

The role of p16 as putative biomarker for cervical neoplasia: A controversial issue?

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INTRODUCTION: Protein p16 has been extensively studied as a potential biomarker for precursor lesions to distinguish cervical Intraepithelial neoplasia (CIN) from their mimics. However, the use of p16 as prognostic biomarker for diagnosis of cervical cancer and precancer is controversial. This study focuses on the assessment of peer-reviewed scientific data related to the use of p16 to predict disease severity and its controversies.

METHODS: We reviewed publications in MEDLINE/PubMed assessing the clinical, diagnostic and prognostic significance of p16 in CIN and cervical cancer; we included publications from 2009 to June 2017.

RESULTS: The use of p16 as a prognostic marker is still unreliable, although it could be a useful tool for diagnosis of Cervical Intraepithelial Neoplasia lesions with undetermined morphology. Moreover, p16 appears to be a specific marker of high-risk oncogenic HPV infection.

CONCLUSION: This review shows the potential utility and drawbacks of p16 for clinical practice and the diagnosis of cervical cancer. Further studies are required to substantiate the role of p16 in conjunction with other more sensitive and specific biomarkers for diagnosing CIN and predicting its progression.

KEYWORDS: p16 protein, HPV infection, cervical cancer, biomarker, cervical intraepithelial lesion.

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INTRODUCTION

Cervical cancer is the second most frequent type of cancer in women in underdeveloped regions;¹ in Brazil, it is the third most frequent cancer in the female population and a public health problem mostly affecting poor women without access to public health services.² The recent introduction of prophylactic HPV vaccination has been successfully implemented in several countries worldwide, although HPV vaccines, like all other ones, may not protect all vaccinated individuals, a reason why regular cancer screening programs must be carried out.³

Pap smear tests are of limited value due to the significant amount of false positive findings in current screening programs and by the high inter-observer variability of smear interpretation. This makes a strong case for the need of a more sensitive and specific test for improving cervical cancer screening and accurately diagnosing precancerous lesions.⁴ The use of biomarkers may improve the positive predictive value of Pap smears. This contribution, in turn, may decrease the incidence of cervical cancer, resulting in more adequate treatment and a lower morbidity associated with unnecessary interventions.

Protein biomarkers, such as p16, may be useful for detecting Cervical Intraepithelial Neoplasia (CIN) because its expression may be affected by P16 mutations

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or hypermethylation,^{5,6} this is the a reason why The Lower Anogenital Squamous Terminology Standardization Project for HPV- associated Lesions (LAST Project) suggested p16 staining as a preferred biomarker for cervical lesions.⁷ HPV oncogenic types associated with high grade lesions are characterized by overexpression of p16, which can be detected by immunohistochemistry.⁸ p16 expression is associated with the grade of cellular alteration observed in the squamous epithelium.⁸ The positivity of p16 in cervical intraepithelial neoplasia (CIN 1), although less frequent, could potentially indicate a high risk of progression,⁷ leading to the unequivocal diagnosis of CIN, a sexually transmitted disease with strong emotional implications for women.

However, even today, the use of the p16 protein as a prognostic biomarker of cervical cancer remains controversial. The present study conducted a review to describe the use of p16 to predict disease severity and as a tool for the definitive diagnosis of CIN.

■ MATERIALS, METHODS AND RESULTS

This study is a review using the PubMed/Medline database, with following keywords: [p16 AND prognostic diagnostic] AND [CIN] AND [cervical cancer] AND [p16]. The studies selected include cervical cancer, human papillomavirus and cervical intraepithelial neoplasia, published from April 2009 to June 2017 and in English language.

After analyzing the recovered references, ten articles related to the use of p16 as a possible prognostic biomarker were selected; five of them demonstrated a strong correlation with p16 overexpression as an indicator of the neoplasia development of high-grade lesions in cervical specimens with CIN 1 (low grade CIN). These findings are depicted in Table 1 and discussed below.

■ DISCUSSION

Several studies have reported p16 overexpression in cervical cancer as well as in high grade cervical intraepithelial neoplasia (CIN2-3),⁹⁻¹¹ although the clinical importance of these findings remains controversial. A meta-analysis showed that p16 overexpression in cervical cancer specimens appeared to increase overall or disease-free survival rates, indicating a more favorable prognosis.¹⁰ One report claimed that CIN 2 diagnosis could only be confirmed by p16 immunostaining¹² and another one showed a five-fold higher risk of cancer progression in positive p16 CIN 2 specimens than negative p16 CIN 2 which may spontaneously regress.¹³ Women have a range of approximately two years of stable CIN development and rarely progress to cancer.¹⁴

Additionally, p16 can be useful for identifying high grade lesion when histological features are equivocal but

cannot to differentiate between CIN 2 from CIN 3.¹⁵⁻¹⁷ These results suggest that monitoring women with CIN 2 diagnosis should be re-evaluated.

