UDC 618.146 -006.6-036.3-07 https://doi.org/10.26641/2307-0404.2021.2.234521

I.Z. Gladchuk,
N.M. Rozhkovska,
N.M. Kashtalian

THE ROLE OF IMMUNOCYTOCHEMICAL BIOMARKERS IN DIAGNOSTICS OF PRECANCEROUS PATHOLOGY OF CERVIX

Odessa National Medical University
Department of Obstetrics and Gynecology
Valikhovskiy lane, 2, Odessa, 65026, Ukraine
Одеський національний медичний університет
кафедра акушерства і гінекології
(зав. – д. мед. н. проф. І.З. Гладчук)
Валіховський провулок, 2, Одеса, 65026, Україна
e-mail: nrozhkovska@ukr.net

Цитування: Медичні перспективи. 2021. Т. 26, № 2. С. 80-87
Cited: Medicni perspektivi. 2021;26(2):80-87

Key words: *cervical dysplasia, intraepithelial cervical neoplasia, diagnostics, immunocytochemistry, biomarkers, p16, Ki-67*
Ключові слова: *дисплазія шийки матки, цервікальна інтраепітеліальна неоплазія, діагностика, імуноцитохімія, біомаркери, p16, Ki-67*
Ключевые слова: *дисплазия шейки матки, цервикальная интраэпителиальная неоплазия, диагностика, иммуноцитохимия, биомаркеры, p16, Ki-67*

Abstract. The role of immunocytochemical biomarkers in diagnostics of precancerous pathology of cervix. Gladchuk I.Z., Rozhkovska N.M., Kashtalian N.M. *The last decades showed a worldwide tendency to find consensus between diagnostics improvement and constant increase in the cost of medical services in conditions of restricted financing. The aim of the article was to analyze the diagnostic value of p16 and Ki-67 biomarkers in diagnostics of precancerous diseases of cervix. Data of 80 patients with cervical dysplasia of varying degree who received excisional treatment were analyzed. It was shown that cytological study has a high sensitivity (79.17%) for the diagnosis of cervical intraepithelial neoplasia (CIN) 2-3, but low specificity (53.57%). The p16 immunocytochemical biomarker has a high sensitivity for the diagnosis of CIN 2 (0.92; 95% CI: 0.76-0.98) with good specificity (0.78; 95% CI: 0.67-0.82), for the diagnosis of CIN 3 both sensitivity (0.93; 95% CI: 0.82-0.98) and specificity (0.93; 95% CI: 0.82-0.98) is high. The immunocytochemical biomarker Ki-67 has a high sensitivity for CIN 2 (0.92; 95% CI: 0.65-0.99), but insufficient specificity (0.62; 95% CI: 0.54-0.64), for the diagnosis of CIN 3 the sensitivity is very high (0.96; 95% CI: 0.80-0.99) as well as specificity (0.78; 95% CI: 0.69-0.81). The combined use of p16 and Ki-67 biomarkers can significantly increase the diagnostic accuracy of the diagnosis of high-grade precancerous pathology of cervix and justify timely surgical intervention. Such an approach for the differential diagnosis of severe dysplasia, on the one hand, may contribute to a decrease in the risk of developing cervical cancer, and on the other hand, it will allow to avoid unnecessary operations and preserve reproductive function of women, reduce the economic costs of treatment.*

Реферат. Роль иммуноцитохимических биомаркеров в диагностике предраковой патологии шейки матки. Гладчук И.З., Рожковская Н.Н., Каштальян Н.М. *На протяжении последних десятилетий по всему миру идет поиск консенсуса между улучшением диагностики и ростом стоимости медицинских услуг в условиях ограниченного финансирования. Целью работы было изучение диагностической ценности использования биомаркеров p16 и Ki-67 в диагностике предраковых заболеваний шейки матки. Исследовано данные 80 пациенток с дисплазией шейки матки различной степени тяжести, получивших эксцизионное лечение шейки матки. В результате показано, что цитологическое исследование имеет высокую чувствительность (79,17%) диагностики ЦИН 2-3, но низкую специфичность (53,57%). Иммуноцитохимический биомаркер p16 имеет высокую чувствительность диагностики ЦИН 2 (0,92; 95% ДИ: 0,76-0,98) при хорошей специфичности (0,78; 95% ДИ: 0,67-0,82), для диагностики ЦИН 3 чувствительность (0,93; 95% ДИ: 0,82-0,98) и специфичность (0,93; 95% ДИ: 0,82-0,98) высокие. Иммуноцитохимический биомаркер Ki-67 имеет высокую чувствительность для ЦИН 2 (0,92; 95% ДИ: 0,65-0,99), но недостаточную специфичность (0,62; 95% ДИ: 0,54-0,64), для диагностики ЦИН 3 чувствительность очень высока (0,96; 95% ДИ: 0,80-0,99), как и специфичность (0,78; 95% ДИ: 0,69-0,81). Сочетанное использование биомаркеров p16 и Ki-67 может значительно повысить диагностическую точность диагностики тяжелой предраковой патологии шейки матки и обосновать проведение своевременного хирургического вмешательства. Такой подход для дифференциальной диагностики тяжелой дисплазии будет способствовать, с одной стороны, уменьшению риска развития рака*

