

Cervical preneoplasia biomarkers: a conundrum for the community based gynecologic surgical pathologist

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The integration of high-risk (HR) human papillomavirus (HPV) in the cell genome is an essential step in the oncogenic pathway of lower ano-genital HPV-related squamous preinvasive and invasive lesions. The expression of HR-HPV surrogate biomarkers of HR-HPV integration by immunohistochemistry (IHC) serves as a diagnostic and/or a prognostic tool of cervical preinvasive lesions. IHC is claimed to decrease the interobserver variability in the diagnosis of histomorphologically equivocal lesions, and to be helpful in evaluating the potentiality of regression, persistence or progression. The most common biomarkers used in cervical pathology are p16^{INK4a}, Ki-67, the HPV capsid L1 antigen, and ProExC. Critical review of the literature shows a great variability in the diagnostic accuracy, risk evaluation, and relative distribution of these biomarkers in low and high grade preinvasive lesions. Review of the literature suggests that currently dual IHC with p16 and L1 provide the best diagnostic and prognostic evaluation of lesions diagnosed histomorphologically as low and high-grade.

Keywords: Biomarkers, Cervix uteri, Preinvasive lesions

Since 1999, it has been firmly established that the human papillomavirus (HPV) is “a necessary cause of invasive cervical cancer” [1]. It has also been shown that the main oncogenic or high-risk (HR) subtypes HPV 16 and 18 are present in up to 99.7% of invasive squamous cell carcinomas of the cervix [2]. In other lower anogenital squamous tumors (i.e., penis, scrotum, vulva, vagina, and perianus), because of alternative HPV-independent carcinogenic pathways, the rate of HR-HPV infection varies between 23 percent and less, and 100 [3].

The HPV-mediated carcinogenic pathway consists of a deregulated expression of the viral oncogenes E6 and E7, leading to genetic instability and alterations in regulatory host cell

genes resulting in cell immortality and invasive growth ability. An increase in E6/7 expression reflects integration of the viral DNA in the host cell genome [4]. E6 and E7 are consistently expressed in HR-HPV cervical cancers. The E6 oncoprotein initiates premature degradation of the p53 tumor suppressor protein. The viral E7 protein binds to the tumor suppressor retinoblastoma protein (pRB) and favors the release of E2F-like transcription factors from their complex with active pRB. Inactivation of pRB through E7 results in enhanced expression of p16^{INK4a} [5].

The p16^{INK4a} (or p16 in brief) tumor suppressor gene, located on chromosome 9p21, is a member of the INK4 class of cell-cycle inhibitors. The binding of p16 protein with the cyclin-dependent kinases 4 and 6 blocks its interaction with the D-type cyclins, leading to the prevention of cell cycle progression. Integration of HR-HPV into the genome is associated with upregulation of the p16 protein and increased transcription of E6/7. This process can be visualized through immunohistochemistry (IHC) where p16 overexpression is evidenced by an anti-p16 antibody reaction. This has been

Received Oct 13, 2013, **Revised** Nov 19, 2013, **Accepted** Nov 20, 2013

This expert opinion was not solicited and has not been peer reviewed.

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shown to be specific for dysplastic and neoplastic cervical epithelial cells [5]. p16 overexpression is now widely recognized as a surrogate biomarker of HR-HPV integration [4].

In the preinvasive stages of cervical neoplasia HPV is predominantly present in the episomal form without changing the nucleotide sequence of DNA that regulates gene expression [4]. The transition of cervical intraepithelial neoplasia (CIN) 2 to CIN 3+ correlates with the degree of viral DNA integration from 5.0% in the former to 88.0% in the latter [6,7]. Some degree of integration may be present in low-grade squamous intraepithelial lesions (LSIL) [8]. This is an important finding suggesting that, contrary to some views, LSIL may well be a preinvasive lesion. This raises the question of the sensitivity, specificity, positive and negative predictive value of cytology and histopathology. All the reported markers of accuracy and predictivity vary considerably and reflect a wide interobserver variability. This situation has led to two positions: doubt about the validity of histocytomorphology and possible supremacy of biomarkers over morphology [9].

