CORRELATION BETWEEN CERVICAL CYTOLOGY AND HISTOPATHOLOGICAL CERVICAL BIOPSY FINDINGS ACCORDING TO THE BETHESDA SYSTEM

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STEPEN KORELACIJE CERVIKALNE CITOLOGIJE PO BETHESDA KLASIFIKACIJI SA PATOHISTOLOŠKIM NALAZIMA CERVIKALNE BIOPSIJE

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ABSTRACT

The Pap test (Pap smear) is a morphological cytodiagnostic test that reveals premorbid and early stages of cervical cancer through the use of cervicovaginal cytology.

The present study was conducted at the Department of Gynecology and Obstetrics, Clinical Center Kragujevac, from January 01, 2013, to December 1, 2013, with patients who were part of the national screening program and who used a secondary cytological examination (Pap test) performed using a conventional method.

The patients were grouped according to the results of a Pap smear and histopathological findings. The classification of cytological smears was performed in accordance with the Bethesda system. The hypotheses established in this study were statistically tested.

The greatest number of cytological findings was NILM. However, the most frequent abnormal cytological findings in terms of percentage were ASC-US. After secondary cervical findings of 8.1% of the total number of women, biopsies were performed. In 68.57% of the biopsies performed, CIN was present in all of them, with the most frequent ones being LSIL (50.6%), HSIL (10.4%) and CA Invasiva (0.5%). This study, using the X^2 test, confirmed that cervical cytology and biopsy results are dependent features (sig. = 0.036), between which there is a medium association (Cramer's V = 0.176). In the ASC-US cytological findings, small percentages of CIN1 and CIN2 were detected. Cervical cytology in this study presented high sensitivity, specificity, positive and negative predictive value. As a relatively inexpensive, painless and easily approachable method, cervical cytology fully substantiates its implementation in diagnostic procedures as well as in organized screening programs.

Keywords: *Cervical cancer, Pap test, Bethesda system*

SAŽETAK

Papa test je morfološki citodijagnostički test kojim se pomoću analize cervikovagnalne citologije otkrivaju predstadijumi i rani stadijumi karcinoma grlića materice.

Studija je sprovedena na Klinici za ginekologiju i akušerstvo, KC Kragujevac u periodu od 01. 01. 2013. god. do 01. 12. 2013. god. na pacijentkinjama kojima je u okviru Nacionalnog skrining programa odrađen sekundarni citološki pregled (PAP test) konvencionalnom metodom.

Pacijentkinje su grupisane prema rezultatima PAP testa i patohistološkim rezultatima. Klasifikacija citoloških briseva je vršena prema Bethesda klasifikaciji. Statističkim testovima su testirane hipoteze postavljene u ovoj studiji.

Najveći broj citoloških nalaza bio je NILM. Procentualno najzastupljeniji abnormalni citološki nalazi su bili ASCUS. Nakon sekundarnog cervikalnog nalaza kod 8,1% od ukupnog broja žena je odrađena biopsija. U 68,57% urađenih biopsija je bio prisutan CIN od kojih je najzastupljeniji bio nalaz LSIL (50,6%), zatim HSIL (10,4%), i CA INVASIVA (0,5%). Ovom studijom, uz primenu Xi^2 testa, potvrđeno je da su cervikalna citologija i rezultati biopsije zavisna obeležja (Sig.=0,036), između kojih postoji veza srednje jačine (Cramer's V=0,176). U citološkom nalazu ASCUS detektovano je mali procenat CIN1 i CIN2. Cervikalna citologija u ovoj studiji pokazuje visoku senzitivnost, specifičnost, pozitivnu i negativnu prediktivnu vrednost. Kao relativno jeftina, bezbolna i lako dostupna metoda u potpunosti potvrđuje svoju primenu, kako u dijagnostičkim procedurama, tako i u organizovanim skrining programima.

Ključne reči: *karcinom grlića materice, PAP test, Bethesda klasifikacija*

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INTRODUCTION

Gynaecological cytodiagnostics are relatively quick, inexpensive and minimally invasive methods that can detect precancerous and cancerous conditions of the vagina, vulva and uterus, which promote adequate and timely treatment (1).

