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p16^{INK4a} immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis

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Abstract

Background—P16^{INK4a} is a biomarker for transforming HPV infections that could act as an adjunct to current cytological and histological assessment of cervical smears and biopsies, allowing the identification of those women with ambiguous results that require referral to colposcopy and potentially treatment.

Material and Methods—We conducted a systematic review of all studies that evaluated the use of p16^{INK4a} in cytological or histological specimens from the uterine cervix. We also estimated the mean proportion of samples that were positive for p16^{INK4a} in cytology and histology, stratified by the grade of the lesion.

Results—Sixty-one studies were included. The proportion of cervical smears overexpressing p16^{INK4a} increased with the severity of cytological abnormality. Among normal smears, only 12% (95% CI: 7–17%) were positive for the biomarker compared to 45% of ASCUS and LSIL (95% CI: 35–54% and 37–57% respectively) and 89% of HSIL smears (95% CI: 84–95%). Similarly, in histology only 2% of normal biopsies (95% CI: 0.4–30%) and 38% of CIN1 (95% CI: 23–53%) showed diffuse staining for p16^{INK4a} compared to 68% of CIN2 (95% CI: 44–92%) and 82% of CIN3 (95% CI: 72–92%).

Conclusion—Although there is good evidence that p16^{INK4a} immunostaining correlates with the severity of cytological/histological abnormalities, the reproducibility is limited due to insufficiently standardized interpretation of the immunostaining. Therefore, a consensus needs to be reached regarding the evaluation of p16^{INK4a} staining and the biomarker needs to be evaluated in various clinical settings addressing specific clinical questions.

Keywords

cancer; dysplasia; SIL; CIN; cervix; p16; cyclin-dependent kinase

INTRODUCTION

Since the Papanicolaou (Pap-test) cytological screening for cervical precancerous lesions was introduced in the 1940s, there has been a significant reduction in the incidence and mortality from cervical cancer (1). However, the efficacy of the Pap test is hampered by high interobserver variability and high false negative and false positive rates that range between 20–30% (2) and 5–70% (3), respectively. Technical improvements of the Pap test such as the Liquid Based Cytology (LBC) have not been shown to improve sensitivity or specificity for detection of high-grade cervical intraepithelial neoplasia (CIN) compared to the conventional cytology (4).

The introduction of human papillomavirus (HPV) DNA testing in clinical practice raised hopes for further improvements in the efficacy of the primary screening, triage and post-treatment surveillance. Randomized clinical trials published recently have demonstrated that HPV testing can be efficiently integrated into primary screening, either as an adjunct to cytology or as a sole primary test (5,6). It has also been shown that HPV DNA testing can be used to triage women with equivocal cytological abnormalities (7) and that it has a potential role in identifying women at risk of residual or recurrent disease after treatment for CIN (8,9). However, it fails in the triage of low-grade lesions (9) and even if implemented as a primary screening test, it would be necessary to have a more disease specific triage marker to identify women that would need to undergo colposcopy. Furthermore, a single HPV DNA test although it could confirm infection by the virus, present in 99% of all cervical cancers (10), it does not discriminate between transient and chronic infection. The discrimination between the two types of infection is crucial as it is the persistent infection that predisposes to progression to cervical neoplasia and not the transient one (11).

Even the histological assessment of cervical biopsies that is often considered as the “gold standard”, can be significantly hampered by intra- and inter-observer variability (12). Novel markers applied on histological specimens could improve the identification of women with ambiguous results that require treatment.

Research nowadays is focused on the development of objective biomarkers that can distinguish transforming from productive HPV infections and predict disease severity. The cellular tumor suppressor protein p16^{INK4a} (p16) has been identified as a biomarker for transforming HPV infections. Physiologically, p16 blocks the activity of cyclin-dependent kinases CDK4/6. In a transforming 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 the irregular cell cycle activation; however, since E2F is not released through 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 (13).

This review represents an attempt to collect, systematically present and analyse the existing evidence on possible clinical applications of p16 in cytological and histological samples from the uterine cervix.

MATERIAL AND METHODS

Search Strategy

We searched two electronic databases - MEDLINE and EMBASE - targeting reports published between January 1998 and September 2007. The search strategy used terms such as “cancer”,

“dysplasia”, “SIL”, “CIN”, “cervix”, “p16” and “cyclin-dependent kinase”. The references of retrieved articles together with the proceedings of relevant conferences were hand-searched in order to identify other potentially eligible studies for inclusion in the analysis missed by the initial search or any unpublished data. Additional cross-searches were performed in MEDLINE using the names of investigators who were the lead authors of at least one eligible study.

The literature search, assessment of inclusion and exclusion criteria, quality of studies and extraction of data were independently undertaken and verified by two investigators (IT, MK). The results were then compared and, in case of discrepancies, a consensus was reached with the involvement of a third investigator (MA). There was no language restriction.

Type of Studies, Inclusion and Exclusion Criteria

All retrospective or prospective studies that assessed p16 immunostaining in cervical cytological samples, in conventional cytology or in LBC, as well as in histological specimens from the uterine cervix were included in this review. We evaluated all methods and interpretation of p16 immunostaining.

We excluded studies that assessed the expression of the biomarker in glandular or invasive cervical lesions. In cases of overlap or duplicate studies, we retained only the most comprehensive one.

Types of Outcome Measures

All outcomes were defined prior to the literature search. The primary outcome was the correlation between cytological or histological degree of cervical abnormality and overexpression of p16 identified by immunochemistry. Other parameters assessed were the role of the biomarker in the cervical cancer screening, its role in the triage of equivocal or low-grade cytological abnormalities compared to HPV testing and its efficacy as a marker of progression risk in low grade cervical lesions.

Data Extraction and Statistical Analyses

For all included studies we generated descriptive tables for population and study characteristics. We recorded the first author, publication year, country of the investigators, sample size and interventions. Furthermore, we described the method of p16 immunostaining, the type of antibody and medium used and the various interpretations of p16 immunoreactivity as adopted by each author.

We applied the 1991 Bethesda reporting system (TBS91) for the cytological classification (14). Three cytological groups were considered, i.e. atypical squamous cells of undetermined significance (ASCUS), low-grade (LSIL) and high-grade squamous intra-epithelial lesions (HSIL). If the cytological abnormalities were presented in different reporting formats, they were converted into TBS91 using published standard translation tables (15). The CIN nomenclature was applied in order to describe histological outcomes (16).

Statistical analysis was performed using STATA (Stata Corp., College Station, Texas, US). Random effects models were used to pool proportions for pooling (17) and analysis and interstudy heterogeneity was assessed with the Cochran's test (18).

In the meta-analysis of cytological studies we adopted the cut-off of p16 positivity proposed by each author, whilst for the analysis of histological studies we included in the meta-analytic pool only those that either adopted the distribution of staining proposed by Klaes and colleagues (19) or could be converted to the above classification system. According to the Klaes

classification the diffuse staining (>25% of cells stained for p16) was considered as the cut-off of positivity.

RESULTS

The electronic search yielded 584 studies that were assessed for inclusion in the review. Of those, 97 were potentially eligible and subsequently scrutinized in full text (Fig. 1).

Excluded Studies

Amongst the relevant studies, thirty-six failed to meet the inclusion criteria and were excluded from this overview. Nine of the studies assessed p16 immunostaining in glandular (20–28) and twenty-one in invasive cervical lesions only (29–49). Another six studies represented duplicate reports and were subsequently excluded (50–55) (Fig. 1).

Included Studies

Sixty-one studies qualified for the overview. Twenty-seven assessed the p16 immunoreactivity in cytological specimens (56–81) and in six amongst them (56,58,59,66,70,72) the biomarker was assessed in histological specimens as well, whereas 34 studies assessed staining only in histological samples (19,83–115). The characteristics of included studies are presented in Tables 1 and 2 respectively.

In cytology, the immunostaining for p16 was performed in selected series of smears of different degrees of cytological abnormality. Cervical smears were selected at random from a screening population in only one study (64); however only those women who were HPV tested were included so even in this study there was selection and disease enrichment.