шейки матки, а с другой – позволит избежать ненужных операций и сохранить репродуктивную функцию женщин при уменьшении экономических затрат на лечение.

Despite the fact that cytological screening effectively reduces the frequency of cases of cervical cancer, precancerous diseases of the cervix – dysplasia, or cervical intraepithelial neoplasia (CIN) associated with a lesion of human papilloma virus (HPV) of high oncogenic risk, continue to represent a significant burden for health systems [3, 4].

During the last decades worldwide, there is an intensive development of pharmacoeconomics in order to find consensus between the improvement of diagnostics due to the emergence of new technologies and an increase in the cost of medical services in conditions of limited funding capabilities, especially in the context of a modern global crisis. Treatment and further follow-up of patients with CIN is of a high cost, in addition, surgical treatment can be accompanied by complications, which also increases both risks for health and reproductive function of women and the cost of health care delivery [10, 11].

Management tactics of women with a mild dysplasia or dysplasia of the 1st degree (CIN 1) causes the greatest contradiction: from follow-up to immediate treatment [11]. Dysplasia of the 1st degree is more common in young women, but despite this, in 15-20% of patients with a cytological diagnosis of a mild dysplasia, more significant precancerous changes (CIN 2-3) [4] are detected histologically. Consequently, unjustified or ungotten treatment in hyper- and hyperdiagnostics of precancerous lesions of the cervix is possible due to a low specificity of screening tests [3, 10].

Expression of host cell genes varies under the influence of oncogenic HPV products, including proliferation markers such as KI-67, and cell cycle control – P16. Revealing of atypical cells of the squamous cervical epithelium expressing simultaneously P16 and Ki-67 indicates the induced cell cycle disregulation, i.e. a higher degree of severity. Consequently, the estimation of the prognostic value of biomarkers P16 and KI-67 is extremely relevant for differential diagnostics of metaplastic and dysplastic changes and determining management tactics.

The purpose of the work was to study the diagnostic value of the use of immunocytochemical biometers P16 and Ki-67 in the diagnosis of precancerous pathology of the cervix.

MATERIALS AND METHODS OF RESEARCH

We examined 80 women with cytologically confirmed cervical dysplasia, which received an excision treatment in a multi-field medical center of the Odessa National Medical University. All women were surveyed in accordance with the current clinical guideliness, the study was conducted in compliance with bioethical norms (minutes of meeting of the Commission on Bioethics of Odessa National Medical University No. 134G dated 8/02/2019). The cytological study of the cervical epithelium of the transformation zone was carried out using a liquid-based cytology (LBC) method by BD SurePath ™ technology (USA) [9].

All patients were surveyed for high-oncogenic HPV types by polymerase chain reaction (PCR) with quantitative definition [8]. The evaluation of the expression of immunocytochemical biomakers P16 and Ki-67 was performed at a diagnostic stage in the cytological material [6]. BD Sure Path (Becton Discinson) tank with fixing transporting medium by means of analyzer and test-systems Cintec Plus (Roche, Switzerland) was used [9]. The research was performed in the European Laboratories Synevo (Ukraine).

Colposcopy was performed according to the standard technique (Colposcope Scaner MK200 with a digital video system, Ukraine). The colposcopic terminology of International Federation of Cervical Pathology and Colposcopy, IFCPC, Rio De Janeiro, 2011) was used.

