# Different patterns of p16 immunoreactivity in cervical biopsies: correlation to lesion grade and HPV detection, with a review of the literature

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**Summary**

p16 is one extensively studied marker in gynecological pathology. However, its routine application in the diagnosis of squamous intraepithelial lesions of the uterine cervix may present difficulties for the general pathologist. The aim of the present study was to examine a series of 100 cervical biopsies/LEEP specimens, with detailed HPV-typing, for patterns of p16 immunoreactivity and possible correlations with morphology and HPV types. Four patterns of immunopositivity were recognized, according to the distribution of positively stained cells, and these correlated to lesion grade. A review of the pertinent literature concerning p16 immunoreactivity in squamous intraepithelial lesions and nonneoplastic epithelia of the uterine cervix is included in an effort to summarize the existing data and the remaining questions at both the practical and theoretical level.

*Key words:* Cervix; CIN; HPV; Immunohistochemistry; p16; Patterns; Review; SIL.

## Introduction

The role of human papilloma virus (HPV) in squamous intraepithelial lesions of the uterine cervix has been investigated extensively in molecular studies, which revealed multiple interactions between HPV oncoproteins and their cellular targets. These result in alterations of cell cycle control and apoptosis [1-4]. Several associated markers have been investigated for their potential utility in assisting the histopathologic classification of preinvasive lesions and in facilitating the distinction from non HPV-induced alterations [5-8].

One extensively studied marker is p16, a cyclin-dependent kinase inhibitor, which affects pRb-mediated regulation of the G1/S transition [9-13]. p16 is strongly expressed in some normal tissues [14], while inactivation or overexpression has been reported in human neoplasms [15-17]. HPV-related precursor lesions are often associated with increased p16 expression. This is considered a result of functional inactivation of pRb by high-risk (HR) HPV E7 protein, affecting a negative transcriptional feedback loop [10-12]. A dramatic enhancement of p16 RNA level has been observed in vitro after immortalization by HPV16 or HPV18 [18] and correlation has been reported between HR-HPV oncogene expression and high scores of p16 positivity [19]. However, despite the presence of high levels of p16 in these lesions, its suppressor function is not normally exerted.

After a few initial reports concerning p16 status in cervical cancers and precancerous lesions [11, 20], several investigators have examined immunohistochemically the expression of p16 in cervical squamous intraepithelial lesions and its possible correlation with HR-HPV types and/or lesion "progression" [6, 7, 21-50]. Different criteria have been used for p16 immunoreactivity evaluation, with some authors reporting any type of immunostaining, some focusing only on diffuse immunoreactivity, and others reporting nuclear and cytoplasmic staining separately, as presented in the following.

In routine evaluation of cervical biopsies, often assisted by p16 immunostaining, we observed different patterns of reactivity which often could not be easily categorized. This led us to a different approach of staining patterns. The aim of the present study was to examine a series of cervical biopsies/LEEP specimens with detailed HPV-typing for patterns of p16 immunoreactivity and possible correlations with morphology and HPV types. A review of the pertinent literature concerning p16 immunoreactivity in biopsy specimens of cervical squamous intraepithelial lesions and nonneoplastic epithelia is included in the following discussion in an effort to summarize the existing data and the remaining questions at both the practical and theoretical level.

## Materials and Methods

The study included 100 specimens from 100 different patients. These specimens included 77 punch biopsies and 23 loop electrosurgical excision procedure (LEEP)/conization specimens retrieved from the archives of the Department of

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Pathology, University Hospital of Larissa, Thessalia, Greece. The samples were fixed in 10% buffered formalin solution, embedded in paraffin blocks and cut at 3 m sections.

The corresponding archived H&E slides were reviewed for the purpose of the study by two pathologists independently. In those cases where there was interobserver variation, a final consensus diagnosis was reached jointly. A prerequisite for every cervical biopsy to be included in the study was the availability of HPV testing with a PCR-based technique in order to verify the presence and/or the type(s) of HPV in the sample. We included 25 high-grade squamous intraepithelial lesions and 55 low-grade squamous intraepithelial lesions. These cases were classified according to previously published criteria [51, 52].

Additionally, we included in the study 20 cervical biopsies from 20 different patients without any diagnostic histopathologic abnormality. These specimens had only minor cytopathologic alterations and most of the cases had negative HPV testing results. Nonetheless, for a variety of unrelated lesions colposcopic biopsies had been obtained.