According to the Cancer Registry of Central Serbia, in 2011, 882 new cases of cervical cancer occurred within the territories of all districts, which placed it fourth in incidence behind newly discovered cases of breast, colon and rectal, lung and bronchial cancer; of those identified, 879, or 99.7%, were verified using cytological and histological methods (2).

In the Sumadia region, 66 new cases of cervical cancer were identified in 2011, second in incidence to breast cancer.

The 2011 cancer incidence rate per 100 000 individuals in the population of all districts in Central Serbia was 32.3 for primarily localized cervical cancer (with a standardized incidence rate of 22.1).

The total number of deaths from cervical cancer in central Serbia in 2011 numbered 347; in the Šumadia region, 15 women died of this type of cancer in 2011, which is the eighth highest compared to other districts (2).

Cervical intraepithelial neoplasms (CINs) are gross abnormalities of the squamous epithelium comprising two groups of lesions:

- LSILs (low-histological-grade squamous intraepithelial lesions), which include flat warts and CIN1; and
- HSIL (high-histological-grade squamous intraepithelial lesions), which involve CIN2 and CIN3.

Human papillomavirus (HPV) plays a significant role in the development of cervical intraepithelial neoplasia. Clinic data show that the HPV DNA sequence can be found in more than 80% of squamous intraepithelial neoplasms and more than 98% of cervical cancers. Less than 2% of cervical carcinomas are negative for HPV DNA, which can be explained by undetected types of virus in the HPV gene or HPV having been lost in the process of oncogenesis (3).

Persistent HPV infection is a precursor for the development of cervical cancer. HPV types with high oncogenic potential (HPV16, HPV18) characteristically exhibit the tendency to provide persistent infection in comparison to low-oncogenic types (4). The emergence of the pathological process begins under the influence of so-called cocarcinogens: low immunity, smoking, genital infections caused by the influence of other viruses (e.g., HSV type 2, HIV, CMV, *Chlamydia trachomatis*), the effect of drugs (e.g., cytostatics, immunosuppressants), etc. A cocarcinogen causes circular viral DNA interruption, which is then integrated into the host cell's genome, and thus begins mutagenesis (3).

The Pap test is a cervicovaginal cytological smear stained using the Papanicolaou method, which combines in itself an exfoliative cell cytology obtained from the ectocervix and abrasive cell cytology obtained using an endocervical brush from the endocervical canal. The Papanicolaou method and classification in clinical practice was introduced by Dr. Georgios Papanicolaou (1954) (1,5). This morphological test uses a cervicovaginal cytology analysis to reveal pre-stages and early stages of cervical cancer; the Pap smear is actually carried out in order to prevent these malignant disease in women and can be used as a screening test in a population of women who have no symptoms of cervical disease or as a diagnostic test for patients with signs of gynaecological diseases resulting from a positive pre-established Pap test or diagnosed precancerous lesions or cancers of the cervix, vagina, vulva or uterus (6).

The first classification of Pap smears was Papanicolaou numerical cell cytology classification system (1954) that divided cervical (smears) swabs into five groups and had significant drawbacks (7,8,9).

A group of experts in cytopathology and histopathology and clinicians held a meeting in Bethesda (Maryland, USA) in 1988 with the aim of redefining the former classification of cytological findings to achieve uniformity and clearer and more relevant communication between cytopathologists and clinicians, which resulted in the establishment of the new Bethesda system. The original Bethesda system from 1988 was revised twice: first in 1991 and then in 2001 (10).

The interpretation of cervical cytology smears according to Bethesda system (in 1988, 1991, 2001) led to the transition from numerical Papanicolaou classification (1954) to descriptive clarification of the Pap test (Table1).

Table 1 Comparison of cytological classification systems (11).

Papanicolaou System	***Bethesda System***
Inadequate sample	Unsatisfactory result/ inadequate sample
PA I Normal result	Negative for intraepithelial lesion or malignancy, NILM
PA II Present inflammation, benign reactive and reparative changes	Present inflammation, benign reactive and reparative changes Negative for intraepithelial lesion or malignancy (no observed abnormality), NILM
IIIa Atypical cells of undetermined significance •squamous • glandular	ASC-US (in favour of reactive changes)) ASC-H (in favour dysplasia) AGC (atypical glandular cells)
IIIb Dyskariosis of a light degree Dyskaryosis of a medium degree	L-SIL (CIN 1) H-SIL (CIN 2)
IV Dyskariosis of a severe degree	H-SIL (CIN 3) AIS
V malignant cells	invasive carcinoma

The 2001 Bethesda classification system includes the following:

1. First, the adequacy of smear is assessed, which determines whether the smear is satisfactory or unsatisfactory.
2. Satisfactory smears are then classified into negative and pathological categories and interpreted.

