VIA MEDICA

RECOMMENDATIONS

Ginekologia Polska
2016, vol. 87, no. 9, 670–676
Copyright © 2016 Via Medica
ISSN 0017–0011

DOI: 10.5603/GP.2016.0066

Polish recommendations regarding diagnostics and treatment of cervical squamous intraepithelial lesions according to the CAP/ASCCP guidelines

Anna Nasierowska-Guttmejer[1, 2], Witold Kędzia [3], Wojciech Rokita[2, 4], Szymon Wojtylak[5], Dariusz Lange[6], Robert Jach[7], Mirosław Wielgoś[8]

[1]Department of Pathology, Central Clinical Hospital, Ministry of Internal Affairs, Warsaw
[2]Faculty of Medicine and Health Science, Jan Kochanowski University in Kielce
[3]Department of Gynecology, Karol Marcinkowski Medical University in Poznan
[4]Department of Obstetrics and Gynecology, Voivodeship Combined Hospital in Kielce
[5]Institute and Laboratory of Pathomorphology, Medical University of Gdansk
[6]Department of Cancer Pathology, Maria Skłodowska-Curie Institute of Oncology, Gliwice
[7]Department of Gynecological Endocrinology, Jagiellonian University Medical College, Krakow
[8]1st Department of Obstetrics and Gynecology, Medical University of Warsaw

INTRODUCTION

This publication contains recommendations regarding terminology, diagnostics, and treatment of cervical squamous intraepithelial lesions. It was composed in cooperation with the Section of Cervix Pathology, Colposcopy, and Cytology of the Polish Gynecological Society and the Polish Society of Colposcopy and Cervix Pathophysiology, and the Polish Society of Pathologists. The objectives of this publication are:

- clarification of terminology of cervical squamous intraepithelial lesion (SIL);
- determination of biomarkers useful for interpretation of unclear microscopic morphology changes requiring further differentiation between a high grade squamous intraepithelial lesion (HIL), which is considered a precancerous condition, and changes that are considered negative for an intraepithelial lesion or malignancy (NILM);
- determination of clinical guidelines for management of cervical SIL;
- promulgation of guidelines, improving communication between pathologists, as well as clinical professionals and contributing to more precise and effective diagnostics and therapy of patients with a precancerous condition of the cervix.

TERMINOLOGY REGARDING SIL

The World Health Organization (WHO) in 1975 proposed unification of terminology used in histopathological reports regarding squamous-cell carcinoma precursor lesions. The term "dysplasia" referred to replacement of normal cells of squamous stratified epithelium with abnormal, dysplastic cells spreading onto the consecutive layers of epithelium. Depending on how many layers were affected, dysplasia was classified into three grades: mild, moderate and severe. The WHO defined dysplasia as carcinoma in-situ affecting the entire or almost entire thickness of the epithelium. At present, the concept that separates dysplasia from carcinoma in-situ in a classification system is criticized. It is commonly known that both changes represent the same process, and they directly transform from one into the other.

In 1980, the *International Society of Gynecological Pathologists* (ISGP) changed the term "dysplasia" into "cervical intraepithelial neoplasia" (CIN) and erased the category "carcinoma in-situ". A conclusion was reached that preinvasive lesions in the epithelium are a constant series of events transforming from one into the other. The term "CIN" was divided into three sub-stages: CIN I, CIN II, CIN III; depending on the level of dysplasia evident. The preinvasive cancer was included in the CIN II category. The aforementioned classification was progressive because it also considered precursor lesions in stratified squamous epithelium as a process of carcinogenesis. It was also noted that CIN I is not entirely a precancerous lesion, because it can regress even without treatment.

