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Role of p16 and Ki67 Immunohistochemistry in the Evaluation of Cervical Dysplasia

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Cervical dysplasia refers to abnormal microscopic changes in the cells on the surface of the cervix. It is usually associated with human papillomavirus (HPV) infection and is frequently detected on Pap smears as "squamous intraepithelial lesions" (SIL). The more common abnormal findings on Pap tests include low- and high-grade SIL (LSIL and HSIL respectively), atypical squamous cells of undetermined significance (ASC-US), and atypical squamous cells, without excluding high-grade SIL (ASC-H). Abnormal Pap smears require follow-up testing that may include repeat Pap tests, colposcopy-directed biopsy, or more extensive biopsies, such as a cone or loop excision electrosurgical procedure (LEEP). Dysplasia that is seen on cervical biopsy specimens is called "cervical intraepithelial neoplasia" (CIN). It is grouped into three categories: 1) CIN 1 (mild dysplasia), 2) CIN 2 (moderate dysplasia), and 3) CIN 3 (severe dysplasia to carcinoma in situ).

According to the guidelines of the American Society of Colposcopy and Cervical Pathology (ASCCP), women with cervical biopsy–confirmed CIN 2 or CIN 3 should undergo an excisional treatment to prevent potential progression to invasive cancerous growth. These therapies may potentially affect reproductive outcomes, especially in young women; and most low-grade squamous intraepithelial lesions (CIN 1) regress spontaneously. Therefore, it is important to have accurate diagnostic interpretation of cervical biopsy specimen to distinguish between low-grade (CIN 1) and high-grade SIL (CIN 2 and CIN 3) to avoid overtreatment of false-positive cases and under treatment of false-negative cases.

The p16 protein (p16) is an INK4a cyclin-dependent kinase (CDK) inhibitor that decelerates the cell cycle by inactivating the CDK that phosphorylate retinoblastoma (Rb) protein. The status of Rb expression strongly affects p16 expression, and p16 overexpression has been demonstrated in cervical cancers because of functional inactivation of Rb by HPV E7 oncoprotein.[1] Immunohistochemical expression of p16INK4a has been associated with dysplastic/neoplastic cells but not seen in normal cervical epithelium, and it is also related to degree of histologic dysplasia.[2,3] Thus, p16INK4a expression appears to be a useful biomarker for HPV-related changes in cervical epithelium. In a strong and diffuse block positive pattern p16 immunohistochemical staining is highly sensitive for CIN 2 and 3 but not for CIN 1. Negative or non–block-positive staining strongly favors an interpretation of low-grade disease or a non–HPV-associated pathology. Diffuse p16 expression can be a surrogate marker of transformed high-risk HPV-related cervical lesions, and it helps pathologists distinguish lesions that require follow-up testing from those that do not.

Recommendations for the Use of p16 Immunohistochemistry
1. Differentiating precancer (CIN 2 or CIN 3) from benign mimics, such as immature squamous metaplasia, atrophy, reparative epithelial changes, or tangential sectioning on routine staining.
2. Differentiating low-grade (CIN 1) lesions from higher grade (CIN 2 or 3) SIL that require further follow-up testing.
3. Identifying high-grade disease with current diagnosis of CIN 1 with high-risk prior screening test results (including HSIL, ASC-H, and ASC-US with positive HPV 16 serotyping). In these cases, any area highlighted by p16 immunostaining should correlate with morphologic criteria for high-grade SIL on routine staining.

Ki-67, a proliferation marker, is elevated in HPV-infected mature squamous epithelia and is useful for confirmation of the diagnosis in equivocal low-grade SIL.[4-6] Ki-67, however, may be positive in HPV-negative squamous metaplasia or regenerating epithelium ; therefore, positivity of this marker in immature squamous epithelium is not specific for HPV infection. In atypical immature squamous metaplasia, Ki-67 staining shows variable results, with a wide range of positivity and significant overlap between HPV-positive and HPV-negative cases.[7] Recommendations from the Lower Anogenital Squamous Terminology (LAST) Project, a collaboration between the College of American Pathologists and the ASCCP, state that Ki-67 should not be *routinely* added to p16 immunohistochemistry but may be considered in cases for which p16 staining is inconclusive or technically inadequate.[8] The role of p16 and Ki67 immunohistochemistry in evaluation of cervical dysplasia is important for both pathologists and clinicians to understand.

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