*Immunohistochemistry (IHC) for p16*

IHC for p16 was performed on deparaffinized 3 m sections in a commercially available automated immunostainer (Bond Max, Vision Biosystems, Australia). For antigen retrieval, Bond Epitope Retrieval Solution 2 (30 min, Vision BioSystems, Mount Waverley, Australia) was used. A monoclonal anti-p16 antibody (6H12, Novocastra, Newcastle upon Tyne, UK) was used at 1:100 dilution and binding of the primary antibody was assessed by the Bond Polymer Refine Detection (Vision Biosystems, Newcastle upon Tyne, UK), with DAB as a chromogen. A light hematoxylin counterstaining was used.

Negative control slides were processed similarly by omitting the primary antibody. Positive control slides were selected from known high-grade squamous intraepithelial lesions associated with high-risk HPV infection.

*Assessment of immunohistochemical staining*

All slides were initially evaluated by two pathologists, whose evaluations were conducted blindly and independently. During a subsequent joint evaluation, a final consensus immunoreactivity evaluation was obtained and used for further analysis.

The reaction was evaluated as positive if nuclear and/or cytoplasmic immunostaining was clearly demonstrated. Weak "blush" staining was not considered positive. After preliminary analysis of the findings, the pathologists involved in the evaluation of the immunohistochemical staining realized that the visualized immunoreactivity differences among various cases were best appreciated by categorizing the observed staining into four different patterns according to the extent of immunoreactivity: A, A-low, B, and C. These are presented schematically in Figure 1. Pattern A included occasional positive cells, dispersed or in small groups, usually above the parabasal layer. Pattern A-low was distinguished from pattern A by the presence of occasional positive cells, observed mainly in the lower epithelial layers, dispersed or in small groups. Pattern B consisted of diffuse positivity in the horizontal plane, which involved the basal, parabasal and intermediate layers, without extending to the upper third of the epithelium. Pattern C consisted of diffuse positivity in all epithelial layers.

For certain comparisons pattern A and pattern A-low were considered together as focal staining, while patterns B and C were considered together as diffuse staining for further analysis.

*HPV detection and typing*

DNA extraction was performed by using the Qiamp DNA mini kit (QIAGEN, Hilden, Germany), as described by the manufacturer. The quality of extracted DNA was assessed by spectrophotometry and DNA integrity for each sample was assessed by PCR amplification of the β-globin gene by aPCO4, GCB primers.

DNA was amplified under standard conditions with the L1 consensus HPV PGMY09/PGMY11 primer set, giving a PCR product of 450 bp. Digestion of PCR products by restriction enzymes (DdeI, BamHI, RsaI, PstI, HinfI, HaeIII, Sau3Al; New England Biolabs) and subsequent agarose gel electrophoresis allowed HPV genotyping.

Samples that were negative for PCR by the PGMY09/PGMY11 primers were checked for high and low risk HPV types (16, 18, 31, 33/6, 11, respectively), by commercial kits (Maxim Biotech, CA, U.S.A.). All the results were confirmed by Innolipa HPV genotyping kit (Innogenetics, Gent, Belgium).

*Statistical evaluation*

Statistical analyses were performed using the Statistical Package SPSS 13.0 for Windows (Chicago, USA); $p$ values < 0.05 were considered indicative of statistical significance.

## Results

Twenty-five high-grade squamous intraepithelial lesions, 55 low-grade squamous intraepithelial lesions, and 20 biopsies without diagnostic histopathologic abnormalities were included in the study (Table 1). The age of the patients based on the pathology reports varied from 17 to 58 years (mean 37.5 years).

Four patterns of immunopositivity were recognized according to the distribution of positively stained cells, as described in the previous section, and these correlated to lesion grade (Table 1). Nuclear as well as cytoplasmic immunoreactivity was usually observed. The different patterns are presented schematically in Figure 1, while representative examples of immunopositivity are presented in Figures 2-3. Overall, p16 positivity correlated to the presence of a squamous intraepithelial lesion ($p < 0.001$) and to the detection of HPV ($p < 0.001$). Sensitivity of p16 immunopositivity for the detection of SIL was 81.2% and specificity 85%.

