© Curr Gynecol Oncol 2019, 17 (2), p. 55–60
DOI: 10.15557/CGO.2019.0006

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Received: 26.06.2019
Accepted: 28.07.2019
Published: 30.08.2019

Diagnostic utility of p16^{INK4a} and Ki-67 immunohistochemistry in cervical biopsy specimens in women with ASCUS cytology

Przydatność diagnostyczna immunohistochemicznej oceny ekspresji białek p16^{INK4a} i Ki-67 u pacjentek z cytologicznym rozpoznaniem ASCUS

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Abstract

Objectives: The aim of the study was to evaluate the usefulness of p16 and Ki-67 immunohistochemistry in women diagnosed with ASCUS in follow-up colposcopy with histology. The relationship between the accuracy of p16, Ki-67 and HR HPV DNA testing as biomarkers of cervical intraepithelial neoplasia was evaluated. **Material and methods:** 272 women with cytological diagnosis of ASCUS underwent colposcopy and cervical biopsy. Sections were processed for immunohistochemistry with mouse anti-human p16 and anti-Ki-67 monoclonal antibodies. The HPV test was performed in these patients without knowledge of cytology results. **Results:** Histopathology revealed 143 patients diagnosed with CIN1, 24 as CIN2 and 34 as CIN3. The HR HPV test was positive in 127 cases (70 CIN1, 24 CIN2 and 33 CIN3 patients). p16 positivity was present in 68 cases of CIN1 and HR HPV positive, in 24 CIN2 and HR HPV positive and in 33 of CIN3 HR HPV positive patients. Ki-67 positivity was present in 69 CIN1, 24 CIN2 and 34 CIN3 cases. The sensitivity of the HR HPV test, colposcopy, p16 and Ki-67 was high. The highest specificity was reported for the HR HPV test. **Conclusions:** Our data show that a combined use of p16^{INK4a} and Ki-67 helps to distinguish true dysplastic transformation from its benign mimics and determine the severity of dysplasia in doubtful cases. The use of both biomarkers may result in better management of women with ASCUS cytology followed by histopathology.

Keywords: cervical intraepithelial neoplasia, ASCUS, p16^{INK4a}, Ki-67, HR HPV

Streszczenie

Cele: Celem niniejszej pracy była ocena immunohistochemicznej ekspresji białek p16^{INK4a} oraz Ki-67 w materiale pobranym celem weryfikacji histopatologicznej u pacjentek z rozpoznaniem cytologicznym ASCUS. Oceniano przydatność ekspresji p16, Ki-67 i HR HPV DNA, jako biomarkerów wewnątrznabłonkowej neoplazji szyjki macicy. **Materiał i metody:** U 272 kobiet z cytologicznym rozpoznaniem ASCUS przeprowadzono badanie kolposkopowe i biopsję szyjki macicy. Wycinki poddano powtórnej ocenie mikroskopowej i wykonano odczyny immunohistochemiczne z użyciem mysich anty-ludzkich przeciwciał monoklonalnych skierowanych przeciw białkom p16 oraz Ki-67. W powyższej grupie pacjentek wykonany był test HR HPV. **Wyniki:** U 143 pacjentek weryfikacja histopatologiczna wykazała zmiany CIN1, u 24 – CIN2, zaś u 34 – CIN3. Test HR HPV był pozytywny w 127 przypadkach (dla CIN1 był dodatni u 70 kobiet, dla CIN2 – u 24, a dla CIN3 – u 33). Odczyny przeciw białku p16 wykazały dodatnią ekspresję u 68 badanych z CIN1 i HR HPV (+), u 24 z CIN2 i HR HPV (+) oraz u 33 z CIN3 i HR HPV (+). Odczyn dla Ki-67 był dodatni w 69 przypadkach CIN1, 24 CIN2 i 34 CIN3. Czułość testu HR HPV, kolposkopii oraz odczynów immunohistochemicznych przeciw p16 i Ki-67 była wysoka, zaś specyficzność była najwyższa dla testu HR HPV. **Podsumowanie:** Badanie wykazało, że zastosowanie odczynów p16^{INK4a} i Ki-67 daje możliwość różnicowania rzeczywistej dysplazji ze zmianami łagodnymi, jak również ustalenia jej stopnia w przypadkach wątpliwych. Wskazuje to na przydatność obu markerów w odpowiednim postępowaniu diagnostyczno-terapeutycznym u pacjentek z rozpoznaniem cytologicznym ASCUS poddanych weryfikacji histopatologicznej.