A negative swab (negative for intraepithelial lesion or malignancy—NILM) may be a normal smear or swab with the presence of non-neoplastic changes.

Pathological smears involve pathological changes in the plate and/or the glandular epithelia or the presence of other cells (of uncertain significance and other types of cancer cells).

Pathological changes in the platelet-layered epithelium include the following:

1. Atypical squamous cells (atypical squamous cells of undetermined significance—ASC-US—and atypical squamous cells in which HSIL cannot be excluded—ASC-H);
2. Squamous intraepithelial lesions (LSIL and HSIL); and
3. Squamous epithelium carcinoma (PCA).

The pathological changes in the glandular epithelium include the following:

1. Atypical glandular cells not otherwise specified (AGC-NOS);
2. Atypical glandular cells, probably neoplastic (AGC-FN);
3. Endocervical adenocarcinoma in situ (AIS); and
4. Endocervical adenocarcinoma (CA-A) (6, 9, 10, 11, 12).

In this study, we tested the sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy of cervical cytology based on histological cervical biopsy as the "gold standard" for diagnosis. Based on the data obtained in this study, descriptive and analytical statistics were performed.

METHOD AND PATIENTS

The study was conducted at the Department of Gynecology and Obstetrics, Clinical Center Kragujevac, for a period of eleven months (from January 0 1, 2013, to December 01, 2013) with patients who responded to the national screening program for cervical cancer prevention at the invitation of selected gynaecologist who performed a gynaecological examination and sampled for a Pap test in the health centre in Kragujevac.

Cervical smears were taken by a conventional method according to an expert examination procedure.

Staining was performed according to the Papanicolaou method, in which the standardized manufactured tinctures were used. According to this dyeing method, three colours—the Harris hematoxylin (PA 1 colour) colour engine, which allows the visibility of chromatin, methyl orange (PA colour 2) and polichrome (PA colour 3), which differently colour the cytoplasm of cells, thereby enabling the differentiation of mature epithelial tissues . A composition of 96% alcohol was used for rinsing; xylene was used at the end of the dyeing process.

Coloured and air-dried preparations were placed on Canada balsam and covered glass measuring 24 mm x 50 mm (at least 24 mm x 40 mm) so that the whole smear was covered.

Microscopic analysis of the cervical Pap smear method was performed on a professional binocular microscope with magnifications of 10x, 20x and 100x.

For each cervical smear, a cervical cytological finding was issued and was evaluated with respect to the following factors :

1. Adequacy of the sample;
2. Whether the smear was negative or pathological;
3. Stage of the morphological changes;
4. Vaginal flora; and
5. The gynaecologist's recommendations on further treatment (12).

Satisfactory smears were classified simultaneously according to the Papanicolaou classification and the Bethesda system or the Bethesda system alone.

Within the screening program, a two-way analysis was carried out, wherein all plates were primarily analyzed by cytoscreeners performing a screening of suspected secondary swabs and were then evaluated by a certified clinical cervical cytology supervisor. To ensure the quality of the second cycle, 10% of normal findings, as well as all of the unsatisfactory ones, were also analyzed by the clinical supervisor. The cytological findings were published in the recommended form, with recommendations to the gynaecologist on further action.

After obtaining the cytological findings, the gynaecologists referred women with abnormal cervical cytological findings to colposcopy and/or further diagnostic procedures according to the professional and methodological recommendations provided in the Guide for the treatment and diagnosis of cervical cancer (11).

Cervical biopsies were taken under colposcopic control (i.e., targeted biopsies), with fields that showed the greatest degree of abnormality. Curettage of the cervical canal (endocervical curettage—ECC) was necessary in order to diagnose endocervical lesions that were not observed through colposcopy in cases of squamocolumnar junction (SCJ) that could be visualized and for which cytological findings were abnormal.

The data obtained in this study were statistically analyzed and compared with the help of the program PASW Statistics 18. For the statistical tests, we applied the Kolmogorov-Smirnov test, Kruskal-Wallis test and the chi-square test.