Liquid based cytology was the preparation method adopted in 19 studies (56–59,61,62,64–66,69,71,73,75,77–82) and conventional cytology in eight (60,63,67,68,70,72,74,76). Immunostaining was performed in cell block preparations in two reports (61,79) and in one study the p16 stain was used with a Papanicolaou counterstain (74). Correlation between cytology, histology and p16 immunoreactivity was assessed in eight studies (62,65,70,74,77, 80–82) (Table 1).

The histological samples included punch, loop large excision of the transformation zone (LLETZ) or cone biopsies and hysterectomy specimens. In four studies (87,90,110,114) women were followed-up in time allowing an evaluation of the longitudinal predictive value of the biomarker (Table 2).

A variety of antibodies were used for immunodetection of p16: CINtec p16^{INK4a} Cytology Kit (clone E6H4, Dako Cytomation, Glostrup, Denmark), clone G175–405 (PharMingen, San Diego, CA, USA), clone 16P07 (Neomarkers, Fremont, CA, USA), Biosource (Camarillo, CA, USA), clone JC8 (Biocare, Boston, MA, USA), clone 6H12 (Novocastra, Newcastle upon Tyne, UK), clone F-12 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), clone #7962 (Abcam, Cambridge, UK), clone SC468 (Santa Cruz, CA, USA). The method of staining was similar amongst cytological (conventional or LBC) and histological specimens, with small alterations indicated by each manufacturer.

P16 positivity in cytological specimens was determined according to the distribution of staining into the cell compartments (cytoplasm or nucleus) and the number of cells overexpressing the biomarker. The cut-offs of positivity varied substantially across the studies. Quantitative criteria (i.e. the number of stained cells) were evaluated in 12 reports (57,60,61,63,67,68,70, 72,75,78,79,81), whereas both quantitative and qualitative parameters (i.e. morphological cytological features) were assessed in nine studies (56,58,62,66,69,71,73,80,82) (Table 3). The

proportion of smears stained positive for p16 per type of cytological abnormality is presented in Table 3.

The majority of histological studies adopted the classification proposed by Klaes and colleagues (19) that scored the distribution of p16 positivity on a semiquantitative scale as follows: negative (<1% of the cells were positive), sporadic (isolated cells were positive, but <5%), focal (small cell clusters, but <25% of the cells were positive), diffuse (>25% of cells stained positive). In order to reduce the heterogeneity of the results, we converted alternative classifications of staining adopted by other studies into the Klaes classification (Table 4). However, this was not feasible in four studies (72,96,111,113). We considered as positive cut-off for p16 immunoreactivity the diffuse stain that includes both basal and parabasal cell layers and indicates hrHPV-induced transformation (13). The proportion of positive p16 biopsies per grade of histological lesion is presented in Table 4.

The biomarker was hardly evaluated in the triage of women with equivocal cytological results. Only four studies (62,70,80,81) allowed assessment of the accuracy of p16 staining and HPV testing with the hybrid capture 2 (HC2) assay Qiagen, city, state, USA) for an underlying CIN2 + in women with ASCUS (70,80) or LSIL (62,80) (Table 5). P16 in general showed lower or equal sensitivity but higher specificity compared to HC2. We could not assess the statistical parameters such as sensitivity and specificity of the biomarker in the fourth study (81).

In histology, the immunohistochemical expression of p16 as a marker of progression risk in low-grade dysplastic lesions of the cervix uteri was evaluated in four studies (87,90,110,114). Negri and colleagues (87) assessed the role of p16 in predicting CIN1 lesions that were likely to progress to CIN3 in a four year follow-up. The authors concluded that although p16 may be expressed in low-grade squamous lesions (CIN1) that undergo spontaneous regression, cases with diffuse staining (>25% cells stained) had a significantly higher tendency to progress to a high-grade lesion than p16-negative cases. Overall, 71.4% and 37.8% of p16-negative and diffusely positive CIN1 regressed at follow-up, respectively, whereas 28.6% and 62.2% negative and diffusely positive CIN1 lesions progressed to CIN3, respectively. In the study by Wang and colleagues (90) CIN1 lesions were followed-up for 5 to 7 years. The positive predictive value (PPV) of diffuse staining for p16 for CIN2+ was 39% and the negative predictive value (NPV) was 85%. Another study (110) demonstrated 96% sensitivity and 39% PPV of diffuse p16 immunostaining in detecting those CIN1 biopsies that could progress to CIN2 in a five year follow-up for a considerably high NPV (96%). Finally, higher risk of progression to CIN3 was observed in CIN2 lesions with diffuse staining for p16 in a 2 year follow-up in a study performed by Omori and colleagues (114).

Meta-analysis

Taking into consideration the aforementioned discrepancies in the interpretation of staining, we estimated and also presented graphically the mean proportion of samples that were positive for p16 in cytology and histology, stratified by the grade of the lesion (Fig. 2, 3).

The pooled average proportion of p16-positive smears increased with the severity of cytological abnormality (Fig. 2). Among normal smears, only 12% (95% CI: 7–17%) were positive for the biomarker compared to 45% of ASCUS (95% CI: 35–54%), 45% of LSIL (95% CI: 37–57%) and 89% of HSIL (95% CI: 84–95%) smears (Fig. 2).

Similarly, in histology, on average only 2% of normal biopsies (95% CI: 0.4–30%) and 38% of CIN1 (95% CI: 23–53%) showed diffuse staining for p16 (Klaes classification) compared to 68% of CIN2 (95% CI: 44–92%) and 82% of CIN3 (95% CI: 72–92%) (Fig. 3).

Results were very heterogeneous in all types of specimen and categories of epithelial neoplasia (p for Q test always <0.001). In particular in the intermediate categories, inter-study variation was extreme. The percentage of smears positive for p16 ranged from 10% (61) to 100% (59) for ASCUS and from 10% (71) to 86% (59) for LSIL (Fig. 2). Similarly, in histology the proportion of CIN1 biopsies exhibiting diffuse staining for p16 varied from 0% (66,93) to 100% (84) (Fig. 3). Only at both ends of the spectrum of lesions (normal specimen or HSIL/CIN3 lesions) the percentages tended to scatter, after exclusion of one or two outlying studies, within a more narrow range near the left and right border of the forest plots.

The meta-analysis was restricted only to the correlation between p16 immunoreactive samples and grade of intra-epithelial abnormality, on cytological or histological specimens, due to lack of adequate number of studies that explored other clinical parameters of p16 immunostaining.

DISCUSSION

The progress in the understanding of HPV- related cervical carcinogenesis promoted the evaluation of various biomarkers that could potentially improve the current methods of cervical cancer screening. The cyclin-dependent kinase inhibitor protein p16 is one of the most promising and most studied of these biomarkers.

Immunostaining for p16 can be easily applied in both cytology and histology specimens. However, assessment of its clinical applications is seriously hampered by lack of standardized methodology. Until now, despite several proposed evaluation strategies of p16 in both cytology and histology, there is no general consensus for establishing threshold values above which a sample becomes “p16-positive”. This is especially a problem in p16 cytology, since the histological criteria of p16 positivity (negative, focal, diffuse) are now widely accepted. Several features of p16 staining have not been analyzed systematically, e.g. nuclear versus cytoplasmic staining, or the intensity of staining. Currently these features are not considered relevant for the assessment of p16 staining by the majority of the authors.

As some non-dysplastic cells may also display p16 immunoreactivity additional criteria that discriminate p16 staining in abnormal cells from atrophic or metaplastic cells may increase specificity. In a recent study (71) a nuclear scoring (NS) system was introduced for p16 in LBC samples. By assessing the nuclear size and nuclear-cytoplasmic ratio as well as other nucleomorphic features, a four-tiered classification of nuclear abnormalities has been proposed to describe p16 positive squamous cells. By applying the NS on p16 positive cells the authors noticed a significant increase in specificity for the detection of CIN2+ as compared to calling every slide with any p16 positive cells positive (from 50% to 80%) (82). However, the morphological assessment of p16-stained cells may be difficult in some cases, thus training and experience are necessary (80). An ideal biomarker should avoid relying on subjective criteria as much as possible.

Taking into consideration the above described limitations in the interpretation of p16 staining, we attempted a meta-analysis of the available data in order to evaluate the correlation of the p16 immunostaining to the degree of cytological or histological abnormality. P16 is related to the severity of cervical neoplasia in both cytology and histology however, there is extremely wide variation of p16 positivity in equivocal and low grade squamous cervical abnormalities reflecting its lack of reproducibility in these categories (Fig. 2, 3).

