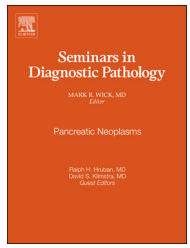
Seminars in Diagnostic Pathology 32 (2015) 409–418

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/semdp



Immunohistochemistry and in situ hybridization for the diagnosis and classification of squamous lesions of the anogenital region

Edyta C. Pirog, MD, PhD

Department of Pathology, Weill Medical College of Cornell University, 525 E 68th Street, ST-1041, New York, NY

ARTICLE INFO

Keywords:
Ki-67
p16
ISH
ProEx C
LSIL
HSIL
HPV

ABSTRACT

Distinguishing anogenital squamous intraepithelial lesions from benign conditions and mimics may be problematic. Immunohistochemistry for surrogate markers of HPV infection, such as Ki-67, p16, and ProEx™ C, may aid the diagnosis in equivocal cases. The main diagnostic pitfall in the diagnosis of LSIL is the occurrence of "pseudokoilocytes" in benign squamous mucosa, which may lead to overdiagnosis. When interpreted correctly, Ki-67 is a sensitive and specific marker for dysplasia in mature squamous epithelium and is therefore useful for confirmation of LSIL and condyloma. A Ki-67 positive result is defined as the presence of a cluster of at least two strongly stained epithelial nuclei in the upper two-thirds of the epithelial thickness. With such a definition, there is almost complete concordance between consensus diagnosis of LSIL/condyloma confirmed by detection of HPV DNA and positive Ki-67. A related proliferation marker, ProEx™ C, has similar staining patterns and utility for the diagnosis of low grade dysplasia. The differential diagnosis of HSIL includes atypical immature squamous metaplasia and atrophy. A marker with high sensitivity and specificity for the detection of HSIL in cervical, vulvar, and anal mucosa is p16. A 2-tier scoring system is used to evaluate p16 staining. No staining or a discontinuous, patchy nuclear and cytoplasmic staining pattern is considered as a negative result. A positive result is defined as diffuse and strong staining of cells of the basal and parabasal layers of the squamous epithelium, with or without staining of superficial cell layers. New markers that are undergoing evaluation for their clinical utility include stathmin-1, phosphorylated S6, and SOX2. Confirmation of the diagnosis of dysplasia by HPV detection in tissue sections using HPV capsid protein immunohistochemistry, HPV DNA or HPV RNA in situ hybridization offers lower sensitivity as compared to immunohistochemistry for surrogate markers and therefore has more limited utility in this context.

Histopathologic interpretation of tissue sections forms the basis for the appropriate management of patients with anogenital squamous intraepithelial lesions (SILs). Overdiagnosis of these Human Papillomavirus (HPV)-associated lesions can lead to psychological stress for the patient, unnecessary treatment and increased medical costs. Despite well-defined criteria, distinguishing SILs from benign, reactive atypia as well as determining the grade of SIL may be

E-mail address: ecpirog@med.cornell.edu

http://dx.doi.org/10.1053/j.semdp.2015.02.015
0740-2570/

problematic. In particular, there is considerable interobserver variation in the diagnosis of low grade squamous intraepithelial lesions (LSIL) or intraepithelial neoplasia 1 (-IN1), as well as intraepithelial neoplasia 2 (-IN2). The subjectivity of (-IN1) diagnosis mainly rests upon difficulty in identification of true HPV cytopathic effect (i.e., koilocytotic atypia). Presence of "pseudokoilocytes" or mimics of koilocytotic atypia is a well-recognized diagnostic pitfall.

The basic definition of -IN2 is a lesion in which the immature basaloid cells extend between one-third and two-thirds of the epithelial thickness, with mitoses present in the lower two-thirds of the epithelium. The inconsistencies in diagnosis of -IN2 may result from subjectivity of assessment of the immature cell expansion when there is no demarcated plane; in addition, tangential tissue sectioning may impede accurate classification.

The problems of diagnostic reproducibility were well-illustrated in a study by Ismail et al.,[1] in which 100 consecutive cervical biopsies with various original diagnoses were examined by a panel of eight experienced pathologists. The agreement between observers was excellent for invasive carcinoma, moderate for CIN3, and poor for CIN1 and CIN2 (kappa = 0.832, 0.496, 0.172, and 0.175, respectively). The pathologists were also asked to mark their degree of confidence about the diagnosis using a linear kappa analog scale. The pathologists expressed confidence in diagnosing CIN3 and invasive carcinoma, but their confidence was relatively weaker for CIN1 and CIN2.