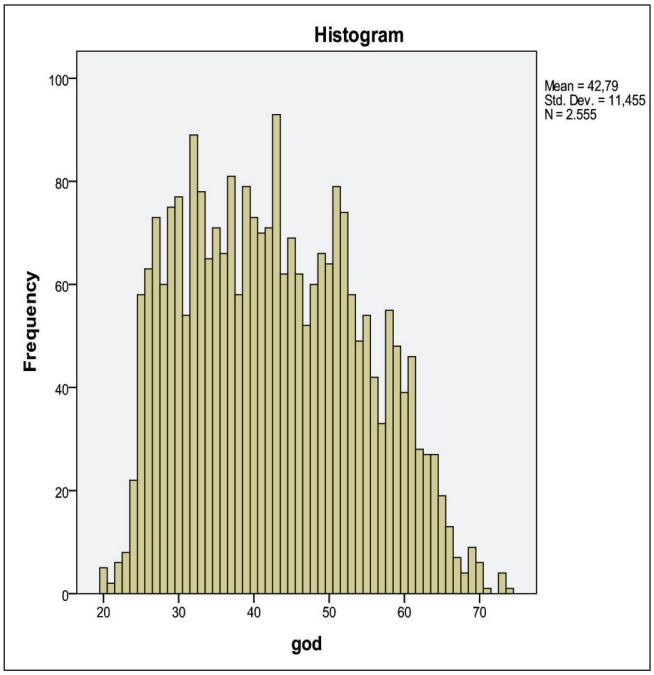

Figure 1. Distribution of the female patients who participated in the screening compared by age

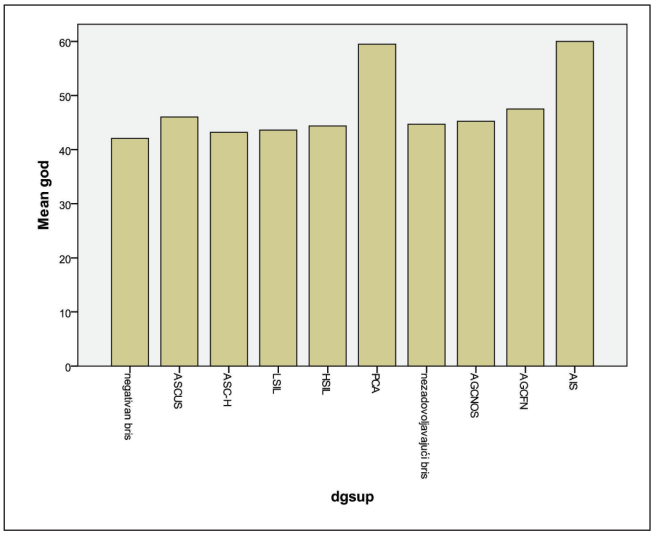

Figure 2. Mean values for the age groups under different cytological results.

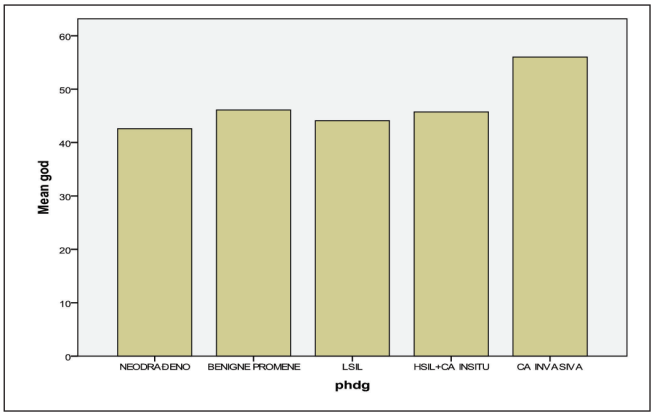

Figure 3. Mean values for the age group according to different biopsy results

For the purpose of testing our previously mentioned hypothesis, we used the following formulae.

Sensitivity = true positives / (true positives + false negatives) x 100

Specificity = true negatives / (true negatives + false positives) x 100

Positive predictive value = true positives / (true positives + false positives) x 100

Negative predictive value = true negatives / (true negatives + false negatives) x 100

Overall accuracy = (true positives + true negatives) / sample size.