Cytologic reporting based on the Bethesda System (TBS) was elaborated and implemented into diagnostics in 1988 (modifications were presented in 1991 and 2001). This report explained the terminology of lesions suspected of

Corresponding author:
Wojciech Rokita
Department of Obstetrics and Gynecology, Voivodeship Combined Hospital in Kielce
Grunwaldzka St. 45, 25–726 Kielce, Poland
e-mail: rokita@kielce.com.pl

Table 1. Bethesda System terminology of 2001

Abnormal stratified squamous epithelium	
ASC-US	Atypical cells of stratified squamous epithelium of unspecified characteristics
ASC-H	Atypical cells of stratified squamous epithelium, high-grade squamous intraepithelial lesion (HSIL) cannot be excluded
LSIL	Mild intraepithelial neoplasia; concerns HPV infections/low-grade dysplasia CIN I
HSIL	High grade squamous intraepithelial lesion; Including moderate (CIN II) and severe neoplasia (CIN III), CIS (*carcinoma in situ*)
Squamous carcinoma	In case of suspected invasion
Abnormal cells of glandular epithelium	
AGC	Atypical cells of the cervical glandular epithelium (AGC) qq or uterine body, or other granular
AIS	Cervical adenocarcinoma *in situ*
Adenocarcinoma	Cells of adenocarcinoma of the cervix, uterine body or an extrauterine tumor

Table 2. Comparison of the CIN and SIL classification systems for cervical precancerous lesions

Former diagnosis dysplasia	CIN classification *"cervical intraepithelial neoplasia"*	Bethesda system, SIL *"squamous epithelial lesion"*
Papilloma	Papilloma	LSIL
Mild dysplasia	CIN I	LSIL
Moderate dysplasia	CIN II	HSIL
Severe dysplasia	CIN III	HSIL
Preinvasive carcinoma	CIN III	HSIL

cancer and CIN based on abnormal morphology of the cells derived from stratified squamous epithelium and glandular epithelium of the cervix.

Cytologic reporting based on the Bethesda System (TBS) was implemented in 2001. The rules of aforementioned classification are presented in Table 1.

In 2012, the College of American Pathologists (CAP) and American Society of Colposcopy and Cervical Pathology (ASCCP) elaborated the Lower Anogenital Squamous Terminology (LAST) regarding changes caused by the Human Papillomavirus (HPV). The lower anogenital region is an area covered by mucous membrane or skin within the cervix, vagina, vulva, penis, and crotch, including the anal canal and perianal region. The cells of nonkeratinized stratified squamous epithelium, mucous membrane or keratinized epithelium of the skin are vulnerable to HPV infection. Genotypes of HPV were assigned into two groups depending on the risk for malignant transformation. The HPV subtypes of low oncogenic potential: 6, 11, 42, 43, 44, and 53 are related to moderate intraepithelial neoplasia and papillary epithelial lesions resembling genital and plain warts. Whereas infections associated with moderate and high risk for neoplastic changes are caused by the HPV genotypes of high oncogenic potential (types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). HPV 16 and HPV 18 type usually coexist with HSIL and squamous invasive cancer of the cervix.

Recommendations of the WHO and AP/ASCCP suggest that histopathological reports describing changes in tissue samples should be written using the terminology based on the two-stage Bethesda System, as in reports from gynecological cytology. According to the recommendations of WHO/ASCCP Intraepithelial Lesion /IN are classified as: *Low Grade Squamous Epithelial Lesion (LSIL), Mild Dysplasia (CIN I) High Squamous Epithelial Lesion (HSIL), Moderate Dysplasia (CIN II), and Severe Dysplasia (CIN III*).

Genital warts, which were not previously classified as CIN, were added to the LSIL group, and CIN II and CIN III were classified into one category - HSIL. In practice, CIN and SIL are often associated together and defined as LSIL (CIN I), HSIL (CIN II) or HSIL (CIN III). Comparison of terminology used in histopathological reports according to different classification systems for cervical preinvasive squamous lesions are presented in Table 2.

SUMMARY. RECOMMENDATIONS FOR DESCRIPTION OF THE TYPE OF SIL

It is recommended to use unified histopathological terminology for description of anogenital lesions caused by HPV.

It is recommended to use two-stage terminology for description of changes in morphology of intraepithelial neoplasia (IN) of the anogenital region caused by HPV such as *low-grade squamous intraepithelial lesion* (LSIL) and *high-grade squamous intraepithelial lesion* (HSIL).