The strong association between the degree of dysplasia and p16 immunoreactivity in the CIN specimens could be explained by cervical malignant transformation associated with high-risk HPV infections due to high levels of the E7 viral oncogene.^{4,12} Cervical p16 staining with high-risk HPV types has been reported as strong, diffuse and located either in nuclei and/or cytoplasm compartments.¹⁸⁻²⁰ Differently, in infections caused by low-risk HPV types, the p16 immunostaining pattern displayed weak and focal staining, located in nuclei and cytoplasm of the intermediate and superficial layers only.¹¹

Amaro-Filho et al.⁹ observed p16 overexpression in invasive cervical cancer, with diffuse patterns and strong intensity, when compared to the control samples. Another study in CIN 1 showed that specimens with diffuse p16 immunostaining had higher chances of progression to CIN 2-3.¹¹

It has not been well established whether non-dysplastic epithelial cells can express the p16 protein. In certain physiological conditions, such as tissues from older women, which show shortening of the telomeres, p16 expression may alter the cell cycle and induce apoptosis. In this case, a focal pattern with weak intensity has been observed. In fact, the diffuse (non-focal) staining pattern, as opposed to the overall signal intensity might be the more important variable in the interpretation of p16.¹⁹ Moreover, a p16 positive reading in normal tissues has been suggested to represent background from immunohistochemistry methodology, which could be resolved by improving the technique, such as using a different enzyme horseradish peroxidase.¹⁹ Additionally, in HIV-1 positive specimens there is a potential reason for caution when interpreting the immunohistochemical staining of p16 in cervical lesions.²⁰

Razmpoosh et al.²¹ conducted a study matching the immunohistochemical staining with DNA-HPV data by polymerase chain reaction, and found that p16 expression with a diffuse staining pattern was associated with high-risk HPV. They suggested that cases with a p16/HPV ratio within the high-risk range should not be considered to have clinical value, because the mere presence of high risk HPV is a poor predictor of CIN 1. However, Mills et al.²² demonstrated that women with the diagnosis of CIN 1 with positive p16 that exhibited diffuse pattern had a higher risk of lesion progression after two years of diagnosis of CIN 1.

It is important to emphasize that for specimens negative for HPV and also negative for p16 the diagnosis of high-grade intraepithelial lesions (HSIL) should be very cautiously dealt with, in order to avoid unnecessary excisional procedures.²³

p16 utility in screening and in conjunction with other markers

The p16 protein can be used as an auxiliary complement for the screening of cases of low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells of undetermined significance (ASC-US) and with pap smear negative results.²³ Moreover, it has been strongly suggested that the p16 protein may be a useful tool to improve cytology and submit the colposcopy to a more detailed analysis.²³ Also, p16 staining can assist in the interpretation of results of Pap Smears or of histology in cases of ambiguous results and/or divergence between observers.²³ Although p16 has been shown to be a good indicator of undetermined cases, the use of this protein as a prognostic tool of lesion progression is restricted, since it is not possible to evaluate the complete lesion of the cervix. There are no widely accepted criteria for an immuno-histochemical staining study that evaluates whether the p16 stain is positive or negative, often causing the stain to be labelled as unspecific and the results to be classified as unreliable.¹⁴

Several studies have shown increased sensitivity and specificity for cervical cancer screening using p16 staining in conjunction with other biomarkers, such as p16/Ki-67^{15,16,24-26} and Survivin, the inhibitor of apoptosis.²⁷

Controversies

It is well known that cervical cancer is preceded by cervical intraepithelial neoplasia (CIN), and that high grade CINs show a greater potential of progressing to cervical cancer compared to low grade CINs. However, it is also recognized that the interpretation of cervical biopsies

is subjective and has a substantial degree of inter- and intra-observer variability. This can lead to both under- and over-reporting of clinically important cervical disease.¹² Previous studies of p16 immunohistochemistry in CIN 1 have suggested the likely utility of p16 in stratification of women at risk for subsequent CIN 2-3. But Ziemke et al¹⁷ have shown that those studies had limitations in statistical power, histologic diagnosis, and disease ascertainment. Moreover, it is still unclear whether p16 is suitable as a prognostic marker for low-grade lesions.¹⁷

Mills et al.²² recommend that p16 should only be used selectively for problematic scenarios, such as CIN 2 because of its inherent lack of reproducibility, especially in cases in which CIN 1 and CIN 2 are benign mimics of CIN3. Tsoumpou et al.¹² considered the reproducibility assays for p16 is still limited due to insufficient standardization and interpretation of the different immunoreactive stain. Furthermore, there is a level of heterogeneity related to the expression of p16 according to the different CIN grades and cervical cancer. In fact, Nuovo¹⁹ recently raised the point that false positive results in diagnostic immunopathology can lead to unnecessary treatment, emphasizing that a false positive level of p16 can be related to variables such as incorrect pretreatment and too concentrated primary antibody as well as the choice of the polymer with the conjugated horseradish peroxidase. Additionally, Liu et al.²⁸ described that the accuracy of ambiguous p16 immunoreactive stain in predicting high risk HPV and HSIL outcome is low, suggesting that specific guidelines for the ambiguous cases should prevent diagnostic errors and help implement p16 IHC in general practice.²⁸