Table 1. — *Immunoreactivity patterns of p16 in different groups of lesions*.

| | Pattern C | Pattern B | Pattern A-low | Pattern A | Negative | Total |
|---|---|---|---|---|---|---|
| HGSIL | 11 | 13 | 0 | 0 | 1 | 25 |
| LGSIL | 1 | 7 | 18 | 15 | 14 | 55 |
| Specimens negative for SIL | 0 | 1* | 2* | 0 | 17 | 20 |

*HPV(+).

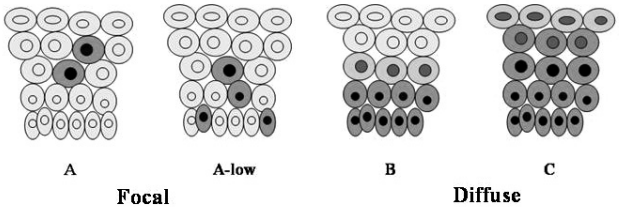
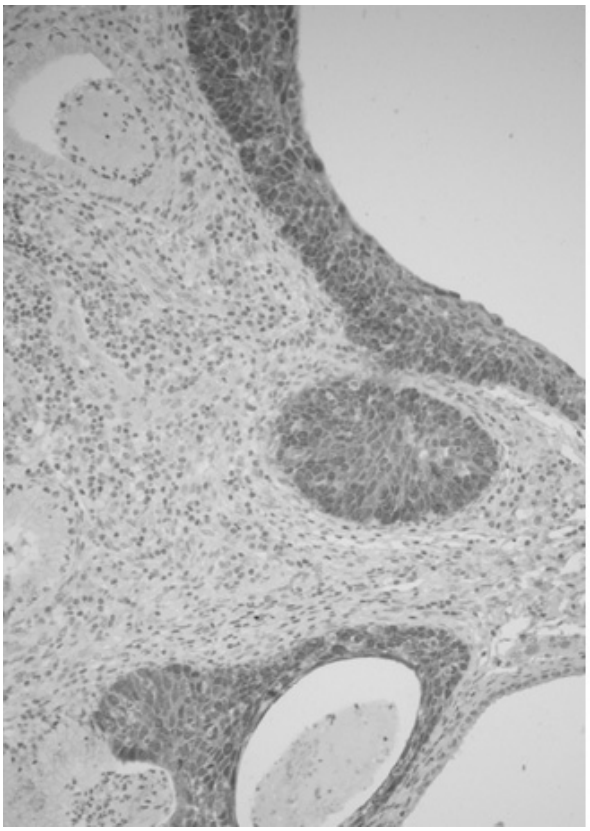



Figure 1. — Patterns of p16 immunoreactivity presented schematically.

Fig. 2a



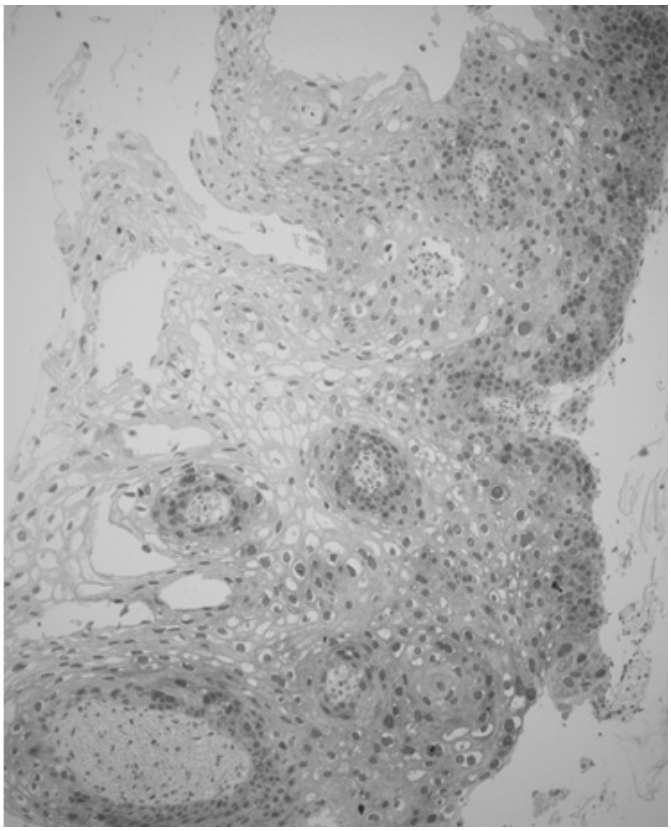


Fig. 2b

Figure 2 (a-b). — Pattern C positivity extending to the underlying glands in a LEEP specimen (a) and pattern B positivity in an exophytic lesion (b).