RULES FOR CONDUCTING CERVICAL CYTOLOGY SCREENING

Necessary data for evaluation of cervical cytology screening

The vast majority of cytologic smears sampled for cervical cancer screening are normal and do not present with abnormalities in the cells of stratified squamous epithelium and glandular epithelium. While formulating the diagnosis, the cytologic presentation should be evaluated in the context of women's age and phase of menstruation cycle. In young women, there are mostly mature cells of the stratified squamous epithelium in the cytologic smears due to the effect of estrogens. Cytologic smears obtained from pregnant women contain mostly intermediate glycogen-rich cells (fusiform cells), as the result of progesterone. Cytologic smears in women after menopause contain mostly basilar and peribasilar cells due to a lack of estrogens. In abnormal and atypical cells as well as in intraepithelial neoplasia, pleomorphism occurs, and the proportion of the nucleus increases relative to the cytoplasm. The silhouette of the nucleus is irregular, the nuclear membrane is thin, and there are protrusions and vacuoles inside the nucleus. The nucleoli are clearly visible with abnormal figures of division. The stage of these changes in the cell determines whether atypia or intraepithelial cervical neoplasia should be diagnosed. Koilocytes are a key cytologic sign of HPV infection. A koilocyte is an abnormal squamous epithelial cell with enlarged, hyperchromatic nucleus surrounded by large, clear space with peripheral cytoplasm.

The algorithm for managing cytologic specimens obtained from the cervix

A smear obtained from the squamocolumnar junction and cervical canal with a brush must be immediately placed onto the glass and preserved with fixatives designed for that purpose (in case of conventional cytology) or placed into a specially dedicated container with a liquid base (in the case of liquid-based cytology). The watch glass with the smear or the container with the probed material on the liquid medium should be labeled. The labelling should contain the diagnostic test number and name of the patient. A referral with the patient's name, personal ID number and/or bar code should be attached to the smear or container. Information about the patient's last menstrual period and potential hormone therapy should be mentioned on the referral.

It is recommended that cytologic smears are performed using the Papanicolaou (PAP smear). Diagnostic test results should be presented according to Bethesda's classification. In cases when the result of the cytologic evaluation is normal, biomarker tests are not recommended.

BIOMARKERS USED IN CERVICAL INTRAEPITHELIAL NEOPLASIA

Biomarkers used in cytologic smears

Cytologic diagnosis of ASC-US or LSIL indicates the presence of abnormal stratified squamous cells. Such morphological changes in the cells may be a manifestation of an inflammatory process or their early transition to a precancerous state after the initiation of carcinogenesis. In these cases differentiation between benign lesions that do not require treatment and actual precancerous changes one of which is HSIL, do require management. Differential diagnosis of these lesions may be performed with a two-colour immunohistochemical test with simultaneous use of p16 and Ki67 antibodies, which are included in the CinTecPlus® test kit. When the process of malignant transformation in cervical epithelial cells is initiated, expression of p16 and Ki67 proteins is elevated. In such clinical cases, the CinTecPlus® test helps select those patients with an abnormal cytologic evaluation, who require further diagnostics. The CinTecPlus® test can be considered positive when the nucleus of the epithelial cell is stained red (Ki 67 protein expression) and the cytoplasm brown (p16 protein expression). A test containing both biomarkers p16/Ki-67 in one set provides a high sensitivity and specificity in detecting actual precancerous lesions and cervical cancer. The ability to perform this test on smears significantly increases the quality and precision of the diagnosis by decreasing the number of false positive and false negative results. Our experiences in Poland show a high accuracy of this test, which in the case of diagnosing ASC-US amounts to 41%, LSIL — 56% and HSIL — 73%.