Słowa kluczowe: wewnątrznabłonkowa neoplazja szyjki macicy, ASCUS, p16^{INK4a}, Ki-67, HR HPV

INTRODUCTION

Cervical cancer is one of the most common cancers in women. Papanicolaou screening contributed to reduced morbidity and mortality from cervical carcinoma. The causative role of distinct types of high-risk human papilloma virus (HR HPV) has been established. Usually HPV infections are transient, lasting not longer than 12–24 months, and resolve spontaneously, with only a few progressing to precancerous lesions or invasive cancer[1–3]. HR HPV can be detected with molecular biological techniques such as polymerase chain reaction (PCR)-based techniques and HPV DNA/RNA hybridization; however, these tests do not distinguish between infections with and without oncogenic transformation.

$P16^{INK4a}$ and Ki-67 are two surrogate markers of HR HPV infection and high grade dysplasia (cervical intraepithelial neoplasia, CIN)[4].

The interaction of viral oncogenes with chromosomal stability as well as inhibition of tumour suppressor proteins such as p53 and pRb (retinoblastoma protein) triggers neoplastic epithelial transformation. This results in the overexpression of viral proteins E6 and E7, with the latter one binding to and inactivating pRb and inducing the overproduction of p16. The value of p16 as a marker of HPV-induced oncogenesis is well established and the use of p16 immunohistochemistry allows precise identification of dysplasia in cervical histology[2,5–8].

Ki-67 is a cell proliferation marker. Its nuclear expression within squamous cervical epithelium increases with increasing grade of dysplasia; however, Ki-67 is not an independent prognostic predictor[9]. Since only persistent HPV infections progress to carcinoma, combinations of p16 overexpression, Ki-67 and HPV testing have been implicated as markers to predict the behavior of cervical squamous and glandular lesions. The use of p16 and Ki-67 has been suggested in assessing the risk of precancerous lesion in cytological smears classified as ASCUS[1,6,9].

The diagnosis of atypical squamous cells of undetermined significance (ASCUS) in cervical screening varies from about 4%[10] to 5%[11]. The estimated prevalence of squamous lesions ≥CIN2 (including CIN2, CIN3, adenocarcinoma *in situ* and invasive carcinoma) in the ASCUS group is from 12.0%[10,11] to 13.61%[12], as confirmed by HR HPV test and colposcopy with a follow-up biopsy[10,12]. Cervical biopsy with microscopic assessment is not a routine management in patients diagnosed with ASCUS. Since about 43% of ASCUS women are HR HPV positive, the use of p16 and Ki-67 in this group to improve diagnostic accuracy seems understandable[13].

In this study we evaluates the usefulness of p16 and Ki-67 in women diagnosed with ASCUS in follow-up colposcopy with histology.

MATERIAL AND METHODS

Study design

The study was approved by the local medical research ethics committee of the Medical University of Lublin (project approval number KE-0255/44/2015). Results of Pap test from one laboratory (Luxmed, Lublin) from years 2010–2015 were reviewed. The data of 3,257 women diagnosed with ASCUS were selected, 272 of these patients had HR HPV test results and follow-up colposcopy and cervical biopsy with histopathological examination in Department of Clinical Pathomorphology, Medical University of Lublin, Lublin. Mean age of these 272 patients was 39 years (19–75 years). Written consent was obtained from all 272 patients.

Colposcopy results were included in referral sheet for histopathological examination.

