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Review Article

P16^{INK4a} immunocytochemistry/immunohistochemistry: need for scoring uniformization to be clinically useful in gynecological pathology

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Abstract High-risk (HR) human papillomaviruses (HPVs) are the main agents involved in the pathogenesis of cervical preinvasive and invasive lesions. Their regression or persistence is paramount in the progression or regression of preinvasive lesions. Therefore, the diagnosis of the presence or absence of HR-HPV is essential in the prognosis and follow-up of low-grade and high-grade squamous intraepithelial lesions. Human papillomavirus DNA and messenger RNA can be identified by a variety of molecular methods; however, their clinical use has limitations. The fact that p16^{INK4a} is a surrogate marker for the presence of HR-HPV is well established. However, the clinical usefulness of p16^{INK4a} is currently limited by the lack of immunohistologic and cytologic standardization of a scoring system. This article presents an overview illustrating this shortcoming based on relevant literature data.

Keywords: Uterine cervix; Preinvasive lesions; Human papillomavirus; p16^{INK4a}; Scoring; Review

1. Introduction

The unraveling of the genetic and molecular events involved in human papillomavirus (HPV) infection has brought about a paradigm shift in the understanding of oncogenic HPV-induced malignancies, including their diagnosis and management. The availability of HPV vaccines has forced us to reconsider the traditional way of screening and the follow-up of cervical preinvasive lesions. Testing for HPV-DNA is now viewed as an integral component in this regard. However, the availability and cost will remain a limiting factor especially in the developing world where the burden of disease and mortality from invasive cervical cancer are the highest in the world.

A more affordable alternative to HPV-DNA testing could be the immunohistochemical identification of high-risk (HR) HPV by a surrogate marker. One of them, p16^{INK4a} (p16 for short), has been widely validated in this regard. It is important to bear in mind that p16 is an important stain in HPV lesions that are due to HR-HPV 16 to 18 but that no such evidence is available for the non–16-to-18 HPV. Furthermore, p16 is not a marker of invasiveness.

To be of clinical value to the diagnostic pathologist as a biomarker for HPV 16/18 in preinvasive lesions and their follow-up, there should be an agreed upon standardized immunohistochemical scoring method. However, there is wide confusion and contradiction between reports that are linked to methodological differences, especially in the immunohistochemical/immunocytochemical scoring systems. To be of value to practicing pathologists and of benefit to the patients, agreement should be reached on a standardized histoscore. This should be achievable as it happened, for example, in hormone receptor scoring for breast cancer.

2. Materials and methods

We reviewed the current literature, with emphasis on the lack of uniformity in p16 scoring systems.

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3. Results and discussion

3.1. The limits of conventional cytology and histology

Conventional Papanicolaou smear screening can identify koilocytes as surrogate markers of HPV infection but is not in a position to distinguish between the low-risk and HR nature of the infectious agent. The presence of koilocytosis is irrespective of HPV type [1]. Moreover, not all HPV infections are morphologically expressed.

The low sensitivity, specificity, and predictive value of the cytologic and histopathologic diagnosis of cervical preinvasive lesions result mainly from interobserver variability, especially at the lower end of cervical intraepithelial neoplasias (CIN1) (including HPV cervicitis) [1,2]. The interobserver agreement kappa (κ) value for low-grade lesions is reported to be as low as 0.45 to 0.60 [3,4]. In addition, the global sharp increase in cervical adenocarcinoma suggests that preinvasive endocervical lesions are often overlooked, both cytologically and histopathologically [5,6].

The immunohistologic/cytologic overexpression of p16 has been shown to improve the interobserver variability of CIN1 to κ values between 0.75 and 0.90, resulting in a significant decrease in false-positive and negative diagnosis [3,4,7].