Table 2. — *HPV detection in different groups of cases*.

| | HPV detection | Most common types |
|---|---|---|
| HGSIL | 25/25 (100%) | 16, 31,18, 33, 58* |
| LGSIL | 51/55 (92.7%) | 16, 6/11, 53, 33, 45* |
| Specimens negative for SIL | 5/20 (25%) | 16, 18, 61, 33, 53 |

*In descending order of frequency.

*High-grade squamous intraepithelial lesions (HGSIL)*

Twenty-five lesions diagnosed as HGSIL were included in the study. All of them had a positive HPV test (100%). High-risk HPV types were detected in all cases, HPV 16 and HPV 31 being the most common types (Table 2).

Immunoreactivity for p16 (Figure 2a) was detected in 24 biopsies diagnosed as HGSIL (96%). Only patterns B and C were encountered.

*Low-grade squamous intraepithelial lesions (LGSIL)*

Fifty-five lesions diagnosed as LGSIL were included in the study. Fifty-one (92.7%) had a positive HPV test. HPV16 and HPV6/11 were the most common types, followed by HPV53, HPV33 and HPV45 (Table 2). Among HPV-positives, 70% of the cases tested for HPV type were associated with high- or probable high-risk virus types. Four cases with a negative HPV test exhibited p16 immunopositivity.

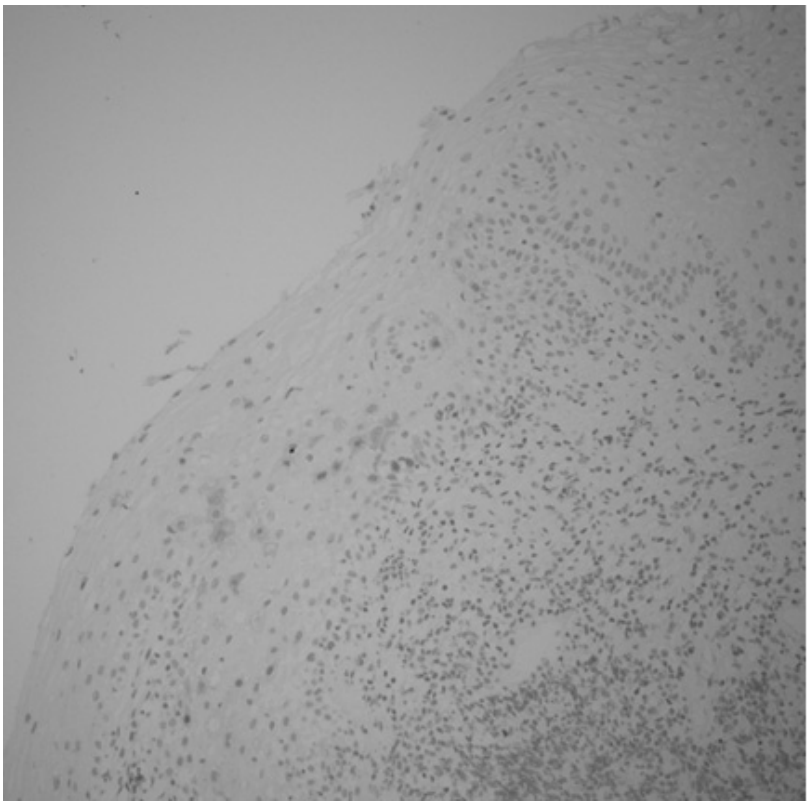
Immunoreactivity for p16 was detected in 41 biopsies diagnosed as LGSIL (74.5%). Pattern A-low was the most common (Table 1, Figure 3b), while pattern C was observed in only one case. This latter case and two of the cases exhibiting pattern B positivity were characterized by markedly increased nuclear dimensions in the upper epithelial layers in comparison to other cases. The percentage of high-risk or probable high-risk HPV associated lesions positive for p16 was 71.4% (25/35). This was not significantly different from immunopositivity observed in low-risk HPV associated lesions.

In cases with an A-low pattern of immunoreactivity HPV6/11 were the most common, followed by HPV16 and HPV53. In cases with pattern A immunoreactivity HPV16 was the most common. Cases with diffuse immunoreactivity (patterns B and C) were mostly associated with HPV types 31, 6/11, 58.