HR HPV

HR HPV was detected using the Xpert® HPV assay (Cepheid AB, Sweden). This test simultaneously detects a total of 14 HR HPV types: HPV-16 individually, HPV-18 individually and 12 pooled HR HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). The samples from the cervix were separately sent to Luxmed Laboratory in containers dedicated for these types of tests with alcohol as a fixative agent. The Xpert® HPV test was performed in all patients in the ASCUS group, without knowledge of cytology results.

Immunohistochemistry

Then, 4 μm sections were cut from paraffin blocks, deparaffinized in xylene, washed in ethanol and finally washed in phosphate-buffered saline, pH 7.4. Sections were pretreated in microwave for 20 minutes on high in Tris/ethylene diamine tetraacetic acid buffer, pH 9.0. After cooling to room temperature and rising in distilled water, endogenous peroxidase activity was blocked with 3% H_2O_2 for 15 minutes. The sections were subjected to immunohistochemistry with either mouse anti-human p16 monoclonal antibody (BD Biosciences, dilution 1:25) or mouse anti-Ki-67 monoclonal antibody (DAKO, dilution 50). For visualization DAKO REAL™ EnVision™ HRP Rabbit/Mouse kit was used (DAKO).

The p16 reaction was considered positive if cytoplasmic and, in some cases, nuclear immunostaining was clearly demonstrated. Staining was considered positive if continuous reaction was present in horizontal plane of squamous epithelium either partial, or full thickness. For Ki-67, only nuclear reaction was considered positive. Since basal staining is a normal finding, only suprabasal staining within the upper two-thirds of the epithelium was scored as positive. To determine the grade of Ki-67 expression, nuclei of 200 epithelial cells located across the suprabasal

DOI: 10.15557/CGO.2019.0006

epithelial layers were examined in a high-power field (×400). The Ki-67 index was defined as the percentage of Ki-67 positive cells: negative, 1+, 2+, 3+ for the Ki-67 index below 5%, 5–25%, 26–50% and greater than 50%, respectively. Sections containing histologically normal epithelia were included as negative control, while positive controls were obtained from slides previously diagnosed as preinvasive carcinoma.

Statistical analysis

We characterized the values of analyzed parameters using frequencies and percentage. Additionally, the diagnostic value of colposcopy, HPV testing and immunohistochemistry were evaluated.
The accuracy of the test was determined based on a comparison between test results and histopathological verification, which describes the actual disease state. The used basic measurements of test accuracy were as follows: sensitivity (SENS), specificity (SPEC), positive predictive value (PPV), negative predictive value (NPV) and accuracy (ACC).

Funding source

The Medical University of Lublin funded all reagents, laboratory equipment and tests that were used in the study. The source of funding had no involvement in the study design, analysis, or discussion of the results.

RESULTS

Histopathology and colposcopy

A total of 71 microscopic slides from 272 patients diagnosed with ASCUS showed no signs of CIN and were considered negative. In 143 patients, CIN1 was initially diagnosed. CIN2 was present in 24 and CIN3 in 34 cases. In total, ≥CIN2 lesions were present in 58 women (Fig. 1).
All microscopic slides were reviewed for a second time by one experienced histopathologist. This revision showed discrepancy in the CIN1 group (Tab. 1). The results of the second revision of slides in groups CIN2 and CIN3 were consistent with the initial diagnosis. Colposcopy was satisfactory in all patients, negative in 70 and positive in 202 cases (summary is presented in Tab. 1).

HPV

The HR HPV (DNA) test was negative in 144 patients (71 with negative histology and 73 cases of CIN1) and positive in 127 cases (70 cases of CIN1, 24 cases of CIN2 and 33 cases of CIN3). Data are summarized in Tab. 1.

Immunohistochemistry

Tab. 1 summarizes the immunohistochemical findings. Continuous (not single cells) either weak or strong p16 positivity correlated with the presence of HR HPV and histopathological diagnosis. p16 positivity was present in 68 HR HPV positive CIN1, 24 HR HPV positive CIN2 and 34 HR HPV positive CIN3 cases (Fig. 1). A similar relationship was observed for suprabasal Ki-67 positivity, with CIN1 positive reaction (graded as +1) in 69 cases, and strong reaction (graded as +2 or +3) in 24 CIN2 cases and 34 CIN3 cases (Tab. 1, Fig. 1).