Statistical processing of the results was carried out on a personal computer using Statistica 6 statistics (AXXR712 D833214fan5) and Medcalc (Version 14.8.1), which also built drawings and copied in Microsoft Word 2010. The difference was considered reliable at $p<0.05$ [1].

RESULTS AND DISCUSSION

The average age of surveyed women was 32±1.18 years. A mild dysplasia of the squamous cervical epithelium was detected in 35 (43.75%; 95% CI: 33.12%-54.87%) patients, moderate – in 30 (37.50%; 95% CI: 25.48%-46.51%) patients and severe dysplasia – in 15 (18.75%; 95% CI: 10.4%-27.6%) patients.

Among the detected HPV genotypes the most widespread were: 16, 33, 31, 18, 45, 39, 35, 58 and 59 genotypes. In the further analysis of virological status of patients, monoinfection of HPV was detected in the minority of patients (24 (30%; 95% CI: 19.95%-40.04%)), most patients (56 (70%; 95% CI: 59.95%-80.04%)) were infected with two or more highly monocogenic HPV genotypes. In addition, most patients (50 (62.50%; 95% CI: 52.42%-73.58%)) had a low viral load, average

indicators in patients with low viral load were 3.45±0.17 Ig HPV/10^5 cells. High viral load was diagnosed in 30 (37.87%; 95% CI: 27.36%-48.63%) patients with averages of 5.83±0.22 Ig HPV/10^5 cells.

According to international classification 43 (53.75%) patients had an atypical colposcopic picture of the 1st degree, 37 (26.25%) – an atypical colposcopic picture of the II degree.

After excisional treatment in each second patient (43 (51.25%; 95% CI: 40.04%-61.95%)), the diagnosis of CINs was not confirmed, but the cytopsychotic effects of HPV were detected: in 15 (18.75%; 95% CI: 10.4%-27.6%) – CIN 1, in 14 (17.50%; 95% CI: 9.58%-26.41%) – CIN 2 and in 10 (12.50%; 95% CI: 5.63%-20.37%) – CIN 3. There were no cases of cervical cancer.

To determine the diagnostic accuracy of the cytological method of research, we analyzed the correspondence of its results to the final histological diagnosis.

When comparing the results of cytological examination and histological diagnosis, hyperdiagnosis of dysplasia of the cervix in 41 (51.25%) was detected. Cases of hypodiagnostics were detected as well (Table 1).

Table 1

Correspondence between cytological and histological diagnoses

Cytological diagnosis	Histological diagnosis				
	without CIN n (%)	CIN 1 n (%)	CIN 2 n (%)	CIN 3 n (%)	In total n (%)
Mild dysplasia	23 (28.75%)	7 (8.75%)	4 (5.00%)	1 (1.25%)	35 (43.75%)
Moderate dysplasia	15 (18.75%)	5 (6.25%)	7 (8.75%)	3 (3.75%)	30 (37.50%)
Severe dysplasia	3 (3.75%)	3 (3.75%)	3 (3.75%)	6 (7.50%)	15 (18.75%)
In total	41 (51.25%)	15 (18.75%)	14 (17.50%)	10 (12.50%)	80 (100%)

In order to analyze the correspondence between cytological and histological diagnosis, the Cohen's coefficient was calculated, being 0.097±0.058 (CI: -0.016-0.210), and is considered as low.

The coincidence of cytological and histological diagnoses for CIN 1 and CIN 2-3 was in 61.25% of cases, in 6.25% of cases CIN 2-3 was hypodiagnosed and in 32.5% – hyperdiagnosed.

Based on these data, indicators of diagnostic value of cytological research in detection of CIN 2-3 degree were calculated (Table 2).

Thus, a cytological study has a high sensitivity of CIN 2-3 diagnostics in low specificity and positive prognostic value. The negative prognostic value of the study is high, and the total accuracy corresponds to the average indicators, that is, the use of only cytological examination in the diagnostics of severe dysplasia is not sufficient.

Analysis of the state of immunocytochemical biomarkers P16 and KI-67 was carried out in a cytological material of smears from the cervix. Among 41 patients with unconfirmed precancerous pathology of the cervix, 39 had a negative status P16 and 40, negative status K-67, out of 15 women with CIN 1 the majority had a negative status of both markers, out of 14 women with CIN 2 more than 70% of women had a positive status P16 and a positive status Ki-67 – more than 40%; out of 10 women with CIN 3, absolute majority had a positive status of both biomarkers (Table 3).