RESULTS

The study included 2570 patients from the national screening program. The youngest patient was 20 years old and the oldest was 74 years old. The descriptive statistics for the age of the population in years were as follows : mean of 42.79, median of 42.00, standard deviation of 11, 455.

Cervical cytology findings were grouped into 10 categories (negative cytology—NILM, ASC-US, ASC-H, LSIL, HSIL, PCA, AGC-NOS, AGC-FN, AIS, and unsatisfactory samples).

Biopsies were grouped into 4 groups: negative (benign changes), LSIL, HSIL (HSIL + in situ CA) and invasive CA.

The Kolmogorov-Smirnov test showed that the values for age in this population did not follow a normal distribution (sig. 0.000). The observed histogram showed a slightly asymmetric distribution, shifted to the left (i.e., to younger ages). The largest group (93 of individuals) was that of 43-year-old women (Figure 1).

The largest number of swabs was negative (1930), with an average age of 42.09, and the positive cytological smears were mostly ASC-US (224), with a mean age of 46.02. The second-highest number of findings was LSIL (149), with a mean of 43.61 years. Two squamous cell carcinomas were detected (mean age of 59.50), and one adenocarcinoma in situ was identified (60 years old). In 15 cases, the data on age were missing.

For the Kruskal-Wallis test, we established the null hypothesis that there were no differences in the median age groups formed on the basis of the results of the cytological testing. Since the value of sig. 0,000 is less than 0.5, the null hypothesis was rejected, and significant differences were noted to exist between groups. From the graph, we can see that there is considerable variation with respect to mean age for the PCA and AIS groups (Figure 2).

The greatest number of biopsy findings fell into the LSIL category (106), with a mean value of 44.08 years. Invasive cancer was confirmed in a woman of 56 years (Figure 3).

Based on the Kruskal-Wallis test's (sig. 0.011 < 0.05), the null hypothesis was rejected, meaning that there was a statistically significant difference in median age between the groups with different histopathologies.

Of the total of 2570 analyzed cytological smears evaluated under the supervision of the National Screening Program, the largest group (1930; 75.1%) were negative cytological smears (Figure 4). Of all pathological smears, the most numerous smear finding was ASC-US (224; 8.7% of the total number of samples taken), followed by smears with LSIL findings (149; 5.8% of the total number of samples taken). ASC-H findings numbered 55 (2.1%), 58 (2.3%) findings were HSIL, PCA consisted of 2 cases (0.08%), AGC-NOS was reported for 39 (1.5%), AGC-FN was found in 8 (0.3%), and AIS was found in 1 (0.04%). Unsatisfactory findings were reported for 104 (4%) smears, and these women were once again invited to examination (Table 2).

The total number of detected abnormal cytological smears was 536. Abnormal smears were divided into eight groups of which the largest group was ASC-US, with 224 (41.8% of all abnormal smears), followed by LSIL, with 149 (27.8%),; the smallest number of findings were for AIS, with 1 (0.2%) (Table 2, Figure 5).

Of the total number of women (2570) who had secondary cytological examinations, 2359 (91.8%) did not undergo further diagnostic procedures, and in 211 (8.1%), biopsy and exploratory curettage of the cervical canal were performed. Of all the women who had histological diagnoses in comparison with the total number of women who underwent cytological smear analyses, 82 women (3.2%) had a negative biopsies, 106 (4.2%) had LSIL, 17 (0.9 %) had HSIL (CIN2: 17 (0.7%); CA in situ : (0.2%)), whereas the biopsies detected 1 (0.04%) invasive CA. Results show that after the successfully biopsies, the highest percentage of women had LSIL findings (Table 3).

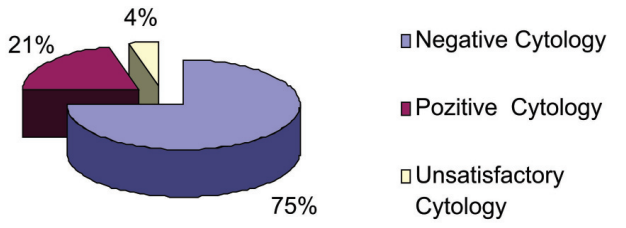

Figure 4. Percentage of negative, positive and unsatisfactory results in the total number of analyzed cytological smears