None of the studies assessed the role of p16 in primary screening for cervical cancer. Although in the study by Sahebali and colleagues (64) cervical smears were randomly obtained from women who underwent routine cervical cancer screening, only those who were tested for HPV were included, so even in this study there was selection and disease enrichment.

Furthermore, the limited number of studies assessing the role of p16 in the triage of LSIL and ASCUS smears versus HC2 (62,70,80,81), combined with the lack of consensus over the interpretation of p16 positivity, hampered our attempt to assess whether the biomarker could outperform the viral testing in these diagnostic categories.

Finally, there is some evidence that diffuse p16 immunostaining in histological specimens could be a predictor of disease progression (87,90,110,114) identifying those low grade lesions (CIN1) that need more intensive follow-up. However, the number of studies is limited.

CONCLUSION

The very large majority of studies on p16 immunostaining focus on the correlation between the biomarker and the degree of cytological or histological abnormality. Only a few address specific clinical questions such as the role of p16 in primary cervical cancer screening, in the triage of equivocal or low-grade smears versus the HPV-DNA test and its role as a marker of progression risk in low-grade dysplastic lesions of the cervix uteri. Furthermore, the discrepancies in the interpretation of p16 immunostaining in both cytology and histology reduce its reproducibility and hamper the interpretation of the data across different studies.

The available evidence does not allow us to formulate any definite recommendations regarding the applications of p16 in clinical practice. The standardization of sample preparation and evaluation is of paramount importance. After a consensus is reached regarding the interpretation of staining, the biomarker needs to be evaluated in various clinical settings addressing specific clinical questions. A good and meaningful study would require a rigorous colposcopy-biopsy workup (with multiple biopsies taken) and at least a two year follow up to detect disease missed in initial colposcopy/biopsy.

REFERENCES

1. Ostor AG. Natural history of cervical intraepithelial neoplasia: a critical review. *Int J Gynecol Pathol* 1993;12:186–92. [PubMed: 8463044]
2. Fahey MT, Irwig L, Macaskill P. Meta-analysis of Pap test accuracy. *Am J Epidemiol* 1995;141:680–9. [PubMed: 7702044]
3. Sherman ME, Schiffman MH, Lorinez AT, Manos MM, Scott DR, Kuman RJ, et al. Toward objective quality assurance in cervical cytopathology: Correlation of cytopathologic diagnoses with detection of high-risk human papillomavirus types. *Am J Clin Pathol* 1994;102:182–7. [PubMed: 8042586]
4. Arbyn M, Bergeron C, Klinkhamer P, Martin-Hirsch P, Siebers AG, Bulten J. Liquid compared with conventional cervical cytology. A systematic review and meta-analysis. *Obstet Gynecol* 2008;111:167–77. [PubMed: 18165406]
5. Naucler P, Ryd W, Törnberg S, Strand A, Wadell G, Elfgrén K, et al. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. *N Engl J Med* 2007;357:1589–97. [PubMed: 17942872]
6. Ronco G, Segnan N, Giorgi-Rossi P, Zappa M, Casadei GP, Carozzi F, et al. Human papillomavirus testing and liquid-based cytology: results at recruitment from the new technologies for cervical cancer randomized controlled trial. *J Natl Cancer Inst* 2006;98:765–74. [PubMed: 16757701]
7. Arbyn M, Buntinx F, Van Ranst M, Paraskevaïdis E, Martin-Hirsch P, Dillner J. Virologic versus cytologic triage of women with equivocal Pap smears: a meta-analysis of the accuracy to detect high-grade intraepithelial neoplasia. *J Natl Cancer Inst* 2004;96:280–93. [PubMed: 14970277]
8. Paraskevaïdis E, Arbyn M, Sotiropoulos A, Diakomanolis E, Martin-Hirsch P, Koliopoulos G, et al. The role of HPV DNA testing in the follow-up period after treatment for CIN: a systematic review of the literature. *Cancer Treat Rev* 2004;30:205–11. [PubMed: 15023438]
9. Arbyn M, Paraskevaïdis E, Martin-Hirsch P, Prendiville W, Dillner J. Clinical utility of HPV-DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN: an update of pooled evidence. *Gynecol Oncol* 2005;99(3 Suppl 1):S7–11. [PubMed: 16154623]

10. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999;338:12–9. [PubMed: 10451482]
11. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. *N Eng J Med* 1998;338:423–8.
12. Stoler MH, Schiffman M. Atypical Squamous Cells of Undetermined Significance-Low-grade Squamous Intraepithelial Lesion Triage Study (ALTS) Group. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. *JAMA* 2001;285:1500–5. [PubMed: 11255427]
13. Wentzensen N, von Knebel Doeberitz M. Biomarkers in cervical cancer screening. *Dis Markers* 2007;23:315–30. [PubMed: 17627065]
14. Luff RD. The Bethesda system for reporting cervical/vaginal cytologic diagnoses: a report of the 1991 Bethesda workshop. *Hum Pathol* 1992;23:719–21. [PubMed: 1612572]
15. Herbert A, Bergeron C, Wiener H, Schenck U, Klinkhamer PJ, Bulten J, et al. European guidelines for quality assurance in cervical cancer screening: recommendations for cervical cytology terminology. *Cytopathology* 2007;18:213–9. [PubMed: 17635161]
16. Richard RM. Cervical intraepithelial neoplasia. *Pathol Annu* 1973;8:301–28. [PubMed: 4583016]
17. Dersimonian R, Laird NM. Meta-analysis in clinical trials. *Controlled Clin Trials* 1986;7:177–88. [PubMed: 3802833]
18. Cochran WG. The combination of estimates from different experiments. *Biometrics* 1954;10:101–29.
19. Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U, et al. Overexpression of p16INK4a as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. *Int J Cancer* 2001;92:276–84. [PubMed: 11291057]
20. Cameron RI, Maxwell P, Jenkins D, McGluggage WG. Immunohistochemical staining with MIB1, bcl2 and p16 assists in the distinction of cervical glandular intraepithelial neoplasia from tubo-endometrial metaplasia, endometriosis and microglandular hyperplasia. *Histopathology* 2002;41:313–21. [PubMed: 12383213]
21. Riethdorf L, Riethdorf S, Lee KR, Cviko A, Loning T, Crum C. Human papillomaviruses, expression of p16^{INK4a}, and early endocervical glandular neoplasia. *Hum Pathol* 2002;3:899–904. [PubMed: 12378514]
22. Negri G, Egarter-Vigl E, Kasal A, Romano F, Haitel A, Mian C. P16^{INK4a} is a useful marker for the diagnosis of adenocarcinoma of the cervix uteri and its precursors. *Am J Surg Pathol* 2003;27:187–93. [PubMed: 12548164]
23. Murphy N, Heffron CCBB, King B, Ganuguapati UG, Ring M, McGuinness E, et al. P16^{INK4a} positivity in benign, premalignant and malignant cervical glandular lesions: a potential diagnostic problem. *Virchows Archive* 2004;445:610–5.
24. Nieh S, Chen SF, Chu TY, Lai HC, Fu E. Expression of p16^{INK4a} in Pap Smears containing atypical glandular cells from the uterine cervix. *Acta Cytol* 2004;48:173–80. [PubMed: 15098536]
25. Tringler B, Gup C, Singh M, Groshong S, Shroyer L, Heinz D, et al. Evaluation of p16^{INK4a} and pRb expression in cervical squamous and glandular neoplasia. *Hum Pathol* 2004;35:659–95.
26. Chen SF, Yang SF, Chu TY, Lai HC, Lin YW, Bai CY, et al. Which test is a better strategy to determine the outcome of atypical glandular cell-categorized Pap smears? Immunocytochemical p16^{INK4a} expression or human papillomavirus test-a retrospective cohort study. *Gynecol Oncol* 2005;99:578–84. [PubMed: 16139346]
27. Pavlakis K, Messini I, Athanasiadou S, Kyrodinou E, Pandazopoulou A, Vrekoussis Th, et al. Endocervical glandular lesions: A diagnostic approach combining a semi-quantitative scoring method to the expression of CEA, MIB-1 and p16. *Gynecol Oncol* 2006;103:971–6. [PubMed: 16876235]
28. Liang J, Mittal KR, Wei JJ, Yee H, Chiriboga L, Shukla P. Utility of p16INK4a, CEA, Ki67, P53 and ER/PR in the differential diagnosis of benign, premalignant, and malignant glandular lesions of the uterine cervix and their relationship with Silverberg scoring system for endocervical glandular lesions. *Int J Gynecol Pathol* 2007;26:71–5. [PubMed: 17197900]