3.2. The natural history of cervical preinvasive lesions

Evidence has accumulated showing that what really matters in the natural history of cervical preinvasive lesions is the presence, persistence, or recurrence of HR-HPV, more specifically (but not exclusively) of the 16 and 18 oncogenic subtypes. The regression, persistence, and progression of cervical preinvasive lesions depend mainly on the persistence or interval new infection with HR oncogenic HPV subtypes. High-risk HPV 16 and 18 are present in nearly all cervical cancers [8]. Undetected and/or untreated high-grade squamous intraepithelial lesions (HGSILs) infected with HR-HPV progress to invasive cancer if the HR-HPV infection persists and if the host immunity is deficient [9]. In addition, the transition time from CIN3 to invasive carcinoma is halved (10-5.4 years) by the presence of HR-HPV [10].

About 90% of low-grade squamous intraepithelial lesions (LGSILs) regress spontaneously; this is attributed to the fact that many LGSILs are induced by low-risk nononcogenic HPV types [11]. That between 10% and 50% LGSIL progresses to high-grade ones over a period of 2 ½ to 7 years highlights the importance of the correct diagnosis and the necessity to follow-up both low- and high-grade lesions [11,12].

3.3. Conventional follow-up practices

Conventional best practice in the follow-up of patients treated by large loop excision of the transformation zone or loop electrosurgical excision procedure for LGSIL/CIN1 and HGSIL/ CIN $\geq$2 includes colposcopy or visual inspection with acetic acid and cytology at 6-month intervals for 2 years. This practice has sparked the debate about the predictive value of the character of the excision margins, that is, free or diseased. Traditionally, the physician expected the pathology report to be clear about the safety or not of the excision margins—the now outdated concept of *conization with therapeutic intent*, that is [13]. The relative risk of posttreatment disease of any grade is 5.5 after incomplete excision [14]. However, it has now been repeatedly shown that recurrence of disease happens even when the excision margins were reported free of disease. This is attributed to the persistence of HR-HPV [15]. It is evidenced that the pretreatment presence of HR-HPV is one of the main predictors of persistence/progression of preinvasive lesions [16,17]. After large loop excision of the transformation zone or loop electrosurgical excision procedure, there is a 35% persistence/recurrence of HR-HPV [18]. The presence or persistence of HR-HPV after 6 months (the time required to give a chance to regression) posttreatment predicts the recurrence/persistence of HGSIL [18,19].

A major benefit of pretreatment HPV testing is to prevent unnecessary treatment by cone in the absence of HR-HPV. This is because HPV negativity eliminates the risk of HGSIL or cancer, at least in the short term [1]. For these reasons, pretreatment workup and follow-up after conization now includes HPV DNA testing before treatment and 3 to 6 months for 1 year after [20–22]. This type of managerial policy has shown to reduce the number of advanced cervical cancers and cervical cancer mortality, including those in the developing world [21].

3.4. Diagnostic methods of HR-HPV

There are many methods to diagnose HPV carriage: messenger RNA polymerase chain reaction, HPV-DNA Hybrid Capture 2 test, rapid HPV-DNA care HPV test, DNA in situ hybridization, and immunohistochemistry (IHC) [23–25]. The limitations, however, are that most methods (IHC excluded) do not distinguish between episomal and integrated HPV, whereas the degree of severity of the disease correlates with the frequency of HPV integration [1].

Our focus is on the diagnostic possibilities offered by IHC, especially in developing world settings, where systematic Papanicolaou screening is virtually inexistent, not to mention treatment and follow-up facilities.

The E6 and E7 oncoproteins of HR-HPV bind to host cell regulatory proteins, in particular, the tumor suppressor gene product p53 and the retinoblastoma protein. This leads to the degradation of the p53 suppressor protein by E6 and the inactivation of the tumor suppressor protein retinoblastoma protein through binding to the E7 gene product [26]. This, in turn, results in the overexpression of the cyclin-dependent kinase inhibitor p16 [1,7]. The p16 is a surrogate marker of HR-HPV because it does not identify the viruses but rather their protein products.