*Negative specimens*

Twenty biopsies considered negative for an HPV-associated squamous intraepithelial lesion on histopathologic examination, even on review, were included in the study. Five of these biopsies had a positive HPV test (25%).

Fig. 3a



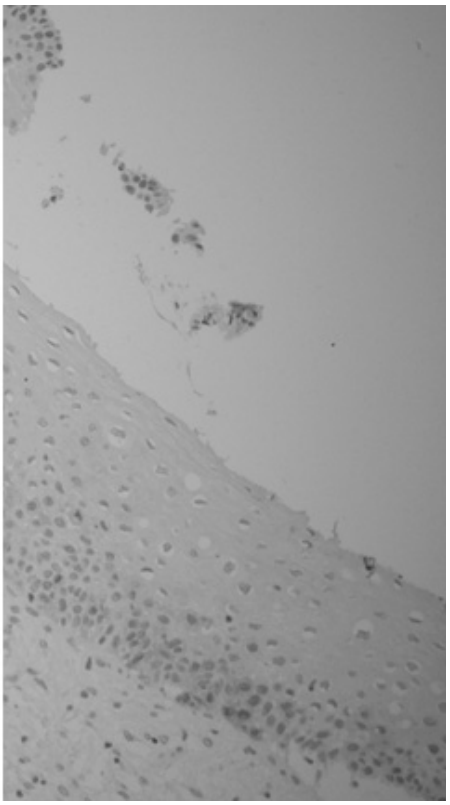


Fig. 3b

Figure 3 (a-b). — Specimens showing pattern A (a) and pattern A-low (b) immunoreactivity.

HPV types detected included 16, 18, 61, 33 and 53. The immunohistochemical stain for p16 was positive in only three of these cases (15%), with an A-low pattern of positivity in two cases and pattern B in one case associated with HPV 53. Careful review of this latter case showed that its histopathologic characteristics could be considered borderline for the diagnosis of a squamous intraepithelial lesion.

## Discussion

Our study, in concurrence with previously published studies, showed that p16 immunoreactivity is increased in cervical squamous intraepithelial lesions. High-grade lesions were characterized in all positive cases by diffuse immunoreactivity in the dysplastic cervical epithelium. Both these findings are in agreement with previous studies which are summarized in Tables 3 and 4.

P16 decelerates the cell cycle; it functions as a tumor suppressor by modulating the responses to hyperproliferative signals [53, 54] and has a role in cellular senescence [55]. The expression of p16 is altered in several human tumors by deletions, mutations, or methylation [15-17, 56], while germline mutation carriers are predisposed to a high risk of pancreatic and breast cancers [57]. Gene alterations have been also described in cervical carcinoma cases [58-62]. However, the increased expression observed in HPV-related intraepithelial squamous lesions is mainly attributed to the presence of a feedback loop, which depends on the status of pRb, and the well-known potential of HR-HPV E7 proteins to inactivate the latter [13, 19, 63-64]. pRb inactivation is a main action of E7 protein, but the interactions are probably influenced by several factors, as presented in the following.

E7 proteins of different HPV types differ in their efficiency for pRb binding and degradation [13, 62-63]. As a consequence, HPV type would be expected to influence the action of the feedback loop that results in increased p16 expression. Integration of the viral genome with associated loss of the inhibitory E2 action [65], might be another important factor in this cascade of events. In addition, alterations of the CDKN2A gene or its promoter(s) might occur in some intraepithelial lesions [59, 66]. Finally, other factors, related or not to the feedback mechanism, may affect the degree of overexpression [10]. As a result, despite the repeatedly reported correlation of p16 immunopositivity with detection of HPV and with detection of SIL, expectations concerning the discovery of a marker showing a positive immunoreaction in every squamous intraepithelial lesion or in every HR-HPV related lesion would probably not be fulfilled, especially considering the additional role of technical factors. A review of previously published studies summarized in Table 3, together with the results of our present study, reveal exactly these limitations, although they also point to spe-