29. Kim JW, Namkoong SE, Ryu SW, Kim HS, Shin JW, Lee JM, et al. Absence of p15INK4B and p16INK4A gene alterations in primary cervical carcinoma tissues and cell lines with human papillomavirus infection. *Gynecol Oncol* 1998;70:75–9. [PubMed: 9698478]
30. Kim JR, Kim SY, Kim MJ, Kim JH. Alterations of CDKN2 (MTS1/p16INK4A) gene in paraffin-embedded tumor tissues of human stomach, lung, cervix and liver cancers. *Exp Mol Med* 1998;30:109–14. [PubMed: 9873831]
31. Munirajan AK, Kannan K, Bhuvarahamurthy V, Ishida I, Fujinaga K, Tsuchida N, et al. The status of human papillomavirus and tumor suppressor genes p53 and p16 in carcinomas of uterine cervix from India. *Gynecol Oncol* 1998;69:205–9. [PubMed: 9648588]
32. Wong YF, Chung TK, Cheung TH, Nobori T, Yu AL, Yu J, et al. Methylation of p16INK4A in primary gynecologic malignancy. *Cancer Lett* 1999;136:231–5. [PubMed: 10355753]
33. Alfsen GC, Reed W, Sandstad B, Kristensen GB, Abeler VM. The prognostic impact of cyclin dependent kinase inhibitors p21WAF1, p27Kip1, and p16INK4/MTS1 in adenocarcinomas of the uterine cervix: an immunohistochemical evaluation of expression patterns in population-based material from 142 patients with international federation of gynecology and obstetrics stage I and II adenocarcinoma. *Cancer* 2003;98:1880–9. [PubMed: 14584070]
34. Ishikawa M, Fujii T, Masumoto N, Saito M, Mukai M, Nindl I, et al. Correlation of p16INK4A overexpression with human papillomavirus infection in cervical adenocarcinomas. *Int J Gynecol Pathol* 2003;22:378–85. [PubMed: 14501820]
35. Masumoto N, Fujii T, Ishikawa M, Saito M, Iwata T, Fukuchi T, et al. P16 overexpression and human papillomavirus infection in small cell carcinoma of the uterine cervix. *Hum Pathol* 2003;34:778–83. [PubMed: 14506638]
36. McCluggage WG, Jenkins D. p16 immunoreactivity may assist in the distinction between endometrial and endocervical adenocarcinoma. *Int J Gynecol Pathol* 2003;22:231–5. [PubMed: 12819388]
37. Negri G, Egarter-Vigl E, Kasal A, Romano F, Haitel A, Mian C. p16INK4a is a useful marker for the diagnosis of adenocarcinoma of the cervix uteri and its precursors: an immunohistochemical study with immunocytochemical correlations. *Am J Surg Pathol* 2003;27:187–93. [PubMed: 12548164]
38. Tripathi A, Banerjee S, Roy A, Roychowdhury S, Panda CK. Alterations of the P16 gene in uterine cervical carcinoma from Indian patients. *Int J Gynecol Cancer* 2003;13:472–9. [PubMed: 12911724]
39. van de Putte G, Holm R, Lie AK, Tropé CG, Kristensen GB. Expression of p27, p21, and p16 protein in early squamous cervical cancer and its relation to prognosis. *Gynecol Oncol* 2003;89:140–7. [PubMed: 12694668]
40. Zielinski GD, Snijders PJ, Rozendaal L, Daalmeijer NF, Risse EK, Voorhorst FJ, et al. The presence of high-risk HPV combined with specific p53 and p16INK4a expression patterns points to high-risk HPV as the main causative agent for adenocarcinoma in situ and adenocarcinoma of the cervix. *J Pathol* 2003;201:535–43. [PubMed: 14648656]
41. Ansari-Lari MA, Staebler A, Zaino RJ, Shah KV, Ronnett BM. Distinction of endocervical and endometrial adenocarcinomas: immunohistochemical p16 expression correlated with human papillomavirus (HPV) DNA detection. *Am J Surg Pathol* 2004;28:160–7. [PubMed: 15043304]
42. Kanao H, Enomoto T, Ueda Y, Fujita M, Nakashima R, Ueno Y, et al. Correlation between p14(ARF)/p16(INK4A) expression and HPV infection in uterine cervical cancer. *Cancer Lett* 2004;213:31–7. [PubMed: 15312681]
43. Li W, Thompson CH, Cossart YE, O'Brien CJ, Liu J, Scolyer RA, et al. The site of infection and ethnicity of the patient influence the biological pathways to HPV-induced mucosal cancer. *Mod Pathol* 2004;17:1031–7. [PubMed: 15143339]
44. Schorge JO, Lea JS, Elias KJ, Rajanbabu R, Coleman RL, Miller DS, et al. P16 as a molecular biomarker of cervical adenocarcinoma. *Am J Obstet Gynecol* 2004;190:668–73. [PubMed: 15041997]
45. Parwani AV, Smith Sehdev AE, Kurman RJ, Ronnett BM. Cervical adenoid basal tumors comprised of adenoid basal epithelioma associated with various types of invasive carcinoma: clinicopathologic features, human papillomavirus DNA detection, and P16 expression. *Hum Pathol* 2005;36:82–90. [PubMed: 15712186]

