The role of immunohistochemistry for p16Ink4 in the diagnosis and management of squamous lesions of the cervix

O papel da imunohistoquímica para p16Ink4 no diagnóstico e tratamento de lesões escamosas do colo do útero

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Over the past few years, the role of the overexpression of the $p16^{INK4a}$ protein, as a substitute marker for high-risk human papillomavirus (hrHPV) associated with the cervix, has been well established[1]. Missaoui et al.[2] demonstrated the role of overexpression of this protein as an additional useful marker for the interpretation of precancerous lesions during the evaluation of suspicious cervical biopsies.

$p16^{INK4a}$ (p16) is a tumor suppressor protein that plays a crucial role in regulating the cell cycle. Overexpression of p16 is considered a substitute marker for unregulated expression of E7, therefore identifying cells undifferentiated by the action of hrHPV. Many molecular markers of the disease can be based on the direct or indirect detection of the expression of the viral oncogenes E6 and E7 in basal keratinocytes transformed by hrHPV; however, only this protein (p16) has demonstrated great utility in clinical practice[3,4].

Immunohistochemistry assays for p16 show that areas with high-grade squamous intraepithelial lesions were identified in histological specimens, in which they had not initially been recognized only in sections stained with hematoxylin-eosin[4].

Eleutério Jr. et al.[5] demonstrated that p16 was detected by immunohistochemistry in the cervix in 92.3% of high-grade squamous intraepithelial lesions (HSIL), in 15.4% of low-grade squamous intraepithelial lesions (LSIL), and in no cases of normal tissues, thus better associated with HSIL than with the presence of hrHPV. It can, therefore, be a tool to identify HSIL and to help defining the diagnosis of inconclusive cases of squamous intraepithelial lesions and mimicking lesions.

This is even more important among young women (<24 years old) who may have mimicking lesions with atypical immature squamous metaplasia, which can be morphologically diagnosed as a HSIL, but in reality, when using p16, it is evident that the lesion has a high chance of regression and it is not pre-invasive[6].

Recently, new targets in animal models have been studied to determine the susceptibility of the squamous-columnar junction (SCJ) to HPV carcinogenesis. Cells, classified as junctional cells, were identified at the SCJ, having an immunophenotype shared with HSIL, adenocarcinoma *in situ*, invasive squamous cell carcinoma, and adenocarcinoma. Interestingly, all HSIL involved SCJ[7]. Thus, it is considered that the whole process of cervical oncogenesis begins there, with the persistent action of hrHPV.

According to Eleutério Jr. et al.[8], p16 expression, more clearly associated with the viral transforming mechanism, could be a marker that should enter the clinical practice in the diagnostic aid of HSIL, sometimes morphologically difficult to be differentiated from intense reparative pictures and minor injuries with important reactive changes, as well as in a probable identification of lesions with greater evolutionary potential for invasion.

More recently, the Lower Anogenital Squamous Terminology project[4], a group of task to better define the diagnostic approaches for squamous genital lesions, classified them as LSIL (non-pre-invasive) and HSIL (pre-invasive). Specifically, in the cervix, the lesions should be classified using p16 for specific conditions, according to Table 1.

It is important to clarify that unlike immunohistochemistry for p16, immunocytochemistry for p16/Ki67, or double staining, is a screening method to only identify women with equivocal cytology, as atypical squamous cells of undetermined significance (ASC-US), which need immediate colposcopy, due to an increased risk for HSIL[3,9].

Histopathological diagnosis directs the gynecologist's conduct in the treatment. Therefore, an accurate diagnosis is very important to trace the behavior of the lesion, using immunohistochemistry for $p16^{INK4a}$.

Table 1 – Approach for the histological diagnosis of squamous lesions of the uterine cervix.

Initial diagnosis	Immunohistochemistry for p16	Final diagnosis
CIN I	Not necessary	LSIL
CIN II	Necessary	Negative p16: LSIL Positive p16: HSIL
CIN III	Not necessary	HSIL
Mimetic lesion (immature squamous metaplasia with atypias)	Necessary	Negative p16: LSIL Positive p16: HSIL

CIN: cervical intraepithelial neoplasia; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion.

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