Utility of Ki-67 (MIB-1) Immunostaining in Cervical Biopsies

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Nearly every pathologist that practices in a community hospital setting struggles with the accurate and reproducible identification of HPV effect and dysplasia in the female genital tract, and recognizes the fact that in certain situations these conditions can be very difficult to distinguish from reactive changes. Last month in the ProPath "Focus on Immunohistochemistry", we reviewed the potential contributions of immunostaining for p16 (INK4a) in addressing this problem, and this month we focus our attention on the use of Ki-67, a well-known marker of cell proliferation, to assist in these differential diagnostic problems.

The Ki-67 antigen is a non-histone protein expressed in all phases of the cell cycle except G0. Because HPV infection leads to increased epithelial cell proliferation in infected tissues, increased Ki-67 staining can be a reflection of HPV infection. As such, it is not surprising that this marker has found utility in recognizing tissues involved by HPV, and the extent of Ki-67 immunostaining generally parallels increasing grades of dysplasia. The most commonly used antibody for immunohistochemical detection of the Ki-67 antigen in paraffin sections is clone MIB-1.

At the 1998 United States and Canadian Academy of Pathology meeting, Tam et al reported a study of 47 cervical biopsies diagnosed as normal (n=20) or low-grade squamous intraepithelial lesion (SIL, n= 27), and performed immunostains for MIB-1, as well as PCR for HPV DNA. All cases were reviewed in a blind fashion by 3 pathologists and consensus diagnosis was defined as agreement by at least 2 reviewers. These investigators noted that Ki-67-positive nuclei were seen in the upper two-thirds of the epithelium in most cases of low-grade SIL, but were confined to the basal or parabasal areas in most normal biopsies.

Mittal and Pallazzo reported a study of 27 cases of cervical condyloma, 11 cases of squamous metaplasia, 11
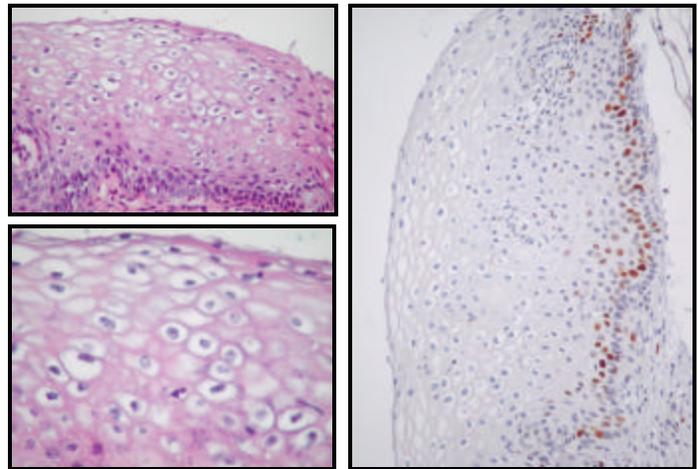

H&E stains (left 2 frames) and Ki-67 immunostain (right) on a cervical bx from a 26 year-old with a pap smear dx of LGSIL. Some pathologists favored HPV effect but others favored reactive changes. The Ki-67 stain showed positive cells restricted to the basal layer, supporting a reactive interpretation. Digene HPV was negative .

cases with cervical inflammation, and 13 cases of normal ectocervix. They found that expression of Ki-67 (MIB-1) in the superficial half of the cervical epithelium (seen in two-thirds of the cases of condyloma but none of the other cases), or the identification of >15% Ki-67-positive basal cells favored the interpretation of condyloma over inflammation or metaplasia.

Keating et al reported a study of 104 epithelial lesions in 99 biopsies in 2001, and defined a "positive" Ki-67 stain as one that showed positive nuclei extending above the basal one-third of the epithelium. When defined in this fashion, positive Ki-67 immunostains were identified in 71.4% of 21 cases of low-grade SIL, and 94.7% of 19 cases of high-grade SIL. 7.7% of 29 histologically normal biopsies showed a positive Ki-67 stain, and 83.3% of 24 biopsies that contained high-risk HPV by PCR analysis were Ki-67 positive. This yielded a positive predictive value of 75% for SIL, and a positive predictive value of 82.4% for HPV. Occasional cases of reactive squamous metaplasia showed Ki-67-positive

nuclei in the upper two-thirds of the epithelium, but these cells were not as numerous as those observed in cases of SIL.

In a review article published in 1999, Mittal et al reported that results of Ki-67 staining in cauterized tissue was similar to the results noted in non-cauterized tissue, making this immunostain particularly useful when morphologic interpretation is compromised by deleterious effects of cautery artifact.
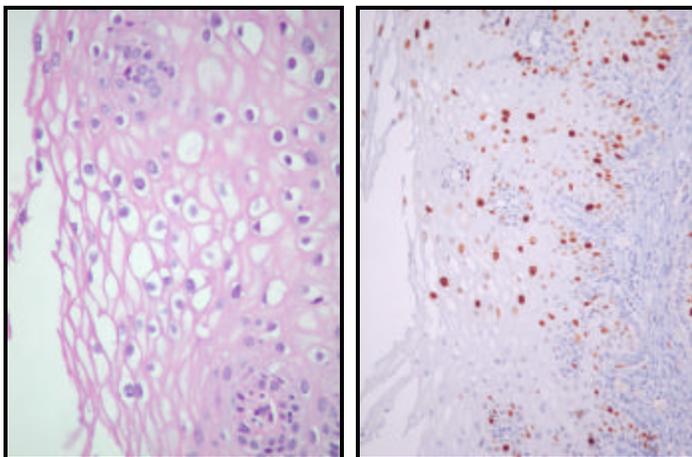
H&E (left) and Ki-67 immunostain (right) on a cervical bx from a 25 year-old with an abnormal pap smear. Several pathologists reviewed the H&E and a consensus diagnosis of HPV vs. reactive changes could not be achieved. The Ki-67 stain showed frequent positive cells in the upper layers of the epithelium, supporting HPV effect

Mittal and associates studied cervical biopsies from women >55 yr. old, including 14 patients with atrophy, 9 with dysplasia, and 12 cases consisting of normal endocervix or squamous metaplasia. They found that Ki-67 was very useful in distinguishing postmenopausal atrophy from dysplasia. In atrophy, Ki-67-positive cells were limited to the basal and parabasal cells, with only rare positive cells identified in the middle third of the epithelium. When compared to normal, the cases of atrophy showed significantly fewer Ki-67-positive nuclei. In contrast, patients with postmenopausal dysplasia showed frequent Ki-67-positive cells in the middle and upper thirds of the epithelium.

In addition to its utility in the uterine cervix, Ki-67 has also been found to useful in the study of vulvar and vaginal lesions. In a study of 24 biopsies from 15 patients , Logani et al. found that the identification of at least 2 Ki-67-positive nuclei in the same microscopic field in the upper two-thirds of the vulvar or vaginal

epithelium correlated well with HPV infection (as determined by PCR analysis).

In summary, Ki-67 (MIB-1) immunostains are a useful adjunct in the interpretation of cervical biopsies, particularly when attempting to distinguish low-grade SIL or HPV effect from reactive changes. Positive Ki-67 staining in the upper two-thirds or upper half of the epithelium argues in favor of an HPV-related lesion. Ki-67 is particularly useful in distinguishing postmenopausal atrophy from dysplasia in postmenopausal patients, and it may also assist in the interpretation of lesions that are difficult to interpret secondary to cautery artifacts. Finally, Ki-67 is useful in the interpretation of vulvar or vaginal biopsies where HPV effect or dysplasia enter into the differential diagnosis.

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