46. Horn LC, Lindner K, Szepankiewicz G, Edelmann J, Hentschel B, Tannapfel A, et al. p16, p14, p53, and cyclin D1 expression and HPV analysis in small cell carcinomas of the uterine cervix. *Int J Gynecol Pathol* 2006;25:182–6. [PubMed: 16633070]
47. Missaoui N, Hmissa S, Frappart L, Trabelsi A, Ben Abdelkader A, et al. p16INK4A overexpression and HPV infection in uterine cervix adenocarcinoma. *Virchows Arch* 2006;448:597–603. [PubMed: 16496173]
48. Ivanova TA, Golovina DA, Zavalishina LE, Volgareva GM, Katargin AN, Andreeva YY, et al. Up-regulation of expression and lack of 5' CpG island hypermethylation of p16 INK4a in HPV-positive cervical carcinomas. *BMC Cancer* 2007;7:47. [PubMed: 17359536]
49. Li C, Rock KL, Woda BA, Jiang Z, Fraire AE, Dresser K. IMP3 is a novel biomarker for adenocarcinoma in situ of the uterine cervix: an immunohistochemical study in comparison with p16^{INK4a} expression. *Mod Pathol* 2007;20:242–7. [PubMed: 17192788]
50. Klaes R, Benner A, Friedrich T, Ridder R, Herringotn S, Jenkins D, et al. P16INK4a immunohistochemisrty improves interobserver agreement in the diagnosis of cervical intraepithelial neoplasia. *Am J Surg Pathol* 2002;26:1389–99. [PubMed: 12409715]
51. Nieh S, Chen SF, Chu TY, Lai HC, Fu E. Expression of p16^{INK4a} in Papanicolaou smears containing atypical squamous cells of undetermined significance from the uterine cervix. *Gynecol Oncol* 2003;91:201–8. [PubMed: 14529682]
52. Murphy N, Ring M, Heffron CCBB, King B, Killalea AG, Hughes C. P16^{INK4a}, CDC6, and MCM5: predictive biomarkers in cervical preinvasive neoplasia and cervical cancer. *J Clin Pathol* 2005;58:525–34. [PubMed: 15858126]
53. Wang JL, Zheng BY, Li XD, Nokelainen K, Angstrom T, Lindstrom MS, et al. P16INK4a and p14ARF expression pattern by immunohistochemistry in human papillomavirus-related cervical neoplasia. *Mod Pathol* 2005;18:629–37. [PubMed: 15502810]
54. Bergeron C, Wentzensen N, Cas F, von Knebel Doeberitz M. La proteine p16^{INK4a} : un marqueur cytologique pour detecter les neoplasies intraepitheliales de haut grade du col uterin. *Ann Pathol* 2006;26:397–402. [PubMed: 17255929]
55. Carozzi FM, Cecchini S, Confortini M, Becattini V, Cariaggi MP, Pontenani G, et al. Role of p16^{INK4a} expression in identifying CIN2 or more severe lesions among HPV-positive patients referred for colposcopy after abnormal cytology. *Cancer* 2006;108:119–23. [PubMed: 1641180]
56. Bibbo M, Klump WJ, DeCecco J, Kovatich A. Procedure for immunocytochemical detection of p16^{INK4a} antigen in Thin-Layer, liquid-based specimens. *Acta Cytol* 2002;46:25–9. [PubMed: 11843554]
57. Saqi A, Pasha TL, McGrath CM, Yu GH, Zhang P, Gupta P. Overexpression of p16^{INK4a} in Liquid-Based specimens (SurePath™) as marker of cervical dysplasia and neoplasia. *Diagn Cytopathol* 2002;27:365–70. [PubMed: 12451568]
58. Bibbo M, DeCecco J, Kovatich A. P16^{INK4a} as an adjunct test in Liquid-Based cytology. *Anal Quant Cytol Histol* 2003;25:8–11. [PubMed: 12630076]
59. Murphy N, Ring M, Killalea AG, Uhlmann V, O'Donovan M, Mulcahy F, et al. P16^{INK4a} as a marker for cervical dyskaryosis: CIN and cGIN in cervical biopsies and ThinPrep™ smears. *J Clin Pathol* 2003;56:56–63. [PubMed: 12499437]
60. Pientong C, Ekalaksananan T, Swadpanich U, Kongyingyoes B, Kritpetcharat O, Yuenyao P, et al. Immunocytochemical detection of p16^{INK4a} protein in scraped cervical cells. *Acta Cytol* 2003;47:616–23. [PubMed: 12920756]
61. Akpolat I, Smith DA, Ramzy I, Chirala M, Mody R. The utility of p16INK4a and Ki-67 staining on cell blocks prepared from residual thin-layer cervicovaginal material. *Cancer* 2004;102:142–9. [PubMed: 15211472]
62. Guo M, Hu L, Baliga M, He Z, Hughson MD. The predictive value of p16^{INK4a} and Hybrid Capture 2 Human Papillomavirus testing for High-Grade cervical intraepithelial neoplasia. *Am J Clin Pathol* 2004;122:894–901. [PubMed: 15539381]
63. Pientong C, Ekalaksananan T, Kongyingyoes B, Kritpetcharat O, Swadpanich U, Pengsa P, et al. Immunocytochemical staining of p16^{INK4a} protein from conventional Pap test and its association with Human Papillomavirus infection. *Diagn Cytopathol* 2004;31:235–42. [PubMed: 15452898]

64. Sahebali S, Depuydt C, Segers K, Moeneclaey LM, Vereecken AJ, van Marck E, et al. P16^{INK4a} as an adjunct marker in Liquid-Based Cervical Cytology. *Int J Cancer* 2004;108:871–76. [PubMed: 14712490]
65. Trunk M, Dallenbach-Hellweg G, Ridder R, Petry KU, Ikenberg H, Schneider V, et al. Morphologic characteristics of p16^{INK4a}-positive cells in cervical cytology samples. *Acta Cytol* 2004;48:771–82. [PubMed: 15581161]
66. Yoshida T, Fukuda T, Sano T, Kanuma T, Owada N, Nakajima T. Usefulness of Liquid-Based Cytology specimens for the immunocytochemical study of p16 expression and Human Papillomavirus testing. *Cancer* 2004;102:100–8. [PubMed: 15098254]
67. Bose S, Evans H, Lantzy L, Scharre K, Youssef E. P16^{INK4a} is a surrogate biomarker for a subset of Human Papilloma Virus-associated dysplasias of the uterine cervix as determined on the Pap Smear. *Diagn Cytopathol* 2005;32:21–4. [PubMed: 15584047]
68. Filho AL, Utagawa ML, Shirata NK, Prereira SM, Namiyama GM, Kanamura CT, et al. Immunocytochemical expression of p16^{INK45a} and Ki-67 in cytologically negative and equivocal Pap smears positive for oncogenic human papillomavirus. *Int J Gynecol Pathol* 2005;24:118–24. [PubMed: 15782067]
69. Moore GD, Lear SC, Wills-Frank LA, Martin AW, Snyder JW, Helm WC. Differential expression of cdk inhibitors p16, p21cip1, p27kip1, and Cyclin E in cervical cytological smears prepared by the ThinPrep method. *Diagn Cytopathol* 2005;32:82–7. [PubMed: 15637682]
70. Nieh S, Chen SF, Chu TY, Lai HC, Lin YS, Fu E, et al. Is p16^{INK4a} expression more useful than human papillomavirus test to determine the outcome of atypical squamous cells of undetermined significance-categorized Pap smears? A comparative analysis using abnormal cervical smears with follow-up biopsies. *Gynecol Oncol* 2005;97:35–40. [PubMed: 15790434]
71. Wentzensen N, Bergeron C, Cas F, Eschenbach D, Vinokurova S, von Knebel Doeberitz M. Evaluation of a nuclear score for p16^{INK4a}-stained cervical squamous cells in liquid-based cytology samples. *Cancer* 2005;105:461–7. [PubMed: 16116604]
72. Ekalaksananan T, Pientong C, Sriamporn S, Kongyingyoes B, Pengsa P, Kleeckaow P, et al. Usefulness of combining testing for p16 protein and human papillomavirus (HPV) in cervical carcinoma screening. *Gynecol Oncol* 2006;103:62–6. [PubMed: 16494930]
73. Holladay EB, Logan S, Arnold J, Knessel B, Smith DG. A comparison of the clinical utility of p16^{INK4a} immunolocalization with the presence of human papillomavirus by hybrid capture 2 for the detection of cervical dysplasia/neoplasia. *Cancer* 2006;108:451–61. [PubMed: 17078096]
74. Negri G, Moretto G, Menia E, Vittadello F, Kasal A, Mian C, et al. Immunocytochemistry of p16^{INK4a} in liquid-based cervicovaginal specimens with modified Papanicolaou counterstaining. *J Clin Pathol* 2006;59:827–30. [PubMed: 16467166]
75. Sahebali S, Depuydt C, Boulet G, Arbyn M, Moeneclaey LM, Vereecken AJ, et al. Immunocytochemistry in liquid-based cervical cytology: analysis of clinical use following a cross-sectional study. *Int. J Cancer* 2006;118:1254–60. [PubMed: 16161049]
76. Bolanca I, Ciglar S. Evaluation of p16INK4a in cervical lesion of premenopausal and postmenopausal women. *Coll. Antropol* 2007;(Suppl 2):107–11.
77. Carozzi FM. Combined analysis of HPV DNA and p16^{INK4a} expression to predict prognosis in ASCUS and LSIL Pap smears. *Coll. Antropol* 2007;(Suppl 2):103–6. [PubMed: 17598511]
78. Carydis VB, Walker T, Wing A, Colgan TJ. Utility of p16 (ink4a) immunocytochemistry in liquid-based cytology specimens from women treated for high-grade squamous intraepithelial lesions. *Acta Cytol* 2007;51:517–22. [PubMed: 17718114]
79. Liu H, Shi J, Wilkerson M, Huang Y, Meschter S, Dupree W, et al. Immunohistochemical detection of p16INK4a in liquid-based cytology specimens on cell block sections. *Cancer* 2007;111:74–82. [PubMed: 17330305]
80. Meyer JL, Hanlon DW, Andersen BT, Rasmussen OF, Bisgaard K. Evaluation of p16^{INK4a} expression in ThinPrep cervical specimens with the CINtec p16^{INK4a} assay. *Cancer* 2007;111:83–92. [PubMed: 17334990]
81. Monsonego J, Pollini G, Evrard MJ, Sednaoui P, Monfort L, Quinzat D, et al. P16^{INK4a} immunocytochemistry in liquid-based cytology samples in equivocal Pap smears. *Acta Cytol* 2007;51:755–66. [PubMed: 17910346]