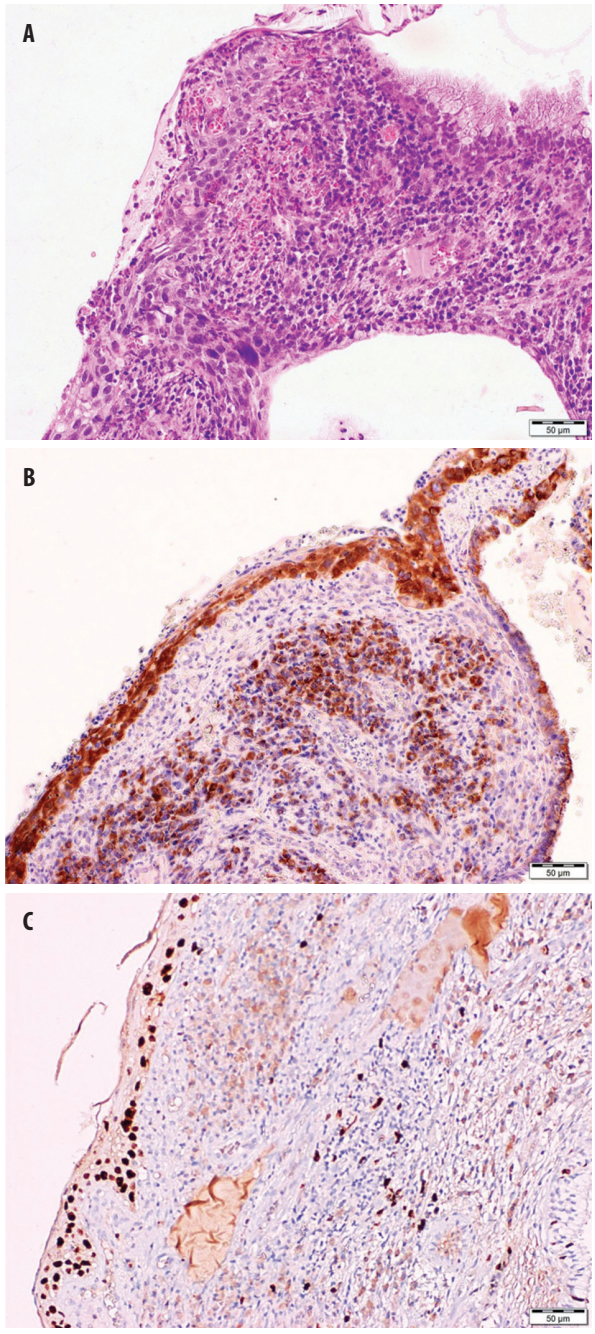

Fig. 1. A case initially diagnosed as CIN1. Colposcopy was negative. Deeper layers revealed CIN1 and focal CIN2 in the epithelium of the squamo-glandular junction. **A** *– H+E,* **B** *– strong immunohistochemical expression of p16,* **C** *– Ki-67, graded +2*

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N = 272	Histopathology			
	Negative *n* = 71	**CIN1** *n* = 143	**CIN2** *n* = 24	**CIN3** *n* = 34
HR HPV	71 neg (100%)	73 neg (51%) 70 pos (49%)	24 pos (100%)	1 neg (2.9%) 33 pos (97.1%)
Colposcopy	59 neg (82.7%) 12 pos (17.3%)	69 neg (48.3%) 74 pos (51.7%)	24 pos (100%)	3 neg (8.83%) 31 pos (91.18%)
p16	69 neg (97.2%) 2 pos (2.8%)	75 neg (52.5%) 68 pos (47.5%)	24 pos (100%)	34 pos (100%)
Ki-67	69 neg (97.1%) 2 pos (2.9%)	74 neg (51.7%) 69 pos (48.3%)	24 pos (100%)	34 pos (100%)
Histopathology Second revision	69 neg (97.1%) 2 pos (2.9%)	72 neg (50.34%) 71 pos (49.66%)	24 pos (100%)	34 pos (100%)
Neg – negative, **pos** – positive.				