Table 3. — *Positive p16 immunostaining in high- and low-grade squamous intraepithelial lesions reported in the literature.*

| Authors | Antibody used | Evaluation of staining | HGSIL positivity | LGSIL positivity | Non-neoplastic epithelia |
|---|---|---|---|---|---|
| Sano *et al*. 1998 [11] | JC8 | > 5% cells | 37/37 (100%) | 20/20 (100%) | negative |
| Keating *et al*. 2001 [6] | G175-405 (Pharmingen) | C | 34/37 (91.9%) | 21/24 (87.5%) | 3/24 (12.5%) |
| Klaes *et al*. 2002 [21] | E6H4 | Diffuse staining | 53/53 (100%) | 15/17 (88.2%) | 7/58 (12.1%) |
| Tsuda *et al*. 2003 [22] | Polyclonal (Pharmingen) | N ≥ 5% cells | 1/9 (11.1%) | 4/33 (12.1%) | |
| Agoff *et al*. 2003 [23] | E6H4 (MTM) | N and C ≥ 5% cells | 163/193 (84.5%) | 43/76 (56.6%) | 24/208 (11.5%) |
| Yoshida *et al*. 2004 [24] | JC8 (Neomarkers) | N and C | 36/37 (97.3%) | 3/8 (37.5%) | 3/38 SM (7.9%) |
| Wang *et al*. 2004 [25] | E6H4 (MTM) | Any immuno-reactivity | 36/38 (94.7%) | 54/75 (72%) | 19/58 (32.7%) |
| Branca *et al*. 2004 [26] | Polyclonal (Abcam) | N and/or C | 95/117 (81.2%) | 7/20 (35%) | 0/9 (0%) |
| Negri *et al*. 2004 [27] | CINtec p16 Histology Kit (DakoCytomation) | N and C ≥ 5% cells in lower third | 31/31 (100%) | 71/96 (74.7%) | ND |
| Tringler *et al*. 2004 [28] | 16PO4 (Neomarkers) | N and C | 46/46 (100%) | 13/18 (72.2%) | 7/108 (6.5%) |
| Volgareva *et al*. 2004 [29] | E6H4 (MTM) | N and/or C | 28/62 (45.2%) | 19/51 (37.2%) | 1/31 (3.2%) |
| Lorenzato *et al*. 2005 [7] | p16INK4A (Dako) | Any immuno-reactivity | 40/43 (93%) | 20/29 (68.9%) | 1/27 (3.7%) |
| Guimaraes *et al*. 2005 [30] | p16/Abs4 (Labvision) | N and C ≥ 1% cells | 13/18 (72.2%) | 15/26 (57.6%) [a] | ND |
| Murphy *et al*. 2005 [31] | p16 (Pharmingen) | N or C | 78/79 (98.7%) | 38/38 (100%) | 0/20 (0%) |
| Dray *et al*. 2005 [32] | JC8 (Biocare Medical) | N and/or C | 74/77 (96.1%) | 20/27 (74.1%) | 6/85 (7.0%) |
| Kalof *et al*. 2005 [33] | CINtec p16 Histology Kit (DakoCytomation) | N and/or C | 17/17 (100%) | 24/25 (96%) | Variable weak C-positivity in the lower half |
| Qiao *et al*. 2005 [34] | G175-405 (Pharmingen) | C Continuous | 16/16 (100%) | ND | 0/15[b] (0%) |
| Lin *et al*. 2005 [35] | E6H4 (MTM) | > 5% cells | 29/30 (96.7%) | | 0/20 (0%) |
| Benevolo *et al*. 2006 [36] | E6H4 (DakoCytomation) | N,C | 20/21 (95.2%) | 17/54 (31.5%) | 0/17 (0%) |
| Ishikawa *et al*. 2006 [37] | E6H4 (MTM) | Moderate and strong | 77/88 (87.5%) | 13/53 (24.5%) | 0/7 (0%) |
| Yildiz *et al*. 2007 [38] | CINtec p16 Histology Kit (DakoCytomation) | N±C | 20/20 (100%) | 12/15 (80%) | ND |
| Hariri and Oster 2007 [39] | p16 Histology Kit (Dako) | Continuous basal and parabasal | 49/49 (100%) | 65/91 (71.4%) | 3/50 (6%) |
| Van Niekerk *et al*. 2007 [40] | E6H4 (DakoCytomation) | N and C ≥ 5% cells in each layer | 124/128 (96.9%) | 32/56 (57.1%) | 50/218 (22.9%) |
| Regauer and Reich 2007 [41] | E6H4 (MTM) | Diffuse intense staining | 48/48 (100%) | 3/30 (10%) | 0/7 (0%) |
| Iaconis *et al*. 2007 [42] | P16 (MTM) | Moderate to strong N and C | 36/36 (100%) | | 1/23[c] (4.3%) |
| Kong *et al*. 2007 [43] | E6H4 (DakoCytomation) | N and/or C ≥ 5% cells | 16/16 (100%) | 11/12 (91.7%) | 2/30 (6.7%) |
| Eleuterio *et al*. 2007 [44] | E6H4 (DakoCytomation) | Moderate or diffuse N and C ≥ 10% cells | 12/13 (92.3%) | 4/26 (15.4%) | 0/57 (0%) |
| Focchi *et al*. 2007 [45] | Ab7 16PO7 (Neomarkers) | C and N ≥ 5% cells | 65/65 (100%) | 80/88 (90.9%) | 9/114 (7.9%) |
| Shi *et al*. 2007 [46] | P16 (Cell Marque) | N and C | 14/14 (100%) | 26/34 (76.5%) | 0/14 (0%) |
| Redman *et al*. 2008 [47] | JC8 (Dako) | N and C > 5% cells | ND | 30/81 (37%) | 0/110[d] (0%) |
| Ozgul *et al*. 2008 [48] | E6H4 | N,C | 22/22 (100%) | 6/13 (46.2%) | 3/25 (12%) |
| Pinto A *et al*. 2008 [49] | G175-405 (Pharmingen) | N and C | 51/61 (84%)[e] | | |
| Present study | 6H12 (Novocastra) | N and/or C | 24/25 (96%) | 41/55 (74.5%) | 3/20[e] (15%) |