82. Wentzensen N, Bergeron C, Cas F, Vinokurova S, von Knebel Doeberitz M. Triage of women with ASCUS and LSIL cytology. *Cancer* 2007;111:58–66. [PubMed: 17186505]
83. Keating JT, Cviko A, Riethdorf S, Riethdorf L, Quade B, Sun D, et al. Ki-67, cyclin E, and p16^{INK4a} are complimentary surrogate biomarkers for human papilloma virus-related cervical neoplasia. *Am J Surg Pathol* 2001;25:884–91. [PubMed: 11420459]
84. Sano T, Oyama T, Kashiwabara K, Fukuda T, Nakajima T. Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. *Am J Pathol* 1998;153:1741–8. [PubMed: 9846965]
85. Agoff SN, Lin P, Morihara J, Mao C, Kiviat NB, Koutsky LA. P16^{INK4a} expression correlates with degree of cervical neoplasia: a comparison with Ki-67 expression and detection of high-risk HPV types. *Mod Pathol* 2003;16:665–73. [PubMed: 12861062]
86. Branca M, Ciotti M, Santini D, Di Bonito L, Giorgi C, Benedetto A, et al. P16^{INK4a} expression is related to grade of CIN and high-risk human papillomavirus but does not predict virus clearance after conization or disease outcome. *Intl J Gynecol Pathol* 2004;23:354–65.
87. Negri G, Vittadello F, Romano F, Kasal A, Rivasi F, Girlando S, et al. p16^{INK4a} expression and progression risk of low-grade intraepithelial neoplasia of the cervix uteri. *Virchows Arch* 2004;445:616–20. [PubMed: 15480761]
88. Volgareva G, Zavalishina L, Andreeva Y, Frank G, Krutikova E, Golovina D, et al. Protein p16 as a marker of dysplastic and neoplastic alterations in cervical epithelial cells. *BMC Cancer* 2004;4:58. [PubMed: 15339339]
89. Wang JL, Zheng BY, Li XD, Angstrom T, Lindstrom MS, Wallin KL. Predictive significance of the alterations of p16^{INK4a}, p14ARF, p53, and proliferating cell nuclear expression in the progression of cervical cancer. *Clin Cancer Res* 2004;10:2407–14. [PubMed: 15073118]
90. Wang SS, Trunk M, Schiffman M, Herreo R, Sherman M, Burk R, et al. Validation of p16^{INK4a} as a marker of oncogenic human papillomavirus infection in cervical biopsies form a population-based cohort in Costa Rica. *Cancer Epidemiology Biomarkers Prev* 2004;23:1355–60.
91. Aoyama C, Liu P, Ostrzega N, Holschneider CH. Histologic and immunohistochemical characteristics of neoplastic and non-neoplastic subgroups of atypical squamous lesions of the uterine cervix. *Am J Clin Pathol* 2005;123:699–706. [PubMed: 15981809]
92. Dray M, Russell P, Dalrymple C, Wallman N, Angus G, Leong A, et al. P16^{INK4a} as a complementary marker of high-grade intraepithelial lesions of the uterine cervix. I: Experience with squamous lesions in 189 consecutive cervical biopsies. *Pathology* 2005;37:112–24. [PubMed: 16028838]
93. Guimaraes M, Goncalves M, Soares C, Bettini J, Duarte R, Soares E. Immunohistochemical expression of p16^{INK4a} and bcl-2 according to HPV type and to the progression of cervical squamous intraepithelial lesions. *J Histochem Cytochem* 2005;53:509–16. [PubMed: 15805425]
94. Hu L, Guo M, He Z, Thornton J, McDaniel LS, Hughson MD. Human papillomavirus genotyping and p16^{INK4a} expression in cervical intraepithelial neoplasia of adolescents. *Mod Pathol* 2005;18:267–73. [PubMed: 15492761]
95. Kalof AN, Evans MF, Simons-Arnold L, Beatty BG, Cooper K. P16^{INK4a} immunoexpression and HPV In Situ hybridization signal patterns. *Am J Surg Pathol* 2005;29:674–9. [PubMed: 15832093]
96. Kim NR, Lin Z, Kim KR, Cho HY, Kim I. Epstein-Barr virus and p16^{INK4A} methylation in squamous cell carcinoma and precancerous lesions of the cervix uteri. *J Korean Med Sci* 2005;20:636–42. [PubMed: 16100457]
97. Lin Z, Gao M, Zhang X, Kim YS, Lee ES, Kim HK, et al. The hypermethylation and protein expression of p16^{INK4a} and DNA repair gene O⁶-methylguanine-DNA methyltransferase in various uterine cervical lesions. *J Cancer Res Clin Oncol* 2005;151:364–70. [PubMed: 15785933]
98. Lorenzato M, Caudroy S, Bronner C, Evrard G, Simon M, Durlach A, et al. Cell cycle and/or proliferation markers: what is the best method to discriminate cervical high-grade lesions? *Hum Pathol* 2005;36:1101–7. [PubMed: 16226110]
99. Qiao X, Bhuiya TA, Spitzer M. Differentiating high-grade cervical intraepithelial lesion from atrophy in postmenopausal women using Ki-67, cyclin E, and p16 immunohistochemical analysis. *J Low Genit Tract Dis* 2005;9:100–7. [PubMed: 15870531]

100. Andersson S, Wangsa D, Flores-Staino C, Safari H, Mints M, Hjerpe A, et al. Expression of p16INK4a in relation to histopathology and viral load of “high-risk” HPV types in cervical neoplastic lesions. *Eur J Cancer* 2006;42:2815–20. [PubMed: 16973350]
101. Benevolo M, Mottotese M, Marandino F, Vocaturo G, Sindico R, Piperno G, et al. Immunohistochemical expression of p16^{INK4a} is predictive of HR-HPV infection in cervical low-grade lesions. *Mod Pathol* 2006;19:384–91. [PubMed: 16415792]
102. Bulten J, van der Avoort I, Melchers W, Massuger L, Grefte J, Hanselaar A, et al. P14^{ARF} and p16^{INK4a}, two products of the same gene, are differently expressed in cervical intraepithelial neoplasia. *Gynecol Oncol* 2006;101:487–94. [PubMed: 16406113]
103. Ishikawa M, Fujii T, Saito M, Nindl I, Ono A, Kubushiro K, et al. Overexpression of p16^{INK4a} as an indicator for human papillomavirus oncogenic activity in cervical squamous neoplasia. *Int J Gynecol Cancer* 2006;16:347–53. [PubMed: 16445657]
104. Lambert AP, Anschau F, Schmitt V. P16^{INK4a} expression in cervical premalignant and malignant lesions. *Exp Mol Pathol* 2006;80:192–6. [PubMed: 16257403]
105. Queiroz C, Silva TC, Alves VA, Villa LL, Costa MC, Travassos AG, et al. P16^{INK4a} expression as a potential prognostic marker in cervical pre-neoplastic and neoplastic lesions. *Pathol Res Pract* 2006;202:77–83. [PubMed: 16376485]
106. Zhang Q, Kuhn L, Denny L, De Souza M, Taylor S, Wright T. Impact of utilizing p16INK4a immunohistochemistry on estimated performance of three cervical cancer screening test. *Int. J. Cancer* 2006;120:351–6. [PubMed: 17066437]
107. Bahnassy AA, Zekri AR, Saleh M, Lotayef M, Moneir M, Shawki O. The possible role of cell cycle regulators in multistep process of HPV-associated cervical carcinoma. *BMC Clin Pathol* 2007;7:4. [PubMed: 17521451]
108. Eleuterio J, Giraldo P, Goncalves A, Cavalcante D, Ferreira F, Mesquita S, et al. Prognostic markers of high-grade squamous intraepithelial lesions: the role of p16INK4a and high-risk human papillomavirus. *Acta Obstet Gynecol Scand* 2007;86:94–8. [PubMed: 17230296]
109. Focchi G, Silva I, Nogueira-de-Souza N, Dobo C, Oshima C, Stavale J. Immunohistochemical expression of p16(INK4A) in normal uterine cervix, nonneoplastic epithelial lesions, and low-grade squamous intraepithelial lesions. *J Low Genit Tract Dis* 2007;11:98–104. [PubMed: 17415114]
110. Hariri J, Oster A. The negative predictive value of p16INK4a to assess the outcome of cervical intraepithelial neoplasia 1 in the uterine cervix. *Int J Gynecol Pathol* 2007;26:223–8. [PubMed: 17581402]
111. Iaconis L, Hyjek E, Ellenson LH, Pirog EC. P16 and Ki-67 immunostaining in Atypical Immature Squamous Metaplasia of the uterine cervix. Correlation with human papillomavirus detection. *Arch Pathol Lab Med* 2007;131:1343–9. [PubMed: 17824788]
112. Kong CS, Balzer BL, Troxell ML, Patterson BK, Longacre TA. P16^{INK4a} immunohistochemistry is superior to HPV in situ hybridization for the detection of high-risk HPV in atypical squamous metaplasia. *Am J Surg Pathol* 2007;31:33–43. [PubMed: 17197917]
113. Nam EJ, Kim JW, Sang WK, Kim YT, Kim JH, Yoon BS, et al. The expression of the pRb pathway in cervical intraepithelial neoplasia; predictive and prognostic significance. *Gynecol Oncol* 2007;104:207–11. [PubMed: 17046054]
114. Omori M, Hashi A, Nakazawa K, Yuminamochi T, Yamane T, Hirara S, et al. Estimation of prognoses for Cervical Intraepithelial Neoplasia 2 by p16^{INK4a} immunoexpression and High-Risk HPV In Situ Hybridization signal types. *Am J Clin Pathol* 2007;128:208–17. [PubMed: 17638654]
115. Yildiz I, Usututun A, Firat P, Ayhan A, Kucukali T. Efficiency of immunohistochemical p16 expression and HPV typing in cervical squamous intraepithelial lesion grading and review of the p16 literature. *Pathol Res Pract* 2007;203:445–9. [PubMed: 17543474]