Table 1- Main articles related to p16 expression

REFERENCES	NUMBER OF SAMPLES	HISTOLOGY	CONCLUSIONS
LIU et al, 2015 ²⁷	380	Normal/CIN 1,2,3 and SCC	p16 positively correlated with the grade of cervical diseases.
MILLS et al, 2015 ²²	524	CIN 1,2,3	p16 should only be used selectively for problematic scenarios, such as CIN2.
KRISHNAPPA et al, 2014 ¹¹	75	Cervicitis / in situ carcinoma	p16 may be useful to diagnose between the low- and high-grade cervix lesions.
ZHANG et al, 2015 ²³	46	Cervices Negative for high risk HPV	p16 may be a useful biomarker for CIN2-3
GUSTINUCCI et al, 2012 ²⁴	578	cythology with	p16 can be considered as a good biomarker
GENOVÉS et al, 2014 ¹³	86	CIN1,2,3	Negative p16 in cervical dysplasia does not always imply regression of the lesion
RAZMPOOSH et al, 2014 ²¹	64	CIN 1	P16 may be useful as prognostic biomarker for CIN1
PACCHIARIOTTI et al, 2013 ¹⁴	369	CIN 1	P16 may be useful as prognostic biomarker for CIN1 (with restrict utilization)
NICOL et al, 2012 ²⁶	326	CIN 2-3/tumor HIV+ and HIV -	P16 may be useful for CIN2-3 and tumor in well-defined situations
AMARO-FILHO et al, 2013 ⁹	130	TMA with cervicitis/normal cervix, CIN 2-3 and tumor	p16 was more expressed in advanced FIGO stages

■ CONCLUSIONS

The use of p16 as a biomarker could become an important tool for the diagnosis of cervical lesions with undetermined morphologies. Despite the clear application of p16 in the diagnostic definition of lesions with difficult morphological interpretation, the use of p16 as a prognosis marker is still a controversial issue and in countries such as the United States it is not typically used as a stand-alone test, but rather in conjunction with other tests such as Ki67. We strongly suggest that further studies are needed to substantiate the role of p16 in conjunction with other markers in large prospective trials in order to design rational and potent clinical prognostic biomarkers for CIN and cervical cancer evaluation.

■ CONFLICT OF INTEREST

The authors declare no conflict of interest.

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■ AUTHORS CONTRIBUTIONS

JESG, AFN (literature review and writing of the manuscript); CVA, GJN (revised and writing the manuscript); MOC, SMAF, FBR (revised the manuscript); AFN (conceived the review and revised the final version); GJN (edited the final version).

REVISÃO: O PAPEL DA PROTEÍNA p16 COMO MARCADOR PUTATIVO PARA NEOPLASIA CERVICAL: UM TEMA CONTROVERSO?

INTRODUÇÃO: A proteína p16 tem sido estudada como um biomarcador potencialmente específico de lesões cervicais precursoras e como uma forma de diferenciar as lesões parecidas com Neoplasia intra-epitelial cervical (NIC). Contudo existem várias controvérsias sobre a utilização de p16 como um biomarcador prognóstico e como uma ferramenta para o diagnóstico de câncer cervical e de lesões pré-câncer. O objetivo deste estudo foi a revisão de dados científicos por pares de bases, relacionados com a utilização da p16 e suas controvérsias.

MÉTODOS: O estudo foi projetado como uma revisão da literatura das publicações do Medline/PubMed que avaliam o significado clínico, diagnóstico ou prognóstico do p16 em lesões de NIC e no câncer cervical no período de janeiro de 2009 a junho de 2017.

RESULTADOS: o uso do p16 como um marcador prognóstico ainda não é confiável, apesar de que a p16 poderia ser uma ferramenta útil para o diagnóstico em lesões de NIC com morfologia indeterminada. Além disso, a p16 parece ser um marcador específico de infecção por HPV de alto risco oncogênico.

CONCLUSÃO: A presente revisão mostra a potencial utilidade da proteína p16, bem como os inconvenientes para uso clínico-patológico e diagnóstico no câncer cervical. Contudo são necessários mais estudos para fundamentar o papel da p16 em conjunto com os outros biomarcadores mais sensíveis e específicos para diagnosticar NIC e prever a sua progressão.

PALAVRAS CHAVES: proteína p16, HPV, câncer cervical, biomarcador, neoplasia intraepitelial cervical.

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