Biomarkers in diagnostics of HPV-dependent lesions of the anogenital area

Histological examination of samples taken under the colposcope from lesions suspected to be precancerous or cancerous is the next step in diagnostics after an abnormal cytologic evaluation result. The p16 protein is a useful biomarker that provides additional information to microscopic evaluation and confirms the diagnosis. The immunohistochemical examination using p16 antibodies helps eliminate or confirm the neoplastic transformation of squamous epithelial cells in the cervix. In normal epithelial cells, there is no p16 expression, or its level is low. P1 protein expression is significantly elevated in epithelial cells in which HPV virus genotypes with a high oncogenic potential initiated the oncogenic transformation and led to their transition into precancerous lesions or cervical cancer. The ability to interpret the immunohistochemical reaction with the use of the p16 antibody by the pathomorphologist is crucial for the accuracy and credibility of this test. A clearly pronounced and extensive color reaction with the p16 antibody confirms the lesion to be precancer-

ous. Non-uniform colouration of the epithelial cells excludes the diagnosis of a neoplastic process.

SUMMARY. RECOMMENDED CLINICAL GUIDELINES

A positive result of the immunocytochemical test with p16 and Ki-67 antibodies (CinTecPlus®) conducted when in case of unclear results of ASC-US cytologic classification suggests the diagnosis of LSIL (eventually ASC-H*) and is an indication for more extensive cervical cancer diagnostics (Fig. 1). The extensive diagnostic process includes a colposcopy and, if necessary, sampling the most suspicious lesions and endocervical curettage. High expression combined with a multicellular colour reaction for p16 indicates an on-going carcinogenic process and suggests that a specialist in cytodiagnostics underestimated morphological changes present in cytologic smears. This may result in omitting medium or high-grade neoplasia (HG SIL). The identification of advanced epithelial lesions under a colposcope is necessary for selective biopsy. Final histopathological diagnosis confirming the presence of HSIL type lesions (CIN II and/or CIN III) is an indication for excision with a margin of healthy tissue. In cases where the lesion was not identified under colposcope or high-grade intraepithelial lesion was not detected in the biopsy sample obtained from a p16/k67 positive patient, the patient should remain under strict cytologic and colposcopic observation supported with virological testing. A cytologic smear should be performed after 3-6 months complemented by a "wide" test covering at least 14 HPV genotypes (DNA HPV HR). A normal result of the cytologic smear obtained in two follow-up tests performed with a 3–6 month interval and a negative result of one test for DNA HPV HR enables the physician to refer the patient for a routine cytologic screening. An abnormal result of a cytologic evaluation and/or a positive DNA HPV HR test result is a ground for repeating a colposcopy in order to identify neoplastic lesions or qualify the patient to a high-risk group.

* Cytologic diagnosis of ASC-H is an indication for extended diagnostics — colposcopy. The results of an immunohistochemical complementary p16/Ki67 test may prove helpful