Table 2

Diagnostic value of cytological study

Indicator	Value	95% CI
Sensitivity	**0.7917**	**0.5729-0.9206**
Specificity	**0.5357**	**0.3986-0.6680**
Positive prognostic value	**0.4222**	**0.2799-0.5776**
Negative prognostic value	**0.8571**	**0.6896-0.9462**

Thus, an increase in the number of positive status of both biomarkers with increasing pathological changes in the cervix was noted, but a significant number of both false-positive and false-negative results for both biomarkers was marked, and statistical reliability was not always achieved.

Table 3

Analysis of the state of immunocystochemical biomarkers p16 and Ki-67 (n=80)

Pathomorphological diagnosis	p16		Ki-67	
	negative n (%) (95% CI)	positive n (%) (95% CI)	negative n (%) (95% CI)	positive n (%) (95% CI)
Norm	**39 (95.12%) (88.32-101.67)**	**2 (4.88%) (-1.67-11.67)**	**40 (97.56%) (93.71-102.28)**	**1 (2.44%) (-2.28-6.28)**
CIN 1	**8 (53.33%) (27.74-78.25)**	**7 (46.67%) (21.74-72.25)**	**13 (86.67%) (69.98-104.01)**	**2 (13.33%) (-4.01-30.01)**
CIN 2	**4 (28.57%) (6.03-51.96)**	**10 (71.43%) (48.03-93.96)**	**8 (57.14%) (31.94-82.05)**	**6 (42.86%) (17.94-68.1)**
CIN 3	**1 (10.00%) (-8.59-28.59)**	**9 (90.00%) (71.40-108.59)**	**3 (30.00%) (1.59-58.40)**	**7(70.00%) (41.59-98.4)**

In conducting an analysis of the state of the immunocytochemical biomarker P16, a significant increase in the odds ratio of the positive status P16 in severe dysplasia of the cervix was detected. The odds ratio of the positive status P16 compared to the absence of CIN increases by 12.25 times in CIN 1, by 38.50 times in CIN 2 and by 196.00 times in CIN 3; this may be a predictor of severe CIN at a pre-excision stage (Fig. 1).

In conducting an analysis of the state of the immunocytochemical biomarker K-67, an increase in the odds ratio of positive status of K-67 in severe dysplasia of the cervix was found, as compared with the absence of CIN by 19.33 times in CIN 2 and by 79.75 times in CIN 3 (Fig. 2). The increase in the chances of the positive status of K-67 in CIN 1 was not statistically significant.

