genitourinary, and gastrointestinal systems. Diffuse p16 positivity was seen in 10 cases outside of the head and neck where HPV cotesting was ordered. Only a subset of these p16 diffusely positive cases were HPV-positive, along with two cases in which p16 was focally positive or negative.

Overall, the data showed that while p16 positivity in the head and neck correlated with HPV status, p16 positivity outside of the head and neck did not. This brings up a diagnostic pitfall with regard to p16 staining in cytology specimens. The data suggest that p16 staining outside of the head and neck should not be used as a surrogate for HPV-related cancers, and the data also draw attention to the incidence of non-HPV–related p16-positive cancers metastasizing to the head and neck. Diffuse p16 staining can be seen in a variety of conditions such as adenocarcinoma, small cell carcinoma, and serous carcinoma irrespective of body site. Focal p16 can also be seen in adenocarcinomas. Therefore, HPV cotesting should be pursued in p16-positive cases outside of the head and neck where an HPV-related primary is suspected, and clinical follow-up is warranted to determine the primary site.

Future studies will be needed to establish a consensus for the interpretation of p16 immunostaining both within and outside of the head and neck. It is possible that differences in percentage of p16 staining may correlate with HPV status in other body sites. As HPV testing is not routinely ordered in non-head and neck specimens that were stained with p16, it will be important to continue to gather more data to come to a consensus on this topic. Therefore, caution is warranted when ordering p16 stains outside of the head and neck as this stain is not specific for a single diagnosis in and of itself. Cytomorphologic correlation with intensity of p16, clinical history, and other ancillary studies such as p40 immunostaining and HPV cotesting can improve diagnostic accuracy and prevent diagnostic pitfalls.n

Dr. Ribeiro, a junior member of the CAP Cytopathology Committee, is the 2021–2022 Dr. Dorothy Rosenthal cytopathology fellow, Department of Pathology, Johns Hopkins Hospital.