Tab. 1. Summary of the HR HPV test, colposcopy and p16 and Ki-67 immunohistochemistry results for the diagnoses of CIN1, CIN2 and CIN3

Statistical analysis

The results of statistical analysis are summarized in Fig. 2 and show that the sensitivity of immunostaining for p16 and Ki-67 was high (0.82 and 0.86). The highest specificity was reported for the HR HPV test followed by p16 and Ki-67 (0.92 each). The lowest specificity was shown for colposcopy (0.46).

We also analyzed the above parameters separately for groups with the following histopathological diagnosis of: lack of dysplasia vs. ≥CIN2. For the diagnosis of ≥CIN2, the best tests were HR HPV followed by both biomarkers equally (Tab. 2).

DISCUSSION

It is important to identify patients with high grade squamous dysplasia among women with ASCUS cytology. Repeated cytology and HPV testing are two basic approaches proposed for these patients. Colposcopy-guided biopsy is performed in HR HPV positive cases with or without positive colposcopy. Several studies showed HPV testing to be more sensitive than repeated cytology[2,3,14,15]; however, the specificity of HPV testing is relatively low, especially in sexually active women[10,16].

Histopathological interpretation of H&E stained slides is the gold standard for the diagnosis of CIN. However, diagnostic variability has been observed in several studies among pathologists[8,17–19]. $P16^{INK4a}$ protein expression in basal and parabasal cells of squamous epithelium is induced by the presence of active HPV oncogenes within the host cells, and thus is a sensitive marker to differentiate transient from persistent HPV infection that have already initiated neoplastic transformation. Several studies have found p16 to be a highly sensitive biomarker in the detection of HPV related true dysplasia in cervical biopsy specimens[8,17,19–21], which increases the interobserver agreement[8,15,22]. Wentzensen et al.[15] showed that p16 is more sensitive in detecting high-grade CIN than HPV testing in ASCUS/low-grade squamous intraepithelial lesions (LSIL) cytology results. Similar results were obtained by Schledermann et al., Yu et al., and Carozzi et al.[1,4,23].

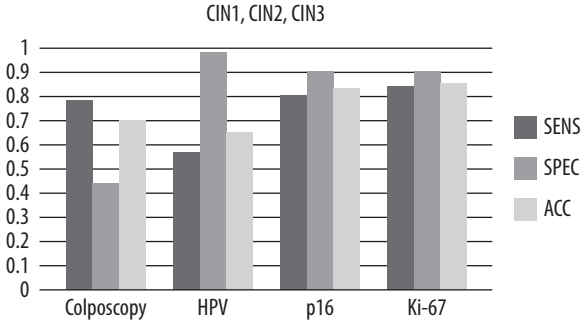

Fig. 2. Statistical analysis for diagnosis of dysplasia (CIN1, CIN2, CIN3)

Lack of dysplasia vs. ≥CIN2	SENS	SPEC	PPV	NPV	ACC
Colposcopy	0.88	0.46	0.60	0.80	0.66
HPV	0.94	1.00	1.00	0.93	0.97
p16	0.96	0.92	0.92	0.96	0.94
Ki-67	0.96	0.92	0.92	0.96	0.94
SENS – sensitivity; **SPEC** – specificity; **PPV** – positive predictive value; **NPV** – negative predictive value; **ACC** – accuracy.					

Tab. 2. Statistical analysis for diagnosis of ≥CIN2

DOI: 10.15557/CGO.2019.0006 CURR GYNECOL ONCOL 2019, 17 (2), p. 55–60

Combining p16 immunohistochemistry with morphologic interpretation of CIN improves the agreement between pathologists for a ≥CIN2 diagnosis[8]. Singh et al.[24], who assessed the variability of the utilisation of p16 and Ki-67 immunostaining in cervical biopsies among pathologists, found that the use of both biomarkers improved cytohistologic correlation frequencies by lowering the ratio of major cytohistologic discrepancies, lowering CIN1 diagnoses and increasing frequencies of ≥CIN2 diagnoses, with a lower range of interpathologists ≥CIN2 frequencies.