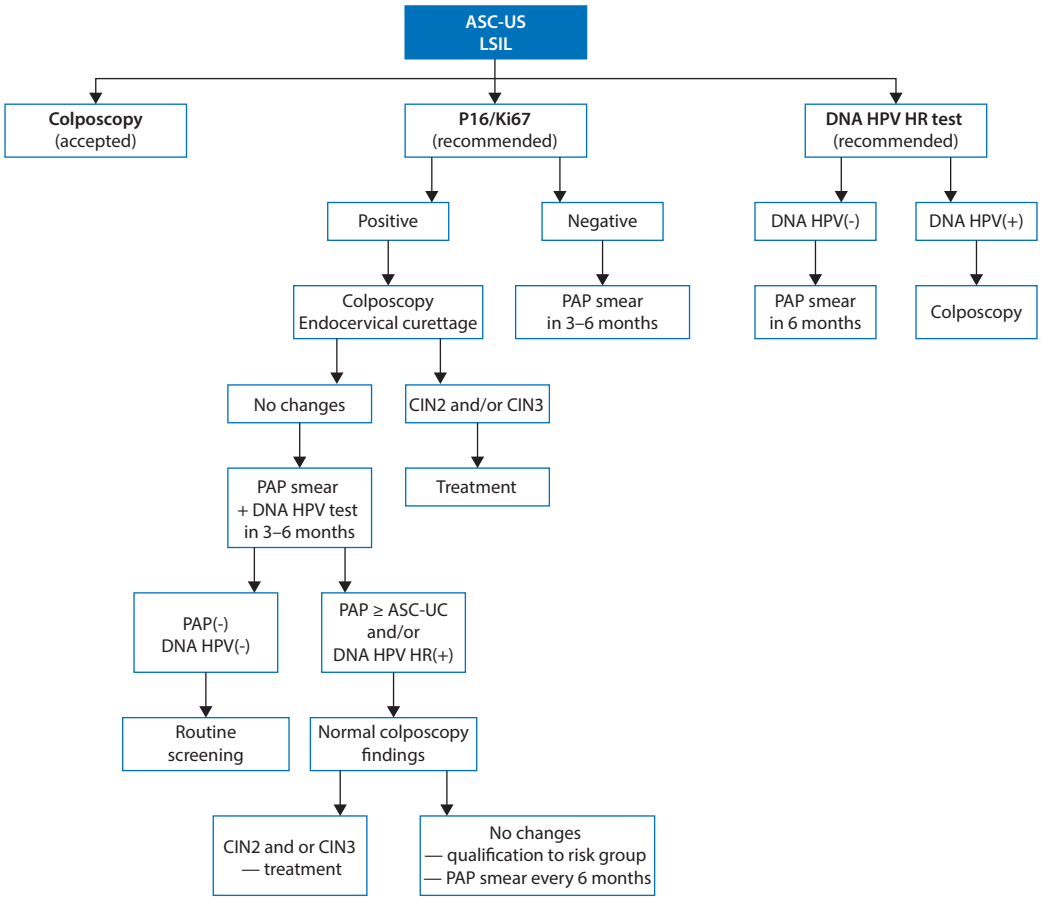

Figure 1. The algorithm for abnormal cytologic result with immunocytodetection of p16/Ki67 proteins

CAP/ASCCP RECOMMENDATIONS FOR USING P16 AS A BIOMARKER OF PRECANCEROUS LESIONS IN CERVICAL SAMPLES

Recommendation 1

The most important diagnostic element of cervical precancerous lesions includes distinguishing actual precancerous changes such as HSIL from morphological changes imitating HSIL described as "*Negative for Intraepithelial lesion or malignancy*" (NILM). The following morphological images fall into this category: early squamous metaplasia, atrophic changes in the epithelium, repair, and regeneration after inflammation. A negative test for p16 enables a NILM diagnosis, whereas a positive p16 test corresponds with HSIL (Fig. 2).

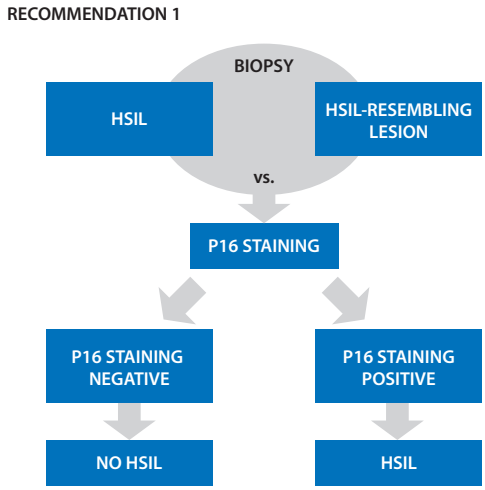

Figure 2. The algorithm for differentiation of HSIL and NILM with histological examination using the p16 biomarker

Recommendation 2

Changes in pap smears recognized in hematoxylin and eosin staining as HSIL (CIN II) classified between LSIL or changes associated with infection with no HPV etiology, not about actual changes HSIL requires p16 staining applications. A negative result of p16 staining indicates the presence of LSIL OR NILM and a change not related to HPV infection , while strong and poured reaction with p16 corresponds to the change of a HSIL (Fig. 3)