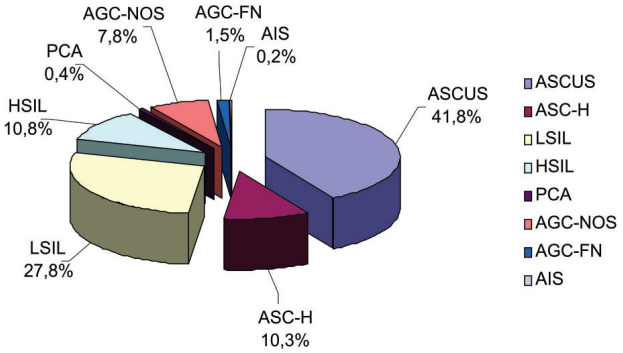

Figure 5. Percentage of the different categories of abnormal cytological smears

Of the total number of successfully completed biopsies, negative results were found for 82 (38.9%), whereas positive histologies were found in 129 (61.1%) (Figure 6). Among the performed biopsies, the largest percentage confirmed LSIL in 106 (50.2%), HSIL (HSIL + in situ CA) in 22 (10.4%) and invasive CA in 1 (0.5%). The histological diagnoses did not detect any of the glandular changes (Figure 7).

Among the positive histological findings, the most frequent finding was LSIL (106 cases; 82.2%) (Figure 8).

After the histopathological test was performed, cytology recorded 62 false-positive results (ASC-US 9, ASC-H 11, LSIL 23 and HSIL 11), and 16 false-negative results (12 LSIL and 4 HSIL that matched CIN2).

From the total of 104 repeated smears, based on dubious colposcopic findings, 9 biopsies were performed (8.56% of repeated smears), of which 6 were negative, and 3 were LSIL (2.89% of the repeated smears).

After colposcopy and/or HPV typing or repeated cytology, biopsy was performed in 12.5% of all cytologies yield-

Table 2 Frequency of cytologic results in the study population

cytological diagnosis of the supervisor	Frequency	Percent	Valid Percent	Cumulative Percent
NILM	1930	75,1	75,1	75,1
ASC-US	224	8,7	8,7	83,8
ASC-H	55	2,1	2,1	86,0
LSIL	149	5,8	5,8	91,8
HSIL	58	2,3	2,3	94,0
PCA	2	0,1	0,1	94,1
Unsatisfactory smear	104	4,0	4,0	98,1
AGC-NOS	39	1,5	1,5	99,6
AGC-FN	8	0,3	0,3	100,0
AIS	1	0,0	0,0	100,0
Total	2570	100,0	100,0	

Table 3 Percentage of biopsies in relation to the total number of women in the study

Histological diagnosis	Frequency	Percent	Valid Percent	Cumulative Percent
UNPROCESSED	2359	91,8	91,8	91,8
BENIGN CHANGES	82	3,2	3,2	95,0
LSIL	106	4,1	4,1	99,1
HSIL+CA INSITU	22	0,9	0,9	100,0
CA INVASIVE	1	0,0	0,0	100,0
Total	2570	100,0	100,0	

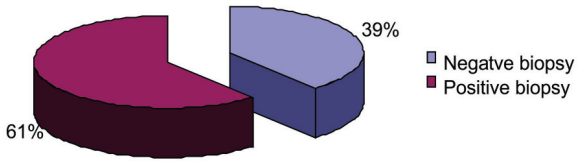

Figure 6. Proportion of the positive and negative biopsy results

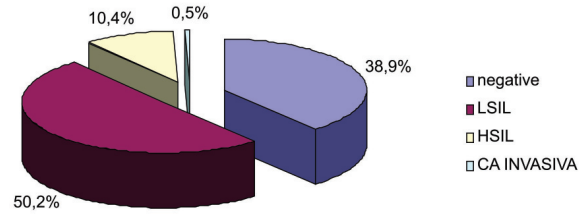

Figure 7. Proportion of histological results after certain biopsies

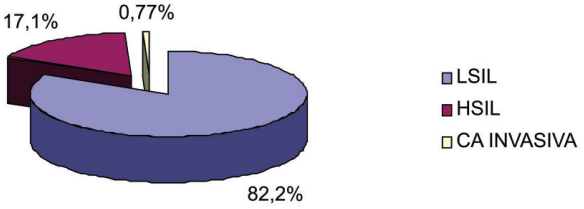

Figure 8. Percentage of categories of histological results in the total number of positive histological results

ing ASC-US findings. Of these, 32.1% of the biopsies had benign findings, and positive biopsies were found in 67.9% (60.7% being LSIL and 7.1% being HSIL). If it is expressed in the total number of cytological diagnoses, 7.6% of the ASC-US diagnoses had LSIL findings, 0.9% had findings of HSIL (of which none were in situ CA), and 8.5% had CIN.