The supporting use of p16 in the interpretation of CIN is suggested by the Lower Anogenital Squamous Terminology Standardization Project[17,25,26]. It was highlighted that combining p16 immunohistochemistry with morphologic interpretation of cervical histology may ensure accuracy.

In our study CIN1 was initially diagnosed in 143/272 ASCUS patients. High grade dysplasia ≥CIN2 was diagnosed in 58/272 ASCUS cases (24 CIN2 and 34 CIN3 cases). These results correspond with those presented in the literature, with high grade dysplasia diagnosed in 5.2% to 18% of ASCUS cases[11]. Statistical analysis showed strong correlation between p16 expression and histopathological diagnosis of ≥CIN2 and HR HPV positivity in our study (Tab. 1). Two cases with negative morphology showed positive staining for p16 and Ki-67. Repeated histology with deep layers cut and assessed by an experienced histopathologist revealed CIN1 with koilocytic atypia in these two cases. High accuracy was achieved for p16 and Ki-67 when assessing ≥CIN2. In one case initially diagnosed as CIN1, strong positivity for p16 as well as for Ki-67 was found. Second revision of deep layers revealed CIN2 parallel with CIN1. The above observations let us conclude that the use of p16 alone or combined with Ki-67 may not only aid CIN diagnosis, but also improve the accuracy of morphologic examination in routine practice. Based on their results on the use of p16 and Ki-67 in cervical biopsies, Galgano et al.[27] concluded that p16 alone is less specific in distinguishing CIN1 from non-dysplastic lesions and that p16 positive CIN1 may be associated with a significant risk of CIN1 subsequently developing into ≥CIN2. These authors agreed that p16 is a reliable diagnostic adjunct for distinguishing biopsies with or without ≥CIN2.

In one HR-HPV-negative patient both p16 and Ki-67 were positive within suprabasal layers of squamous epithelium. The initial microscopic diagnosis (blind of HR HPV results) was CIN1 in this case. The possible explanation for this discrepancy is infection with a different type of HR HPV than those mapped by the test used in the study. An opposite situation was observed in 3 cases diagnosed with CIN1, with HR HPV positivity and very weak staining for p16 only. Keating et al.[28] suggested that not all HR HPVs have the same potential for cell cycle disruption or altered gene expression that leads to p16 upregulation.

One case of CIN3 in a 73-year-old woman was HR HPV negative with p16 and Ki-67 positivity. These findings may suggest that other type of HR HPV than the one matched by the test used in the study was the causative agent in this case. This hypothesis may be supported by p16 positivity. Other mechanisms, different from HR-HPV induced oncogenesis, cannot be excluded.

A discrepancy was found between histopathology and HR HPV test as well as p16 results in the CIN1 group. The HR HPV test was negative in 73 CIN1 cases. Immunostaining for p16 and Ki-67 confirmed the absence of HPV infection in 72 of these cases. Tab. 1 presents low sensitivity with relatively high specificity for HR HPV test and both biomarkers, p16 and Ki-67, in this group. Revision of serial cuts of H+E slides by a histopathologist experienced in cervical pathology revealed that CIN1 was overdiagnosed in these 72 patients (Tab. 1). Most of them (69) showed metaplastic and inflammatory reactive changes of the squamous epithelium. In 3 other cases, repeated histology revealed immature squamous metaplasia. Ki-67 was negative in all of these 3 cases (Tab. 1). Moreover, the co-expression of both biomarkers with a continuous pattern of p16 seems to be a better indicator of true dysplasia as p16 alone with focal and weak pattern of single cells may be present in intermediate or superficial layers of squamous epithelium[21].