These diagnostic difficulties prompted a search for objective tests that may aid in the diagnosis of equivocal cases. Since SILs are caused by HPV infection, HPV detection in lesional tissue could serve as a confirmatory test. However, HPV detection in histologic sections proved to be technically difficult and tests such as HPV DNA in situ hybridization (ISH) and immunohistochemistry (IHC) for HPV capsid protein have only limited use. Novel HPV RNA ISH offers higher sensitivity and may become more applicable. Thus far, IHC for surrogate markers of HPV infection such as Ki-67, p16, and ProEx C™, are used widely, despite some limitations to their sensitivity and specificity.

Immunohistochemistry and in situ hybridization tests

Ki-67 immunohistochemistry

Ki-67 is a nuclear nonhistone protein expressed throughout the mitotic cycle with the exception of the G0 phase. Since HPV infection causes increased proliferation of the squamous epithelium, it has been suggested that IHC with anti-Ki-67 antibody may be useful in the diagnosis of SIL. In normal squamous mucosa or skin, Ki-67 positivity is found exclusively in the nuclei of parabasal squamous epithelium.[2–4] In cases with productive or transforming HPV infections, that is, in condyloma, LSIL, and HSIL, there is increased mitotic activity of the parabasal keratinocytes with extension of the proliferating cells to the intermediate and superficial epithelial layers. Accordingly, in such cases Ki-67-positive nuclei are seen in the parabasal area, as well as in the upper two-thirds of the epithelial thickness.[2–5] It is important to note that the overall count of Ki-67 positive nuclei in the entire epithelial thickness is not useful as a diagnostic parameter, as it may overlap with benign or reactive lesions.[6] The assessment of Ki-67 staining that proved to have diagnostic utility is qualitative and not quantitative. Studies of anogenital lesions, including LSIL, HSIL and condyloma of the cervical, vaginal, vulvar, and anal mucosa[2–5,7–9] have shown an excellent correlation between histological features of dysplasia/condyloma confirmed by HPV detection and the presence of Ki-67-positive nuclei in the upper two-thirds of the epithelial thickness. In these studies, any Ki-67 positivity in the upper two-thirds of the epithelium correlated with detection of HPV and consensus diagnosis of dysplasia/condyloma. Therefore, Ki-67 positivity useful in the diagnosis of squamous dysplasia/condyloma was defined as a cluster of at least two strongly stained epithelial nuclei present in the upper two-thirds of the epithelium anywhere within the lesion. This qualitative assessment of Ki-67 positivity is simple and reproducible, provided that tissue sectioning allows for assessment of the full epithelial thickness. The testing may be performed with either polyclonal anti-Ki-67 antibodies or with MIB-1, a monoclonal antibody recognizing a Ki-67 epitope and yielding the same staining results.

An important point about Ki-67 IHC is that it is not helpful in distinguishing between LSIL and HSIL, as the pattern is similar in both type of lesions (Fig. 1A and B). Furthermore, Ki-67 stain cannot distinguish between low and high oncogenic HPV infection. A similar staining pattern is seen in condylomas caused by low oncogenic HPVs and HSILs caused by high oncogenic HPV types (Fig. 1B and C).

There are several limitations to Ki-67 use. Ki-67 positivity is not specific for dysplasia in immature or regenerating epithelium.[9,10] Since Ki-67 is a marker of proliferation, Ki-67 positivity may be seen in the upper layers of the epithelium in benign immature squamous metaplasia as well as on the edge of an ulcer or erosion.[9,10] In addition, diffuse Ki-67 positivity has been reported in vulvar squamous cell hyperplasia.[7] Positive Ki-67 staining in such cases may not be reflective of the presence of HPV.

Even though the evaluation of Ki-67 positivity is usually straightforward, there may be rare interpretive difficulties. In cases of cervicitis or vulvitis, Ki-67-positive lymphocytes may be present throughout the epithelial thickness. High-power examination helps to identify these cells as nonepithelial. In addition, tangential sectioning through stromal papillae may result in the appearance of positive nuclei in superficial layers of the epithelium. Assessment of the overall tissue architecture, as well as obtaining deeper levels from the paraffin block, may prevent erroneous interpretation of the stain in such cases. In some instances, the epithelium may be extremely thin precluding evaluation of the extension of Ki-67 positivity to the upper layers of the epithelium. The staining may be very focal in longstanding condylomas characterized by marked keratinization and few koilocytes. It was also reported that HSIL may have only focal Ki-67 positivity, suggesting a regressing lesion.[9,10] In cases where the tissue is tangentially sectioned, Ki-67 positivity in between the papillae and away from the basal cells can be used as a confirmation of the diagnosis of dysplasia (Fig. 1D).