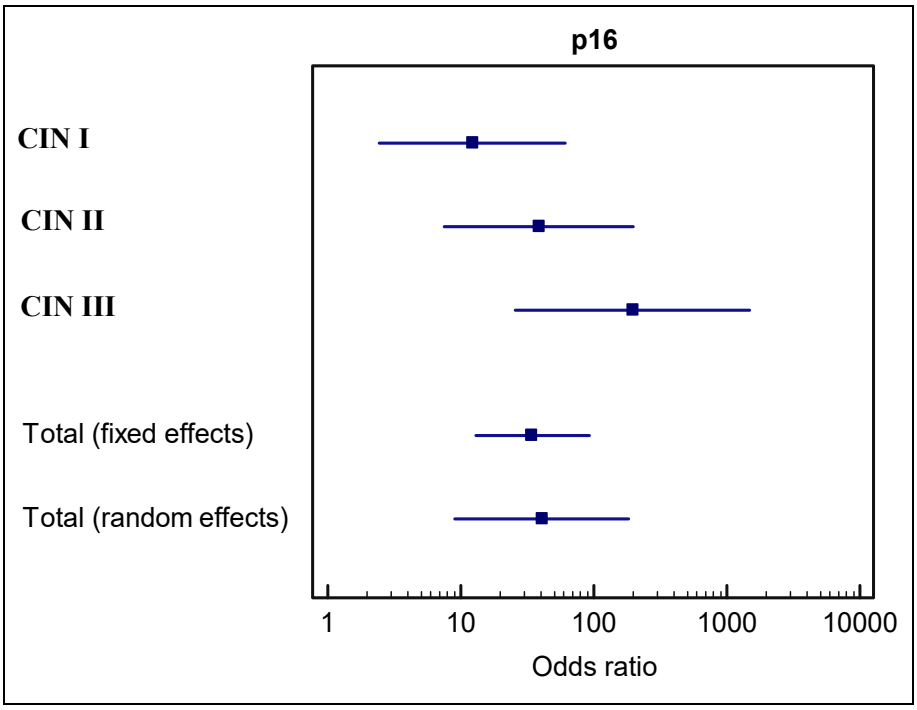

Fig. 1. Odds ratio of positive status of biomarker Ki-67 in CIN

Thus, the biomarker Ki-67 is not an indicant in diagnosis of CIN 1 but despite lower than P16 odds ratio of a positive status in severe dysplasia, biomarker Ki-67 is a valuable prognostic marker of CIN 3 at the pre-excision stage.

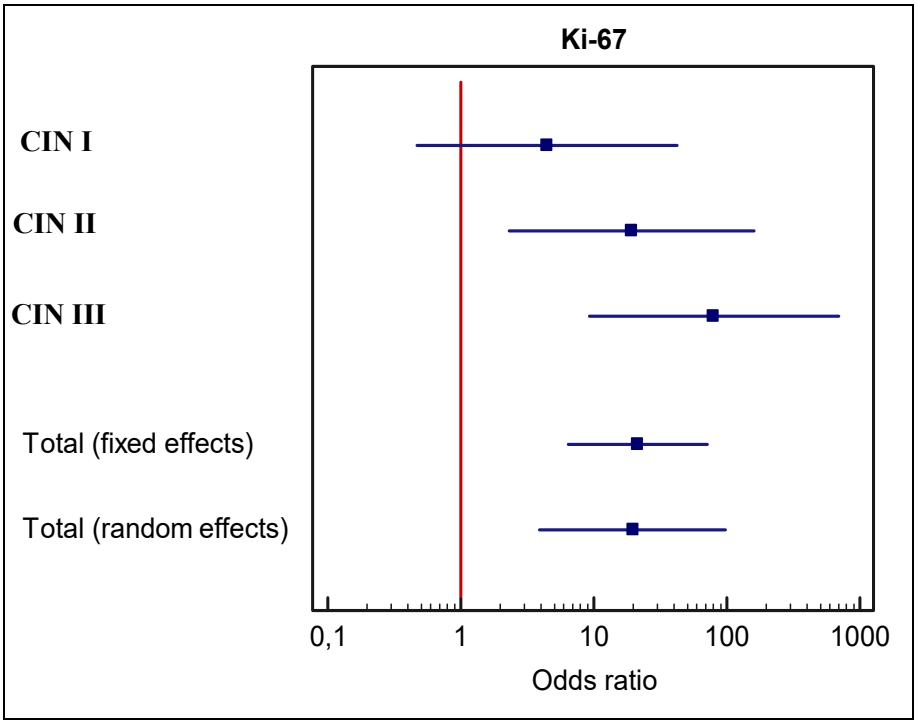

Fig. 2. Odds ratio of positive status of biomarker Ki-67 in CIN

We also analyzed the diagnostic value of combined use of immunocytochemical biomarkers P16 and Ki-67 in diagnostics of CIN (Table 4).

According to the analysis, the immunocyto-chemical biomarker P16 has high sensitivity, but insufficient specificity for CIN 1 diagnostics, for

CIN 2 diagnostics sensitivity is high with sufficient specificity, for CIN 3 diagnostics sensitivity and specificity of biomarker P16 is high with the best indicators. The immunocystochemical biomarker Ki-67 has insufficient sensitivity and specificity for CIN 1 diagnostics, for CIN 2 diagnostics sensitivity is high, but the specificity is still insufficient, for CIN 3 diagnostics sensitivity is very high with sufficient specificity.

