The role of p16^{INK4a} immunostaining in the risk assessment of women with LSIL cytology: a prospective pragmatic study

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Summary

Background: The detection of high-grade cervical intraepithelial neoplasia (CIN2 or worse) among patients with low-grade cytology (LSIL) is challenging. The aim of this study was to assess the efficacy of p16^{INK4a} in the risk assessment of women with LSIL cytology. *Methods:* Consecutive liquid-based cytology specimens of 95 LSIL smears were selected and stained for p16^{INK4a}. All patients had colposcopically directed punch biopsies or large loop excision of the transformation zone of the cervix. The endpoint was detection of a biopsy-confirmed CIN2 or worse. *Results:* The overall sensitivity and specificity of p16^{INK4a} for diagnosis of CIN2+ among LSIL smears were 41% and 86%, respectively. The positive predictive value of the biomarker was 62% and the negative predictive value 72%. *Conclusions:* The study shows that p16^{INK4a} has low sensitivity but acceptable specificity for evaluation of LSIL smears harbouring high-grade lesions. The marker needs to be further assessed as an adjunct to other tests in an attempt to improve the triage of LSIL cytology smears.

Key words: p16^{INK4a}; Immunostaining; Cervix; Liquid-based cytology; LSIL, CIN.

Introduction

The majority of low-grade intraepithelial lesions (LSIL) represent minor cellular changes due to acute or transient human papilomavirus (HPV) infection that tends to regress. However, there are lesions that harbour high-grade histology and are at risk of progressing to cervical cancer if left untreated [1].

The current management options for women presenting with LSIL are repeat cytology, and referral for colposcopic evaluation if the abnormality persists, or immediate referral to colposcopy, or finally, triage with high-risk HPV DNA (hr-HPV) testing. The main disadvantage of cytological surveillance only, is the risk of women defaulting on follow-up. On the other hand, women referred immediately to colposcopy are at risk of overtreatment with potential adverse pregnancy outcomes [2, 3]. High-risk HPV DNA testing has been shown to have a role in the triage of atypical squamous cells of undetermined significance (ASCUS) smears [4] however, its value in the triage of LSIL is limited [5]. The test's role and limitations were presented in a recent review that concluded that future research should focus on methods that will improve the test's specificity [6].

Numerous epidemiologic and molecular studies have demonstrated that hr-HPV genotypes are etiologic agents for the overwhelming majority of cases of invasive cervical squamous cell carcinoma [7, 8]. New biomarkers associated with infection by hr-HPV could be used to distinguish those cases that are at risk of progression. p16^{INK4a} is one of the biomarkers for transforming HPV infection because it accumulates in the nucleus and cytoplasm of affected cells and can be detected by immunostaining [9].

This present study aimed to assess the efficacy of p6^{INK4a} (p16) immunocytochemistry to predict a histological diagnosis of CIN2 or worse (CIN2+) in women with LSIL cytology.

Materials and Methods

This was a prospective diagnostic pragmatic study that assessed women referred to colposcopy for the first time, from October 2008 to February 2010, with a pap test or liquid-based cytology (LBC) sample of LSIL. We excluded women who had had treatment (loop excision of the transformation zone or cone biopsy of the cervix) in the past or had been previously reviewed in colposcopy for abnormal smears. We also excluded women who had had no cervical biopsies taken.

Eligible participants were referred to colposcopy with a sample showing LSIL in the context of cervical cancer screening and were included in the study after giving informed consent. A LBC specimen was obtained prior to the colposcopic examination for p16 immunostaining. A single experienced operator performed all colposcopies and was blinded to the results of the p16 immunostaining. Women with normal colposcopic impressions did not have biopsies taken and were referred for a repeat cytological and colposcopic assessment in

Revised manuscript accepted for publication July 6, 2010

six months. Women with high-grade colposcopy and those women with low-grade colposcopy who had completed their family or were too anxious to return just for repeat cytological and colposcopic surveillance, had either punch biopsies or loop excision of the transformation zone (LLETZ). The remaining women with low-grade colposcopy, after being fully informed about the options, were referred for repeat cytology and colposcopy in six months.

The primary outcome was the sensitivity of p16 to detect CIN2+ in a population of LSIL samples and it was defined prior to the start of the study.

p16 immunostaining was performed using the Dako CINtec cytology kit (Dako Cytomation, Glostrup, Denmark) according to the manufacturer's instructions. All slides were counterstained with hematoxylin (Dako Cytomation) to allow for assessment of the nucleus. We considered as positive p16 staining if at least one dysplastic cell was stained for the marker.

We correlated the accuracy parameters of p16 immunostaining such as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) to the study gold standard (colposcopically directed punch cervical biopsies or LLETZ biopsies), on the basis of detecting CIN2 or worse [10].