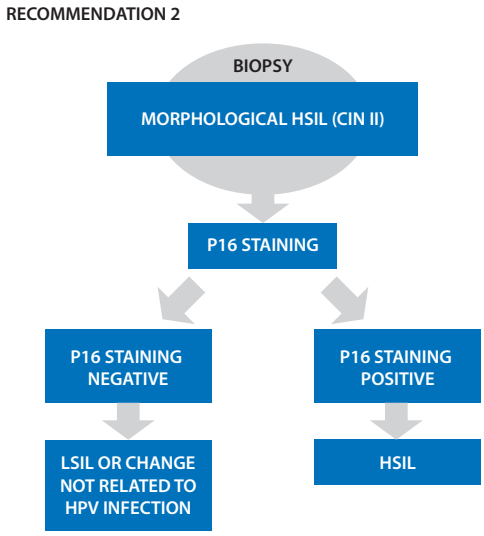

Figure 3. The algorithm for histological differentiation between HSIL (CIN II) with real premalignant conditions (e.g. HSIL)

Recommendation 3

Immunohistochemical testing for p16 is also useful for differentiation between LSIL and HSIL in case of any inconsistency in opinions of the specialists regarding assessment of the samples obtained from the cervix in haematoxylin and eosin staining (Fig. 4).

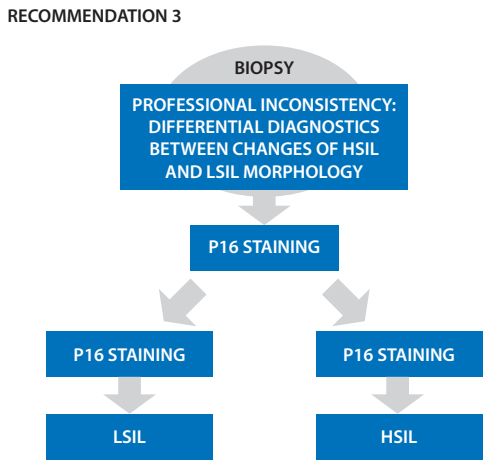

Figure 4. The algorithm for diagnostic inconsistency between specialists in pathology after diagnosing LSIL and HSIL

Recommendation 4

Immunohistochemical staining p16 is not recommended as a routine diagnostic test for lesions that present with clear morphology of either HSIL (CIN II) or LSIL (Fig. 5).

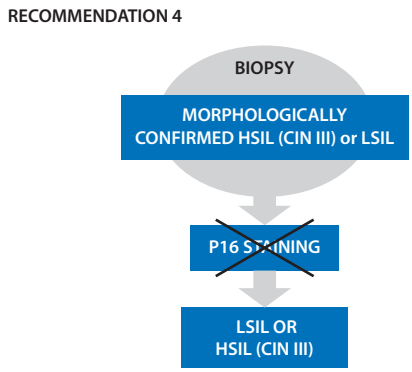

Figure 5. The diagnostic algorithm when the histological diagnosis of LSIL and HSIL is clear in haematoxylin and eosin staining

Recommendation 5

It is recommended to perform immunohistochemical testing for p16 in patients with a cervical lesion less advanced than LSIL, in whom previous cytologic evaluation detected HSIL, ASC-H, ASC-US/HPV16 or AGC. A negative result for p16 testing indicates the presence of LSIL or a lesion induced by HPV infection whereas a positive p16 reaction corresponds with HSIL. Immunohistochemical expression of p16 also confirms cervical adenocarcinoma in situ (AGC).

SUMMARY. RECOMMENDATIONS FOR USING THE P16 BIOMARKER FOR HISTOPATHOLOGICAL EXAMINATION OF SPECIMENS OBTAINED FROM THE CERVIX

1. The result of immunohistochemical reaction for p16 should be interpreted as a supplement for histopathological analysis of the specimen stained with haematoxylin and eosin.
2. Strong, homogeneous and spread colour reaction is interpreted as a positive result of p16.
3. A negative result of p16 testing eventually enables the diagnosis of lesions such as NILM, LSIL and lesions unrelated with HPV infection.
4. A positive result of p16 should be interpreted as HSIL.
5. Every positive result of p16 in the immunohistochemical reaction requires further diagnostics such as colposcopy and diagnostic abrasion.