Table 4

Sensitivity and specificity of biomarkers p16 and Ki-67 in diagnostics of CIN

Diagnosis	p16		Ki-67	
	sensitivity (95% CI)	specificity (95% CI)	sensitivity (95% CI)	specificity (95% CI)
CIN 1	0.86 (0.62-0.98)	0.62 (0.54-0.66)	0.75 (0.22-0.98)	0.52 (0.48-0.54)
CIN 2	0.92 (0.76-0.98)	0.78 (0.67-0.82)	0.92 (0.65-0.99)	0.62 (0.54-0.64)
CIN 3	0.93 (0.82-0.98)	0.93 (0.82-0.98)	0.96 (0.80-0.99)	0.78 (0.69-0.81)

Thus, according to the data obtained, the sensitivity and specificity of the immunocytochemical analysis of P16 status was: in the diagnosis of CIN 1 – 86% and 62%, CIN 2 – 92% and 78%, CIN 3 – 93% and 93% respectively. Sensitivity and specificity of the immunocytochemical analysis of K-67 status was: in the diagnosis of CIN 1 – 75% and 52%, CIN 2 – 92% and 62%, CIN 3 – 96% and 78% respectively.

Literary data of recent years also confirm a linear correlation between the positive status of P16 and the growth of the degree of CIN, greater informativeness of the marker, rather than coloscopy, and high sensitivity and specificity of the detection of CIN 2+ in positive immunocytochemical staining P16, which significantly increases values almost to 100% in a double-positive staining P16/KI-67 [2, 5, 7, 12, 13]. In meta-analysis of 2016 it is found that the determination of coexpression of P16/Ki-67 is a reliable additional method of confirming CIN 2-3 in women with pathological results of cytomorphological examination [6]. In the literary review Ziemke and Gresser indicate that, despite different research design and statistical differences in their results, the consensus on a significant increase in specificity and positive prognostic value for detecting precancerous pathology of the cervix in P16/Ki-67 positive immunocytochemical staining in comparison with cytomorphological examination and detecting DNA of HPV is unchanged; this is particularly justified in cases of persistence of mild dysplasia and in uncertain results of cytomorphological examination (ASC-H) [14].

Thus, a combined determination of coexepression of both biomarkers P16 and Ki-67 in one cell increases sensitivity and specificity of CIN 2-3 detection and differential diagnosis of light and severe dysplasia of the cervix.

CONCLUSIONS

1. The cytological study has a sufficiently high sensitivity (79.17%) of CIN 2-3 diagnostics, but low specificity (53.57%). In general, the correspondence between cytological and histological diagnosis is low (K=0.097), which reduces the diagnostic capabilities of the method to detect a severe dysplasia of the cervix.
2. Immunocytochemical biomarker P16 has a high sensitivity of CIN 2 diagnostics (0.92; 95% CI 0.76-0.98) with good indicators of specificity (0.78; 95% CI: 0.67-0.82), for diagnostics of CIN 3, sensitivity (0.93; 95% di: 0.82-0.98) and specificity (0.93; 95% CI: 0.82-0.98) are high. Immunocytochemical biomarker Ki-67 has a high sensitivity of CIN 2 diagnostics (0.92; 95% CI: 0.65-0.99), but insufficient specificity (0.62; 95% CI: 0.54-0.64), for diagnostics of CINs 3 sensitivity is very high

(0.96; 95% CI: 0.80-0.99) with good specificity indicators (0.78; 95% CI: 0.69-0.81).

3. The combined use of immunocytochemical biomarkers P16 and Ki-67 can significantly increase the diagnostic accuracy of the detection of severe cervical pathology, substantiate approaches to timely surgical intervention. Such an approach for differential diagnosis of mild and severe dysplasia will contribute to, on the one hand, reducing the risk of developing a cervical cancer, and on the other – to avoid unnecessary surgeries and preserve the reproductive function of women with a decrease in economic costs for treatment.

Conflict of interest. The authors declare no conflict of interest.