According to Castle et al. [10], CIN 2 is an equivocal diagnosis of cervical precancer and includes both CIN 1 and HPV effects as well as some precancerous lesions. Based on this view, the Lower Anogenital Squamous Terminology (LAST) Standardization Project recommends a two-tier classification system for preinvasive lesions: LSIL (or -intraepithelial neoplasia [IN]1) and high-grade squamous intraepithelial lesion (HSIL) (or -IN2+). It also recommends p16 immunophenotyping of equivocal LSIL/IN1 by morphological diagnosis. They, however, caution against the overuse of p16 IHC [9]. This recommendation is questionable in view of the fact that p16 IHC has been shown to decrease interobserver variability [11]. On the other hand, they rightfully warn that the clinical utility of p16 IHC is directly related to the performance characteristics of a specific clone. Full validation of the anti-p16 antibody is needed to ensure its specificity and sensitivity to the targeted p16 antigen. The large array of available antibodies, not to mention the lack of standardization of p16 scoring, may explain the wide variability in reported p16 positivity in preinvasive and invasive cervical lesions. On the one hand, if LSIL/CIN 1 is to be viewed as a transient self-limited and innocuous HPV effect one may wonder why the average p16 positivity is around 50% (range, 0% to 100%). LSIL/CIN 1 is either a precursor of CIN 2+ or it is not. On the other hand, if p16 is a surrogate marker of *integrated* HR-HPV then the p16-confirmed diagnosis of LSIL/CIN 1 must trigger on further management of cervical preinvasive lesions (i.e., "see-and-treat" in its broadest sense). On average, there is some correlation between p16 positivity and the degree of severity of cervical lesions. This correlates with the increase in viral DNA integration during the transition

from preinvasive into invasive lesions [6,7]. However, this does not tally with the fact that only 5% of integration was found in CIN 2 and that p16 has been reported to be overexpressed in 46% to 100% of CIN 2+ [6].

It has been shown repeatedly that between 18% and 44% of p16 overexpressing CIN 1 progressed to CIN 2+ in a much shorter time span (2.5 to 7.0 years) than p16 negative CIN 1. This casts doubt on the view that CIN 1/LSIL is innocuous and not potentially a preinvasive lesion provided that the value of p16 as a biomarker of progression is solidly established. This then raises also the question of the accuracy of p16 overexpression. The risk of overtreatment of HR-HPV positive preinvasive lesions needs to be averted through the ability to identify potentially progressive lesions. Unfortunately, the poor specificity of HR-HPV triage results in missing precursor lesions and the variable accuracy of p16 IHC may lead to overtreatment of lesions misdiagnosed as potentially progressive.

This leads to the conundrum that either histomorphology remains the "gold standard" in the diagnosis of preinvasive lesions or that it has lost its traditional stance. Giving priority to histomorphology runs the risk of missing occult lesions and unusual high-grade lesions that are picked up by IHC. Giving too much credit to p16 is also hazardous. There is limited but growing concern about the use of p16 as "solitary" marker for risk assessment of preinvasive lesions [12].

Other biomarkers have been tested, though not as extensively as p16. Ki-67, L1, and to a lesser extent involucrin, Cyclin E and ProExC have been tested as biomarkers of cervical preinvasive lesions. Ki-67 expresses the active phases of the cell cycle. ProExC is a cocktail of monoclonal antibodies against proteins associated with aberrant S phase cell cycle induction (i.e., topoisomerase II alpha, minichromosome maintenance protein 2). No really convincing benefit in risk assessment has been reported, except with L1. The HPV L1 capsid protein is associated with the productive or episomal phase of HPV infection where it does not alter the cell cycle and has a low, if any, malignant potential. The loss of L1 expression reflects the non-productive or integrated state of HPV where it alters the cell cycle and has a malignant potential [13-16]. In this regard, L1 expression appears to be a useful biomarker that expresses the risk of progression of preinvasive lesions. Literature data show that less than a third of preinvasive lesions expressing L1 do progress, and that more than two thirds of cases with loss of L1 expression do progress. The combination of p16 and L1 IHC looks promising but intriguing. For instance, Yoshida et al. [13] reported that 82.0% of LSIL exhibited a p16 (+)/L1 (-) pattern (almost the same as HSIL). This would mean that more than two thirds of LSIL would carry a risk of progression of similar magnitude to HSIL. If this were confirmed, the current

views on management of LSIL may need reconsideration.

The literature on p16 is vast and sometimes conflicting. Some of the reasons are the variety of monoclonal antibodies, and the lack of standardization of the scoring methods [17]. None the less, p16 IHC is useful in reducing interobserver variability and as a risk indicator of potential progression of intraepithelial neoplasia. Like p16 overexpression, loss of HPV L1 capsid expression has been shown to correlate with the severity of disease. The combination of HPV L1 capsid and p16 IHC appears to be more promising than any of the other single antibody IHC. The L1 (+)/p16 (+) and L1 (-)/p16 (-) profiles are puzzling since they cast some doubt on the validity of p16 as a biomarker of integrated HR-HPV. L1 (+) cases may still be in the productive phase (possibly regardless of p16 overexpression). This means that the community-based gynecological histopathologist needs to exert caution with the use of IHC and view it as “useful” but perhaps not yet as the “holy grail”.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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