Ki-67 expression on its own is associated with the grade of dysplasia. Cabibi et al.[29] and Nam et al.[30] showed a correlation between p16 and Ki-67 immunoexpression and the grade of dysplasia. Our observation showed increased accuracy of p16 and Ki-67 immunostaining in cases of discriminating ≥CIN2 from CIN1. These observations suggest that the combined use of both biomarkers, p16 and Ki-67, may be helpful not only in distinguishing dysplasia from its reactive mimics, but also in determining the true grade of dysplasia and increase the accuracy in detecting CIN2.

CONCLUSION

Our data show that p16^{INK4a} and Ki-67 can be useful markers in routine diagnosis of cervical intraepithelial neoplasia in patients with colposcopy-biopsy follow-up. The data presented in the study suggest that immunostaining for p16^{INK4a} allows distinguishing between transformed and non-transformed cervical intraepithelial lesions in women diagnosed with ASCUS.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Funding/Support and role of the sponsor

The Medical University of Lublin funded all reagents, laboratory equipment and tests that were used in the study. The source of funding had no involvement in the study design, analysis, and discussion of the results.

References

1. Schledermann D, Andersen BT, Bisgaard K et al.: Are adjunctive markers useful in routine cervical cancer screening? Application of p16^{INK4a} and HPV-PCR on ThinPrep samples with histological follow-up. Diagn Cytopathol 2008; 36: 453–459.
2. von Knebel Doeberitz M, Reuschenbach M, Schmidt D et al.: Biomarkers for cervical cancer screening: the role of p16^{INK4a} to highlight transforming HPV infections. Expert Rev Proteomics 2012; 9: 149–163.
3. Gultekin M, Zayifoglu Karaca M, Kucukyildiz I et al.: Initial results of population based cervical cancer screening program using HPV testing in one million Turkish women. Int J Cancer 2018; 142: 1952–1958.
4. Yu L, Wang L, Zhong J et al.: Diagnostic value of p16INK4A, Ki-67, and human papillomavirus L1 capsid protein immunochemical staining on cell blocks from residual liquid-based gynecologic cytology specimens. Cancer Cytopathol 2010; 118: 47–55.
5. Cuzick J, Bergeron C, von Knebel Doeberitz M et al.: New technologies and procedures for cervical cancer screening. Vaccine 2012; 30 Suppl 5: F107–F116.
6. Moriarty AT: p16^{INK4a} – is the future of cervical cancer screening rosy? Cancer Cytopathol 2012; 120: 291–293.
7. Killeen JL, Dye T, Grace C et al.: Improved abnormal Pap smear triage using cervical cancer biomarkers. J Low Genit Tract Dis 2014; 18: 1–7.
8. Reuschenbach M, Wentzensen N, Dijkstra MG et al.: P16^{INK4a} immunohistochemistry in cervical biopsy specimens: a systematic review and meta-analysis of the interobserver agreement. Am J Clin Pathol 2014; 142: 767–772.
9. Longatto Filho A, Utagawa ML, Kasumi Shirata N et al.: Immunocytochemical expression of p16^{INK4A} and Ki-67 in cytologically negative and equivocal Pap smears positive for oncogenic human papillomavirus. Int J Gynecol Pathol 2005; 24: 118–124.
10. Stoler MH, Wright TC Jr, Sharma A et al.; ATHENA (Addressing THE Need for Advanced HPV Diagnostics) HPV Study Group: High-risk human papillomavirus testing in women with ASC-US cytology: results from the ATHENA HPV study. Am J Clin Pathol 2011; 135: 468–475.
11. Lee H, Lee EJ: HPV infection and p16 promoter methylation as predictors of ASC-US/LSIL progression. Cancer Cytopathol 2016; 124: 58–65.
12. Pouliakis A, Karakitsou E, Chrelias C et al.: The application of classification and regression trees for the triage of women for referral to colposcopy and the estimation of risk for cervical intraepithelial neoplasia: a study based on 1625 cases with incomplete data from molecular tests. Biomed Res Int 2015; 2015: 914740.