N: nuclear; C: cytoplasmic; SM: squamous metaplasia; ND: no data.
[a] In the first biopsy; [b] Atrophy, with or without atypia; [c] HPV(+); [d] Including cases suspicious for HPV presence; [e] Mainly HSIL and diagnostically challenging cases.

cific applications of p16 IHC, which can be invaluable in the case of certain diagnostic dilemmas.

Table 3 summarizes the results of previously published studies, the antibodies and the evaluation criteria used. Positivity varied from 10% for low-grade squamous intraepithelial lesions to 100%. It is of note that, despite the undoubted influence of technical problems and geographic differences in HPV-type distribution, with increasing numbers of cases in each study there often appears a small group of HGSILs that do not show any immunoreactivity. In the three largest series [23, 40, 45] reporting more than 200 cases (SILs and controls) each, sensitivity of p16 for the detection of SIL varied from 76.6% to 94.8%, with a value of 83.7% calculated in the total number of their cases, while specificity varied from 77.1% to 92.1%, with a value of 84.6% calculated for the total number of cases. In the same studies the positive predictive value varied from 75.7% to 94.1%. In the present study sensitivity of p16 immunopositivity for the detection of SIL was 81.2% and specificity 85%, while the positive predictive value was 95.6%. The results point towards the use of p16 immunostain as a surrogate test in conjunction with histopathologic evaluation. Addition of a consecutive p16-stained slide to the HE-stained slides has been shown to significantly improve interobserver agreement for both punch and cone biopsies [21, 67].

By focusing only on diffuse immunopositivity, differently defined by different groups, the percentage of positively stained lesions varies, as summarized in Table 4. It has been suggested that this type of immunoreactivity is associated with integration of the viral genome, but there is still no direct proof of this [33]. The alternative explanation of monoclonality associated with other (epi)genetic alterations that might lead to p16 overexpression also lacks support. In our material diffuse positivity was observed in all p16-positive high-grade lesions, its sensitivity for HSIL being 96% and its specificity 88%. In the group of low-grade lesions there was no significant difference in HR-HPV detection between cases with or without diffuse positivity. Although this might be partly due to the

Table 4. — *Percentage of high- and low-grade squamous intraepithelial lesions showing diffuse p16-immunopositivity as reported in the literature.*