REFERENCES

1. Antomonov M. [Mathematical processing and analysis of biomedical data]. 2nd ed. Kyiv: MIC «Medinform». 2018. p. 579. Russian. ISBN 978-966-409-202-6
2. Hnatko OP, Skuriatina NH, Berezhna TA. [Diagnostic value of markers of proliferation in determination of the degree of severity of cervical precancerous lesions]. Aktalni pytannia pediatrii, akusherstva ta hinekologii. 2018;1:56-61. Ukrainian. Available from: https://ojs.tdmu.edu.ua/index.php/act-pit-pediatr/article/-view/8736
3. Zaporozhan VM, et al. [National consensus in management of patients with cervical intraepithelial neoplasia caused by papillomaviral infection]. Zdorovia zhinky. 2017;123(7):16-24. Ukrainian. Available from: https://ecopharm.ua/wp-content/uploads/2016/10/ZZH-7_Consensus-2.pdf
4. Singer A, Khan A. [Cervical and lower genital tract precancer: diagnosis and treatment]. Moskow: HEOTAR-Media; 2017. p. 312 Russian. ISBN: 978-5-9704-4296-8
5. Tumanskii VA, Pyrohova ZA. [Specifics of Ki-67, p16ink4a, HPV16v immune histochemical expression in cervical intraepithelial neoplasia and cervical carcinoma]. Patolohiia. 2017;14(2):202-8. Ukrainian. Available from: http://pat.zsmu.edu.ua/article/view/109298
6. Chen CC, Huang LW, Bai CH, Lee CC. Predictive value of p16/Ki-67 immunocytochemistry for triage of women with abnormal Papanicolaou test in cervical cancer screening: a systematic review and meta-analysis. Annals of Saudi medicine. 2016;36(4):245-1. doi: https://doi.org/10.5144/0256-4947.2016.245
7. Khazaei S, Izadi B, Mirbahari SG, et al. Comparison Between Two Detection Methods for HPV16, HPV18 and P16Ink4a Biomarkers in Diagnosis of Abnormal Cervical Cytology. Asian Pac J Cancer Prev. 2016;17(12):5223-7. Published 2016 Dec 1. doi: https://doi.org/10.22034/APJCP.2016.17.12.5223
8. Zhang L, Dai Y, Chen J, et al. Comparison of the performance in detection of HPV infections between the high-risk HPV genotyping real time PCR and the PCR-reverse dot blot assays. J Med Virol. 2018;90(1):177-83. doi: https://doi.org/10.1002/jmv.24931
9. Rozemeijer K, Penning C, Siebers AG, Naber SK, Matthijsse SM, van Ballegooijen M, van Kemenade FJ, de Kok IM. Comparing SurePath, ThinPrep, and conventional cytology as primary test method: SurePath is associated with increased CIN II+ detection rates. Cancer causes & control: CCC. 2016;27(1):15-25. doi: https://doi.org/10.1007/s10552-015-0678-1
10. Kyrgiou M, Kalliala IEJ, Mitra A, Fotopoulou C, Ghaem-Maghami S, Martin-Hirsch PPL, Cruickshank M, Arbyn M, Paraskevaidis E. Immediate referral to colposcopy versus cytological surveillance for minor cervical cytological abnormalities in the absence of HPV test. Cochrane Database of Systematic Reviews 2017;1:CD009836. doi: https://doi.org/10.1002/14651858.CD009836.pub2
11. Kyrgiou M, Athanasiou A, Kalliala IEJ, et al. Obstetric outcomes after conservative treatment for cervical intraepithelial lesions and early invasive disease. Cochrane Database Syst Rev. 2017;11(11):CD012847. Published 2017 Nov 2. doi: https://doi.org/10.1002/14651858.CD012847
12. Das D, Sengupta M, Basu K, Tirkey M, Datta C, Chatterjee U. Role of p16/Ki-67 Dual Immunostaining in Detection of Cervical Cancer Precursors. J Cytol. 2018;35(3):153-8. doi: https://doi.org/10.4103/JOC.JOC_4_17
13. Verma L, Shamsunder S, Malik S, Arora R. To Evaluate the Role of p16Ink4a Immunocytochemistry for Detection of CIN2+ in Women Detected Screen Positive by Visual Inspection Using Acetic Acid. J Cytol. 2020;37(2):82-86. doi: https://doi.org/10.4103/JOC.JOC_89_18
14. Ziemke P, Griesser H. p16-/Ki-67 in der Zervix-Zytologie: Indikationen [Indications for p16/Ki-67 in cervical cytology]. Pathologe. 2017;38(1):38-44. doi: https://doi.org/10.1007/s00292-016-0262-9

СПИСОК ЛІТЕРАТУРИ

1. Антомонов М. Ю. Математическая обработка и анализ медико-биологических данных. 2-е изд. Київ: МИЦ «Мединформ». 2018. 579с. ISBN978-966-409- 202-6
2. Гнатко О. П., Скурятіна Н. Г., Бережна Т. А. Діагностичне значення маркерів проліферації у визначенні ступеня важкості передракових станів шийки матки. *Актуальні питання педіатрії, акушерства та гінекології.* 2018. № 1. С. 56-61. URL: https://ojs.tdmu.edu.ua/index.php/act-pit-pediatr/article/view/8736