Figure 1. Flowchart of study selection

Figure 2. Meta-analysis of the proportion of samples stained positive for p16 per cytological category
WNL: within normal limits, **ASCUS:** atypical cells of undetermined significance, **LSIL:** low-grade intraepithelial lesion, **HSIL:** high-grade squamous intraepithelial lesion

Figure 3. Meta-analysis of the proportion of biopsies stained positive for p16 per grade of cervical lesion

CIN: cervical intraepithelial neoplasia

Table 1

Characteristics of cytological studies

Study	Country	Setting/Population	Sample size	C/H correlation	Medium	p16 antibody
Bibbo 2002	USA	ND	47	No	LBC	E6H4
Saiki 2002	USA	ND	40	No	LBC	Neomarkers
Bibbo 2003	USA	ND	30	No	LBC	E6H4
Murphy 2003	Ireland	ND	27	No	LBC	Clone G175-405 PharMingen
Pientong 2003	Thailand	Routine screening	150	No	CP	Biosource
Akpolat 2004	USA	ND	77	No	LBC- Cell blocks	Neomarkers
Guo 2004	USA	Routine screening	210	Yes	LBC	Novocastra 6H12
Pientong 2004	Thailand	Routine screening	165	No	CP	Biosource
Sahebali 2004	Belgium	Routine screening	291	No	LBC	E6H4
Trunk 2004	Germany	ND	90	Yes	LBC	E6H4
Yoshida 2004	Japan	Abnormal smears	98	No	LBC	Neomarkers
Bose 2005	USA	ND	107	No	CP	E6H4
Filho 2005	Brazil	Routine screening	199	No	CP	E6H4
Moore 2005	USA	ND	34	No	LBC	Biosciences
Nieh 2005	China	ND	66	Yes	CP	E6H4
Wentzensen 2005	Germany	ND	210	No	LBC	E6H4
Ekakaksananan 2006	Thailand	Abnormal smears	186	No	CP	ND
Holladay 2006	USA	ND	400	No	LBC	E6H4
Nesri 2006	Italy	ND	81	Yes	CP	E6H4
Sahebali 2006	Belgium	Routine screening	200	No	LBC	E6H4
Bollanca 2007	Croatia	Routine screening	137	No	CP	E6H4
Carozzi 2007	Italy	Abnormal smears	283	Yes	LBC	E6H4
Caradis 2007	Canada	Abnormal smears	46	No	LBC	E6H4
Liu 2007	USA	ND	75	No	LBC- Cell blocks	16P04
Meyer 2007	USA	ND	394	Yes	LBC	E6H4
Monsonogo 2007	France	Abnormal smears	248	Yes	LBC	E6H4
Wentzensen 2007	Germany	ND	225	Yes	LBC	E6H4

C/H: cytology/histology, CP: conventional Pap-test, ND: not documented

Table 2

Characteristics of histological studies

Study	Country	Setting/Population	Material	Sample size	Follow-up	p16 antibody
Keating 2001	USA	ND	Punch/cone biopsies Endocervical curettage	85	No	GI75-405 PharMingmin
Klaes 2001	Germany	ND	ND	310	No	E6H4
Bibbo 2002	USA	ND	ND	47	No	E6H4
Sano 2002	Japan	ND	ND	64	No	JC8
Agoff 2003	USA	Routine screening	Punch / LLETZ biopsies	569	No	E6H4
Bibbo 2003	USA	ND	ND	30	No	E6H4
Murphy 2003	Ireland	ND	ND	153	No	PharMingmin
Branca 2004	Italy	ND	Biopsies NOS/Hysterectomy	292	No	polyclonal Ab #7962
Nesri 2004	Italy	ND	ND	96	Yes	E6H4
Volgareva 2004	Russia	ND	ND	197	No	E6H4
Wang JL 2004	Sweden	Pt who developed cervical cancer	ND	40	No	Monoclonal F-12
Wang S 2004	Germany	Costa Rica cohort study	Punch/LLETZ /cone biopsies	171	Yes	E6H4
Yoshida 2004	Japan	ND	ND	98	No	Neomarkers
Aoyama 2005	USA	ND	ND	37	No	PharMingmin
Drav 2005	Australia	ND	Punch biopsies	189	No	JC8
Guimaraes 2005	Brazil	ND	Biopsies NOS	28	No	Freemont
Hu 2005	USA	Adolescents	Punch biopsies	142	No	Novocastra
Kalof 2005	USA	ND	Punch / LLETZ biopsies	39	No	E6H4
Kim 2005	Korea	ND	ND	82	No	SC 468
Lin 2005	Korea	ND	ND	117	No	E6H4
Lorenzato 2005	France	hrHPV (+) samples	Punch biopsies	99	No	E6H4
Nieh 2005	China	ASCUS smears	Punch biopsies	66	No	E6H4
Qiao 2005	USA	Postmenopausal women	Punch / cone biopsies/Hysterectomy	31	No	PharMingmin
Andersson 2006	Sweden	ND	ND	80	No	E6H4
Benevolo 2006	Italy	ND	ND	86	No	E6H4
Bullen 2006	The Netherlands	ND	ND	39	No	Neomarkers
Ekalaksananan 2006	Thailand	ND	Punch biopsies	39	No	ND
Ishikawa 2006	Japan	ND	ND	164	No	E6H4
Lambert 2006	Brazil	ND	Biopsies NOS	54	No	Novocastra
Queiroz 2006	Brazil	ND	Biopsies NOS	60	No	E6H4
Zhang 2006	USA	Routine screening	Punch / LLETZ biopsies	329	No	Novocastra
Bahnassy 2007	Egypt	Abnormal smears	Punch / LLETZ biopsies	130	No	ND
Eleuterio 2007	Brazil	ND	ND	96	No	E6H4
Focchi 2007	Brazil	ND	Biopsies NOS/Hysterectomy	314	No	Neomarkers
Hariri 2007	Denmark	ND	Punch / LLETZ biopsies Cervical curettage / Hysterectomy	200	Yes	K5336 Dako
Iaconis 2007	USA	ND	ND	96	No	E6H4
Kong 2007	USA	ND	Punch / LLETZ biopsies Endocervical curettage/Hysterectomy	28	No	E6H4
Nam 2007	Korea	ND	Punch / cone biopsies	256	No	E6H4
Omori 2007	Japan	ND	Biopsies NOS	107	Yes	E6H4
Yildiz 2007	Turkey	ND	LLETZ	35	No	E6H4