Results

A series of 95 women with LSIL cytology who underwent either punch [4] or LLETZ biopsies (91) at the Department of Colposcopy of the University Hospital of Ioannina in Ioannina, Greece were included during the period assessed.

The characteristics of the study population are shown in Table 1. All women had histological specimens taken after the colposcopic assessment. Among the 95 LSIL samples 34 (36%) harboured high-grade histology (CIN2+).

In Table 2 the correlation between the results of immunostaining for p16 and the histological diagnosis are presented. The vast majority of those within normal limits (WNL) and low-grade biopsies (CIN1, HPV) were negative for p16. The results were equivocal for CIN2+ biopsies as 58% of CIN2 lesions stained negative for the biomarker. Moreover, the number of CIN3 lesions that stained positive for p16 was similar with those non-stained for the marker (Table 2). We furthermore assessed the accuracy parameters for the value of p16 in detecting CIN2+ in LSIL cytology. As shown in Table 3, the marker had low sensitivity (41%) but better specificity (86%) for CIN2+ in LSIL smears.

Table 1. — *Patient characteristics*.

Variables	No. of subjects (%)
Age at study entry	
18-24	10 (10%)
25-35	50 (53%)
≥ 36	35 (37%)
Number of life time sex partners	
0-5	76 (80%)
≥ 6	19 (20%)
Current smoker	
Yes33 (35%)	
No	62 (65%)
Parity	
Nil	34 (36%)
≥ 1	61 (64%)

Table 2. — *p16 immunostaining for 95 patients with LSIL cytology*.

	Histology			
	WNL	CIN1, HPV	CIN2	CIN3
p16 (+)	2 (20%)	6 (12%)	9 (35%)	4 (44%)
p16 (-)	8 (80%)	41 (82%)	15 (58%)	4 (44%)
Inconclusive p16 staining	0 (0%)	3 (6%)	2 (7%)	1 (11%)
Total	10	50	26	9

CIN: cervical intraepithelial neoplasia, LSIL: low-grade squamous intraepithelial lesion, WNL: within normal limits.

Table 3. — *Accuracy parameters of p16 immunostaining for CIN2+ histology*.

	Sensitivity	Specificity	PPV	NPV
p16	41% (95% CI: 25% to 58%)	86% (95% CI: 75% to 92%)	62%	72%

NPV: negative predictive value, PPV: positive predictive value.

Discussion

Since hr-HPV is consistently associated with premalignant and malignant lesions of the uterine cervix, the use of molecular techniques to detect infection by hr-HPV has been proposed as a way to improve the results of conventional diagnostic strategies. The use of p16 marker has been suggested to compensate for the lack of specificity of the HPV DNA test and advocated as an adjunct tool for routine use [11]. Physiologically, p16 blocks the activity of cyclin-dependent kinases CDK4/6. In a transforming hr-HPV infection the viral oncogenes E6 and E7 interfere substantially with apoptosis and cell cycle regulation. Most importantly, E7 disrupts the protein of retinoblastoma (pRb) from its binding to E2F transcription factor and thereby promotes cell cycle progression, a molecular switch that is usually activated by CDK4/6. Affected cells strongly express p16 to counteract irregular cycle activation; however, since E2F is not released though CDK4/6 action, but by E7, p16 expression has no effect on cell cycle activation. Over time, p16 accumulates in the nucleus and cytoplasm of affected cells and can be detected by immunostaining [9].

Our work was an attempt to assess whether p16 immunostaining is useful for the evaluation of the malignant potential of LSIL smears. We conducted a pragmatic study choosing a primary outcome that is relevant to everyday life. The greatest strength of such a study is that it can deliver evidence of effectiveness in everyday clinical context.

There are several reports in the literature that assessed p16 in LSIL samples. The sensitivity of the marker to detect CIN2+ varies from 35% [12] to 100% [13] in different studies. In our study the sensitivity of the marker for detection of CIN2+ was low (41%) for a gain in specificity (86%). We have shown in a previous review that there are significant discrepancies among various authors on the interpretation of p16 immunostaining [14]. The discrepancies on the interpretation of p16 positivity, as

well as the variations on the population investigated by each study, add to the difficulty in assessing the clinical effectiveness of the biomarker.

Although the present study was based on a selected number of cases, it correlates p16 immunostaining to the gold standard, which is the histological diagnosis. Furthermore, the quality of the cells stained positive for the biomarker was assessed. p16 immunostaining was considered positive only if dysplastic cells stained for the marker, thus reducing the false-positive results.

Conclusion

The current study does not support the use of p16 as a single marker for the assessment of LSIL smears due to its low sensitivity. The biomarker could however have a place as an adjunct test to other markers in order to increase the specificity in the triage of LSIL cytology.

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