13. Arbyn M, Martin-Hirsch P, Buntinx F et al.: Triage of women with equivocal or low-grade cervical cytology results: a meta-analysis of the HPV test positivity rate. J Cell Mol Med 2009; 13: 648–659.
14. Jeffrey AT: Human Papillomavirus Testing in Cervical Cancer Screening. The California Technology Assessment Forum, San Francisco 2004. Available from: https://www.scribd.com/document/15028102/Human-Papillomavirus-Testing-in-Cervical-Cancer-Screening.
15. Wentzensen N, Bergeron C, Cas F et al.: Triage of women with ASCUS and LSIL cytology: use of qualitative assessment of p16^{INK4a} positive cells to identify patients with high-grade cervical intraepithelial neoplasia. Cancer 2007; 111: 58–66.
16. Solomon D, Schiffman M, Tarone R; ALTS Study group: Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. J Natl Cancer Inst 2001; 93: 293–299.
17. Bergeron C, Ordi J, Schmidt D et al.; European CINtec Histology Study Group: Conjunctive p16^{INK4a} testing significantly increases accuracy in diagnosing high-grade cervical intraepithelial neoplasia. Am J Clin Pathol 2010; 133: 395–406.
18. Stoler MH: Toward objective cervical cancer screening: maybe the eyes do have it. Am J Clin Pathol 2010; 134: 5–6.
19. Lee S, Kim H, Kim H et al.: The utility of p16INK4a and Ki-67 as a conjunctive tool in uterine cervical lesions. Korean J Pathol 2012; 46: 253–260.
20. Cuschieri K, Wentzensen N: Human papillomavirus mRNA and p16 detection as biomarkers for the improved diagnosis of cervical neoplasia. Cancer Epidemiol Biomarkers Prev 2008; 17: 2536–2545.
21. Reuschenbach M, Seiz M, von Knebel Doeberitz C et al.: Evaluation of cervical cone biopsies for coexpression of p16^{INK4a} and Ki-67 in epithelial cells. Int J Cancer 2012; 130: 388–394.
22. Horn LC, Reichert A, Oster A et al.: Immunostaining for p16^{INK4a} used as a conjunctive tool improves interobserver agreement of the histologic diagnosis of cervical intraepithelial neoplasia. Am J Surg Pathol 2008; 32: 502–512.
23. Carozzi F, Cecchini S, Confortini M 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: 119–123.
24. Singh C, Manivel JC, Truskinovsky AM et al.: Variability of pathologists' utilization of p16 and Ki-67 immunostaining in the diagnosis of cervical biopsies in routine pathology practice and its impact on the frequencies of cervical intraepithelial neoplasia diagnoses and cytohistologic correlations. Arch Pathol Lab Med 2014; 138: 76–87.
25. Dijkstra MG, Heideman DAM, de Roy SC et al.: p16^{INK4a} immunostaining as an alternative to histology review for reliable grading of cervical intraepithelial lesions. J Clin Pathol 2010; 63: 972–977.
26. Darragh TM, Colgan TJ, Cox JT et al.; Members of LAST Project Work Groups: The Lower Anogenital Squamous Terminology Standardization Project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. Arch Pathol Lab Med 2012; 136: 1266–1297.
27. Galgano MT, Castle PE, Atkins KA et al.: Using biomarkers as objective standards in the diagnosis of cervical biopsies. Am J Surg Path 2010; 34: 1077–1087.
28. Keating JT, Cviko A, Riethdorf S et al.: Ki-67, cyclin E, and p16^{INK4} are complimentary surrogate biomarkers for human papilloma virus-related cervical neoplasia. Am J Surg Path 2001; 25: 884–891.
29. Cabibi D, Giovannelli L, Martorana A et al.: Predictive role of histological features and Ki67 pattern on high-risk HPV presence in atypical cervical lesions. Histopathology 2007; 51: 713–716.
30. Nam EJ, Kim JW, Hong JW et al.: Expression of the p16 and Ki-67 in relation to the grade of cervical intraepithelial neoplasia and high-risk human papillomavirus infection. J Gynecol Oncol 2008; 19: 162–168.

DOI: 10.15557/CGO.2019.0006 CURR GYNECOL ONCOL 2019, 17 (2), p. 55–60