In this study, of the 279 ASC findings, 55 were ASCH (19.71%). Of the total findings of ASCH, 29.1% were confirmed for LSIL and 5.5% were confirmed for HSIL (or from all confirmed CIN in the ASC-H category, 84.21% were LSIL and 15.79% were HSIL).

In our study, in patients with LSIL, cytological findings confirmed LSIL lesions in 41 (59.42%), and 5 (3.4%) were confirmed with HSIL findings. The percentage of benign HP findings in the category of LSIL was 33.33%.

Of all patients who participated in this study, 2.3% had HSIL cytology findings. Among the positive cytology findings, 10.8% were HSIL. By biopsy, 16 (45.7%) LSIL findings were confirmed, as were 7 (20%) HSIL and 1 (2.86%) invasive carcinoma.

In this study, of the six biopsies performed for AGC-NOS, one confirmed LSIL and AGC-FN in 3 cases showed benign characteristics, whereas the AIS cytology findings with clear cytological elements with respect to this cancer were confirmed as HSIL on biopsy (Table 4).

To test the sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy, we did not take into account patients with unsatisfactory smears nor patients with positive cytology findings on whom we did not perform biopsies. We have divided the results by patients, healthy cytology, abnormal cytology and negative cytology. Within the group are the patients who are positive after the negative biopsy findings (16) and abnormal cytologies (110). Within the healthy group are those with negative biopsies and NILM cytologies (a priori groups without disease), within normal cytologies (1914), and those with negative biopsy findings but with abnormal cytologies (62). The total number of patients in this test was 2102 (Table 5, Table 6).

Table 4 Ratio of cytologic results and histopathological results based on different diagnostic categories

Cytological results	Pathohistological diagnosis					Total
	UNPROCESSED	BENIGN CHANGES	LSIL	HSIL+CA INSITU	CA INVASIVE	
NILM	1900	14	12	4	0	1930
ASC-US	196	9	17	2	0	224
ASC-H	25	11	16	3	0	55
LSIL	80	23	41	5	0	149
HSIL	23	11	16	7	1	58
PCA	2	0	0	0	0	2
Unsatisfactory smear	95	6	3	0	0	104
AGC-NOS	33	5	1	0	0	39
AGC-FN	5	3	0	0	0	8
AIS	0	0	0	1	0	1
Total	2359	82	106	22	1	2570

Table 5 The numeric ratio of negative/positive/repeat cytologies in relation to the histological results

		Pathohistology					Total
		UNPROCESSED	BENIGN CHANGES	LSIL	HSIL+CA INSITU	CA INVASIA	
Cytology	negative	1900	14	12	4	0	1930
	positive	364	62	91	18	1	536
	repeat	95	6	3	0	0	104
Total		2359	82	106	22	1	2570

Table 6 Relationship of abnormal/negative cytologies to the number of patients/healthy patients

	ILL	HEALTHY	TOTAL
Abnormal Cytology	110	62	172
Negative Cytology	16	1914	1930
Total	126	1976	2102

The obtained values were as follows.

- Sensitivity = 110/126 x 100 = 87.3%
- Specificity = 1914/1976 x 100 = 96.86%
- Positive predictive value = 110/172 x 100 = 63.95%
- Negative predictive value = 1914/1930 x 100 = 99.17%
- Overall accuracy = (110 +1914) / 2102 x 100 = 96.28%

Using a Chi-square test, we tested the null hypothesis H_0 that there was no correlation between the two variables (the cytological and histological tests).

In order to meet the requirement that at least 80% of the expected frequencies would exceed 5 and that all of the expected frequencies would exceed 1, we grouped findings as follows:

1. Negative and unsatisfactory in one group (neg/unsat.);
2. ASC-US, ASC-H, AGC-NOS, AGC-FN in the group of atypical cells;
3. LSIL in the low-grade group (LOW G); and
4. HSIL, PCA, AIS in the high-grade group (HIGH G).