3. Національний консенсус щодо ведення пацієнток з цервікальними інтраепітеліальними неоплазіями, зумовленими папіломавірусною інфекцією / В. М. Запорожан та ін. *Здоров'я жінки.* 2017. Т. 123, № 7. С. 16-24.
URL: https://ecopharm.ua/wp-content/uploads/2016/10/ZZH-7_Consensus-2.pdf

4. Сингер А., Хан А. Предраковые заболевания шейки матки, влагалища, вульвы: диагностика и лечение. Москва: ГЭОТАР-Медиа, 2017. 312 с. ISBN: 978-5-9704-4296-8

5. Туманський В. А., Пирогова З. А. Особливості імуногістохімічної експресії Ki-67, p16INK4a, HPV16 у цервікальній інтраепітеліальній неоплазії та карциномі шийки матки. *Патологія*. 2017. Т. 14, № 2(40). С. 202-208.
URL: http://pat.zsmu.edu.ua/article/view/109298

6. Chen C. C., Huang L. W., Bai C. H., Lee C. C. Predictive value of p16/Ki-67 immunocytochemistry for triage of women with abnormal Papanicolaou test in cervical cancer screening: a systematic review and meta-analysis. *Annals of Saudi medicine.* 2016. Vol. 36, No. 4. P. 245-251. DOI: https://doi.org/10.5144/0256-4947.2016.245

7. Comparison Between Two Detection Methods for HPV16, HPV18 and P16Ink4a Biomarkers in Diagnosis of Abnormal Cervical Cytology / S. Khazaei et al. *Asian Pac J Cancer Prev.* 2016. Vol. 17, No. 12. P. 5223-5227. DOI: https://doi.org/10.22034/APJCP.2016.17.12.5223

8. Comparison of the performance in detection of HPV infections between the high-risk HPV genotyping real time PCR and the PCR-reverse dot blot assays / L. Zhang et al. *J Med Virol*. 2018. Vol. 90, No. 1. P. 177-183. DOI: https://doi.org/10.1002/jmv.24931

9. Comparing SurePath, ThinPrep, and conventional cytology as primary test method: SurePath is associated with increased CIN II+ detection rates / K. Rozemeijer et al. *Cancer causes & control*: CCC. 2016. Vol. 27, No. 1. P. 15-25.
DOI: https://doi.org/10.1007/s10552-015-0678-1

10. Immediate referral to colposcopy versus cytological surveillance for minor cervical cytological abnormalities in the absence of HPV test / M. Kyrgiou et al. *Cochrane Database of Systematic Reviews 2017*. No. 1. Art. No.: CD009836.
DOI: https://doi.org/10.1002/14651858.CD009836.pub2

11. Obstetric outcomes after conservative treatment for cervical intraepithelial lesions and early invasive disease / M. Kyrgiou et al. *Cochrane Database Syst Rev*. 2017. Vol. 11, No. 11. CD012847. Published 2017. 2 Nov.
DOI: https://doi.org/10.1002/14651858.CD012847

12. Role of p16/Ki-67 Dual Immunostaining in Detection of Cervical Cancer Precursors / D. Das et al. *J Cytol.* 2018. Vol. 35, No. 3. P. 153-158. DOI: https://doi.org/10.4103/JOC.JOC_4_17

13. Verma L, Shamsunder S, Malik S, Arora R. To Evaluate the Role of p16Ink4a Immunocytochemistry for Detection of CIN2+ in Women Detected Screen Positive by Visual Inspection Using Acetic Acid. *J Cytol*. 2020. Vol. 37, No. 2. P. 82-86.
DOI: https://doi.org/10.4103/JOC.JOC_89_18

14. Ziemke P, Griesser H. p16-/Ki-67 in der Zervix-Zytologie: Indikationen [Indications for p16/Ki-67 in cervical cytology]. *Pathologe*. 2017. Vol. 38, No. 1. P. 38-44. DOI: https://doi.org/10.1007/s00292-016-0262-9

The article was received
2020.04.29