Conflict of interest

The authors declare that there is no conflict of interest in the presented recommendations.

REFERENCES

1. Benevolo M, Mottolese M, Marandino F, [et al.]. Immunohistochemical expression of p16(INK4a) is predictive of HR-HPV infection in cervical low-grade lesions. *Mod Pathol.* 2006,19, 384–391.
2. Benevolo M, Terrenato I, Mottolese M, [et al.]. Comparative evaluation of nm23 and p16 expression as biomarkers of high-risk human papillomavirus infection and cervical intraepithelial neoplasia 2(+) lesions of the uterine cervix. *Histopathology* 2010, 57, 580–586.
3. Bergeron C, Ordi J, Schmidt D, Trunk MJ, Keller T, Ridder R. Conjunctive p16INK4a testing significantly increases accuracy in diagnosing high-grade cervical intraepithelial neoplasia. *Am J Clin Pathol.* 2010, 133, 395–406.
4. Darragh TM, Colgan TJ, Cox JT, [et al.]. 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.
5. Del Pino M, Garcia S, Fuste V, [et al.]. Value of p16(INK4a) as a marker of progression/regression in cervical intraepithelial neoplasia grade 1. *Am J Obstet Gynecol.* 2009, 201, 88.e1–7.
6. 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.
7. Klaes R, Benner A, Friedrich T, [et al.]. p16INK4a immunohistochemistry improves interobserver agreement in the diagnosis of cervical intraepithelial neoplasia. *Am J Surg Pathol.* 2002, 26, 1389–1399.
8. Kurman RJ, Carcangiu ML, Herrington CS, Young RH (eds).WHO Classification of Tumors of Female Reproductive Organs. IARC Lyon 2014.
9. Negri G, Vittadello F, Romano F, [et al.]. p16INK4a expression and progression risk of low-grade intraepithelial neoplasia of the cervix uteri. *Virchows Arch.* 2004, 445, 616–620.
10. Ozaki S, Zen Y, Inoue M. Biomarker expression in cervical intraepithelial neoplasia: potential progression predictive factors for low-grade lesions. *Hum Pathol.* 2011, 42, 1007–1012.
11. Riethdorf S, Neffen EF, Cviko A, Loning T, Crum CP, Riethdorf L. p16INK4a expression as biomarker for HPV 16–related vulvar neoplasias. *Hum Pathol.* 2004, 35, 1477–1483.
12. Rokita W, Skawiński D, Zmelonek-Znamirowska A. Wyniki badań cytologicznych i immunocytochemiczna identyfikacja białek p16 i Ki67 u kobiet ze śródnabłonkową neoplazją i rakiem szyjki macicy. *Ginekol Pol.* 2012, 83, 822–826.
13. Schmidt D, Bergeron C, Denton KJ, Ridder R. p16/ki-67 dual-stain cytology in the triage of ASCUS and LSIL papanicolaou cytology: results from the European equivocal or mildly abnormal papanicolaou cytology study. *Cancer Cytopathol.* 2011, 119, 158–166.
14. Singh M, Mockler D, Akalin A, Burke S, Shroyer A, Shroyer KR. Immunocytochemical colocalization of P16(INK4a) and Ki-67 predicts CIN2/3 and AIS/adenocarcinoma. *Cancer Cytopathol.* 2012, 120, 26–34.
15. Petry KU, Schmidt D, Scherbring S, [et al.]. Triaging Pap cytology negative, HPV positive cervical cancer screening results with p16/Ki-67 Dual-stained cytology. *Gynecol Oncol.* 2011, 121, 505–509.
16. Nasierowska-Guttmejer A, Górnicka B (ed.). Zalecenia do diagnostyki histopatologicznej nowotworów. Centrum Onkologii, Oddział Gliwice, Polskie Towarzystwo Patologów, Warszawa 2013.