| Authors | Evaluation of diffuse staining | HGSIL | LGSIL | Non-neoplastic epithelia |
|---|---|---|---|---|
| Sano *et al.* [11] | > 80% | 100 | 65 | 0 |
| Keating *et al.* [6] | Continuous in the horizontal plane | 70.3 | 37.5 | 0 |
| Klaes *et al.* [21] | Diffuse staining | 100 | 88.2 | 12.1 |
| Agoff *et al.* [23] | > 75% | 57 | 11.8 | 3.8 |
| Yoshida *et al.* [24] | > 30% | 86.5 | 0 | 2.6 |
| Wang *et al.* [25] | Diffuse staining | 81.6 | 36 | 5.2 |
| Branca *et al.* [26] | Diffuse intense | 5.1 | 5 | 0 |
| Tringler *et al.* [28] | > 80% | 80.4 | 0 | 0 |
| Volgareva *et al.* [29] | > 25% | 6.5 | 0 | 0 |
| Guimaraes *et al.* [30] | > 30% | 33.3 | 26.9 [a] | ND |
| Murphy *et al.* [31] | > 50% | 55.7 [b] | 60.5 [b] | 0 |
| Dray *et al.* [32] | Diffuse, at least parabasal | 94.8 | 25.9 | 1.2 |
| Kalof *et al.* [33] | Diffuse, 2/3 to full thickness | 88.2 | 12 | 0 |
| Yildiz *et al.* [38] | Full thickness | 50 | 0 | ND |
| Hariri and Oster [39] | Continuous basal and parabasal | 100 | 71.4 | 6 |
| Regauer and Reich [41] | Diffuse staining | 100 | 10 | 0 |
| Kong *et al.* [43] | > 80% | 100 | 58.3 | 0 |
| Focchi *et al.* [45] | > 25% | 100 | 90.9 | 0 |
| Shi *et al.* [46] | > 50% | 92.8 | 64.7 | 0 |
| Redman *et al.* [47] | > 80% | ND | 23.4 | 0 |
| Ozgul *et al.* [48] | > /=25% | 77.3 | 15.4 | 0 |
| Present study | Patterns B+C | 96 | 14.5 | 5[c] |

[a]In the first biopsy; [b]All associated with high-risk or unknown HPV types; [c]HPV[+].

Table 5. — *Positive p16 immunostaining in HR-HPV positive LGSIL (tested by PCR or Hybrid Capture.*

| Authors | p16 positivity | Method of HR-HPV detection |
|---|---|---|
| Agoff *et al.* (23) | 6/7 (85.7%) | HC2 |
| | 25/30 (83.3%) | PCR |
| Wang *et al.* (25) | 36/44 (81.8%) | PCR |
| Kalof *et al.* (33) | 17/18 (94.4%) | PCR |
| Benevolo *et al.* (36) | 14/19 (73.6%) | PCR |
| Ishikawa *et al.* (37) | 12/37 (32.4%) | PCR |
| Van Niekerk *et al.* (40) | 28/42 (66.6%) | HC2 |
| Ordi *et al.* (50) | 39/50 (78%) | HC2 |
| Present study | 25/35 (71.4%) | PCR |

HC2: Hybrid Capture II HPV test.

small number of cases, it is noteworthy that in several studies presented in Table 5 a significant percentage of LGSILs associated with HR-HPV, as detected by PCR or HC2, does not exhibit any p16 immunopositivity.

Another aspect of p16 immunostaining is the possible correlation with lesion "progression". It has been suggested [6] that certain phases of a given HR-HPV-associated neoplastic process may have different indices of p16 expression. Increased p16 immunopositivity has been reported to correlate with progression to high-grade lesions [25, 27, 30]. In a more recent study evaluating diffuse p16 immunostaining the negative predictive value in predicting the outcome of CIN1 cases was as high as 96% [39]. In a study including conization specimens with coexisting CIN1 and CIN3 areas, all CIN1 were p16 positive [68]. p16 staining did not predict persistence or clearance of HR-HPV after treatment for CIN in a study by Branca *et al.* [26]. In our material follow-up information was limited and correlation to outcome was not possible.

An interesting finding of our study was the difference in HPV-type distribution between cases showing pattern A positivity and those showing pattern A-low, that is between two patterns of sporadic/focal positivity. To the best of our knowledge, this distinction has not been made in previous studies, although staining patterns corresponding to our patterns B and C have been described by different groups of investigators [34, 42, 43]. The above difference might reflect an earlier/increased sporadic expression of E7 in certain lesions, but also underlines a relative lack of recent studies correlating basic biological events and their morphologic appearances.

In summary, the results of our study support the use of p16 as an adjunctive test, in conjuction with careful morphologic evaluation. Although p16 immunohistochemistry has emerged in the last few years as a helpful, inexpensive test that might be used instead of HPV testing in diagnostically problematic biopsies, it lacks in most large studies 100% sensitivity or specificity. Awareness of its patterns of positivity and its limitations might allow for a most proper use in certain clinicopathological settings, aided by standardization of staining and evaluation protocols.

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