Ab: antibody, hrHPV: high-risk HPV, LLETZ: large loop excision of transformation zone, ND: not documented, NOS: not otherwise specified, Pt: patients

Table 3

p16 positivity in cervical cell samples and percentage of p16-positive samples per cytological category

Study	n or c n+c	p16 positivity cut-off(+) >10 AC	WNL 0%	ASCUS NA	% of p16 positive samples LSIL 74%	HSIL 96%
Bibbo 2002	n+c	>>10 cells	21%	50%	74%	90%
Saqui 2002	n+c	>10 AC	NA	NA	NA	63%
Murphy 2003	ND	ND	0%	100%	86%	100%
Pientong 2003	n+c	≥3 cells	0%	57%	33%	93%
Akpola 2004	n+c	≥10% cells	0%	10%	NA	42%
Guo 2004	n+c	≥5 AC	NA	NA	58%	97%
Pientong 2004	n+c	≥10 cells	0%	53%	54%	100%
Sahebali 2004	n+c	ND	NA	NA	NA	NA
Trunk 2004	ND	ND	11%	67%	79%	100%
Yoshida 2004	n+c	≥1 AC	NA	13%	58%	100%
Bose 2005	n+c	>>10 cells	13%	29%	21%	81%
Filho 2005	ND	Any staining	49%	56%	NA	NA
Moore 2005	ND	>30% AC	0%	14%	44%	100%
Nieh 2005	ND	Any staining	NA	61%	NA	NA
Wentzensen 2005	n+c	NS>2	1%	NA	10%	98%
Ekalaksananan 2006	ND	≥10 cells	7%	63%	60%	100%
Holladay 2006	n or c	≥1 AC	0%	35%	42%	80%
Negri 2006	n or c	ND	13%	56%	67%	100%
Sahebali 2006	n+c	ND	NA	NA	NA	NA
Bollanca 2007	n+c	>1% cells	2%	NA	35%	96%
Carozzi 2007	ND	ND	35%	50%	40%	80%
Carydis 2007	ND	>10 cells	10%	NA	NA	NA
Liu 2007	n+c	>10 cells	20%	NA	50%	82%
Meyer 2007	n+c	≥1 AC	9%	18%	42%	81%
Monsonogo 2007	n or c	Any staining	36%	55%	28%	88%
Wentzensen 2007	n	NS>2	NA	27%	24%	NA
Pooled value (random effect model) (95% CI)			12% (7-17%)	45% (35-54%)	45% (37-57%)	89% (84-95%)

AC: atypical cells, ASCUS: atypical squamous cells of undetermined significance, c: cytoplasmic staining, cut-off (+): number or percentage of cells stained positive for p16, HSIL: high-grade squamous intraepithelial lesion, CI: confidence interval, LSIL: low-grade squamous intraepithelial lesion, n: nuclear staining, NA: not-applicable, NS: nuclear scoring, WNL: within normal limits

Table 4

P16 positivity and percentage of positive biopsies per histological grade of intraepithelial neoplasia

Study	Distribution Klaes*	P16 positivity		cut-off(+) Diffuse+	WNL 0%	% of p16 positive samples		
		n/c	c±n			CIN1 38%	CIN2 NA	CIN3 NA
Keating 2001	Klaes*			Diffuse+				
Klaes 2001	Klaes	n+c		Diffuse+	1%	62%	100%	100%
Bibbo 2002	Klaes	n+c		Diffuse+	0%	58%	91%	100%
Sano 2002	Klaes*	n+c		Diffuse+	0%	100%	88%	100%
Agoff 2003	Klaes	n+c		Diffuse+	8%	38%	64%	86%
Bibbo 2003	Klaes	n+c		Focal+	0%	40%	100%	100%
Murphy 2003	Klaes*	ND		Focal+	0%	93%	73%	91%
Branca 2004	Klaes*	ND		Diffuse+	0%	10%	24%	54%
Tringler 2004	Klaes*	n+c		Poor+	6%	72%	NA	NA
Volgareva 2004	Klaes	n+c		Diffuse+	0%	2%	3%	17%
Wang JL 2004	Klaes	n±c		Diffuse+	13%	50%	83%	75%
Wang S 2004	Klaes	ND		Diffuse+	5%	36%	63%	100%
Yoshida 2004	Klaes	n+c		Diffuse+	3%	0%	88%	86%
Aoyama 2005	Klaes*	ND		Diffuse+	NA	17%	NA	NA
Dray 2005	Klaes*	n±c		Diffuse+	NA	45%	NA	NA
Guimaraes 2005	Klaes	n+c		Diffuse+	NA	0%	NA	NA
Hu 2005	Klaes	n±c		Diffuse+	NA	9%	63%	84%
Kalof 2005	Klaes	ND		Diffuse+	NA	50%	NA	NA
Kim 2005	NA	ND		ND	0%	36%	NA	NA
Lin 2005	Klaes	ND		Focal+	0%	90%	NA	NA
Lorenzato 2005	Klaes	ND		Diffuse+	0%	52%	NA	NA
Nieh 2005	Klaes*	ND		Poor+	5%	NA	79%	95%
Qiao 2005	Klaes	c±n		Diffuse+	0%	NA	NA	NA
Andersson 2006	Klaes	n±c		Diffuse+	NA	47%	65%	77%
Benevolo 2006	ND	ND		ND	0%	31%	90%	100%
Bullen 2006	Klaes*	n+c		Focal+	NA	67%	100%	100%
Ekalaksananan 2006	ND	ND		ND	37%	13%	NA	NA
Ishikawa 2006	Klaes*	ND		Diffuse+	14%	25%	80%	94%

Study	Distribution	P16 positivity n/c	cut-off(+)	WNL	% of p16 positive samples		
					CIN1	CIN2	CIN3
Lambert 2006	Klaes	n+c	Focal+	NA	50%	NA	NA
Queiroz 2006	Klaes*	n+c	Diffuse+	9%	53%	70%	93%
Zhang 2006	Klaes*	ND	Poor+	33%	52%	70%	80%
Bahnassy 2007	Klaes*	n±c	Focal+	0%	0%	36%	63%
Eleuterio 200	Klaes	n+c	Diffuse+	0%	15%	NA	NA
Focchi 2007	Klaes	n+c	Diffuse+	0%	91%	NA	NA
Hariri 2007	Klaes*	ND	Diffuse+	6%	71%	NA	NA
Iaconis 2007	ND	n±c	ND	27%	NA	NA	NA
Kong 2007	Klaes*	n±c	Focal+	NA	NA	91%	100%
Nam 2007	NA	n±c	ND	NA	27%	37%	62%
Omori 2007	Klaes	ND	Diffuse+	NA	36%	73%	100%
Yildiz 2007	Klaes*	n±c	Focal+	NA	80%	NA	NA
Pooled value (random effect model) (95% CI)					38% (23-53%)	68% (44-92%)	82% (72-92%)

C: cytoplasmic staining, CI: confidence interval, CIN: cervical intraepithelial neoplasia, Klaes*: translated to Klaes distribution, N: nuclear staining, NA: not-applicable, ND: not-documented, SCC: squamous cell carcinoma, WNL: within normal limits Pooled values given for studies adopting the Klaes/ Klaes* distribution for diffuse+ staining

Table 5

P16 versus HC2 in cytology

Study	Cut-off	End-point	Test	Sensitivity	Specificity	PPV	NPV
Guo 2004	LSIL	CIN2	P16	92%	49%	33%	96%
			HC2	100%	7%	21%	100%
Nieh 2005	ASCUS	CIN2	P16	95%	56%	50%	96%
			HC2	86%	31%	37%	82%
Meyer 2007	ASCUS	CIN2	P16	0%	93%	0%	93%
			HC2	100%	21%	8%	100%
Meyer 2007	LSIL	CIN2	P16	80%	52%	27%	92%
			HC2	100%	17%	21%	100%

ASCUS: atypical squamous cells of undetermined significance, **CIN:** cervical intraepithelial lesion, **HC2:** hybrid capture 2, **LSIL:** low-grade squamous intraepithelial lesion, **NPV:** negative predictive value, **PPV:** positive predictive value