Histopathological findings were classified into three groups:

1. Negative;
2. LSIL; and
3. HSIL + CA + in situ CA in the invasive group, consisting of the three findings together.

The sig. value for the Pearson chi-square test was 0.036, which is less than 0.05, so the null hypothesis was rejected, and this confirms that cervical cytology and histopathological diagnosis are dependent features.

Based on Cramer's indicators (Cramer's V) for 2 degrees of freedom, the range of influence 0,179 was considered a medium-strength correlation between the variables (cervical cytology and cervical histology).

DISCUSSION

Diagnostic cytology is the art and science of analyzing human cells. The significance of cervical cytology is based on the premise that most cervical cancer lesions develop gradually such that cancer cell precursors and localized intraepithelial carcinomas can be detected by this method, making it is possible to treat a disease and fully cure it. A definite cytological diagnosis must be supported with relevant clinical history. Only when a smear is adequate and all clinical data are in support can you then offer a definite cytological evaluation. Of the utmost importance for the maintenance of satisfactory cytological results is uniformity in the technical preparation of products between laboratories (13,14).

In our study, preparations were decentralized in several primary cytology laboratories within the hospital, and the minimum of variances in the quality of smears was respected. Each technically unsatisfactory smear was returned with a recommendation on the required repetition. The largest number of unsatisfactory smears was attributed to technical reasons. The results of our study showed that, among unsatisfactory smears, low-grade lesions (LSIL) were detected in fewer than 3%, which is in accordance with the results of other authors (8).

It is known that in a certain percentage of unsatisfactory smears, significant cervical lesions can be present, and the cervical cytology can detect or lower the degree of cervical lesions' weight (15). In some cases, highly invasive tumours, known as tumour diathesis, appear (granular amorphous precipitate with nuclear waste and red blood cells), in which cancer cells cannot be identified (16); in such cases, the smears can be classified as false negatives. Hindering factors included technically unsatisfactory smears, low frequencies of abnormal cells in the smears, difficulty in distinguishing immature squamous metaplasias and cohesive fragments of HSIL, difficulty in distinguishing small HSIL cells, small cell carcinomas and some AIS of endometrial cells. Additionally, false positive cytology results are possible: for example, cells of the lower uterine segment or endometrial tissue, because of its high N / C ratio, hyperchromasia and mitotic activity, can be replaced with squamous or glandular lesions. Lymphocytes and plasma cells in the cervical smears of postmenopausal women (follicular cervicitis) may be replaced with HSIL, lymphoma, or endometrial cells, among others (16).

Table 7 H^2 test—Testing hypotheses about the relation between the cytological and histological tests

			DGPHX2 (Histological test)			Total
			NEGATIVE	LSIL	HSIL+CA INSITU+CA INVASIVA	
DGSUX2 (Cito-Test)	Neg/unsat	Count	20	15	4	39
		% within DGSUPX2	51,3%	38,5%	10,3%	100,0%
		% within DGPHX2	24,4%	14,2%	17,4%	18,5%
		% of Total	9,5%	7,1%	1,9%	18,5%
	Atypical cells	Count	28	34	5	67
		% within DGSUPX2	41,8%	50,7%	7,5%	100,0%
		% within DGPHX2	34,1%	32,1%	21,7%	31,8%
		% of Total	13,3%	16,1%	2,4%	31,8%
	Low G	Count	23	41	5	69
		% within DGSUPX2	33,3%	59,4%	7,2%	100,0%
		% within DGPHX2	28,0%	38,7%	21,7%	32,7%
		% of Total	10,9%	19,4%	2,4%	32,7%
	High G	Count	11	16	9	36
		% within DGSUPX2	30,6%	44,4%	25,0%	100,0%
		% within DGPHX2	13,4%	15,1%	39,1%	17,1%
		% of Total	5,2%	7,6%	4,3%	17,1%
Total		Count	82	106	23	211
		% within DGSUPX2	38,9%	50,2%	10,9%	100,0%
		% within DGPHX2	100,0%	100,0%	100,0%	100,0%
		% of Total	38,9%	50,2%	10,9%	100,0%

The database in this study included patients after secondary cytological examination. Compared with the pilot project in the Branicevo region, which included patients with primary and secondary cytological screenings, we received expected data on the percentages for the presence of cytological findings: slightly lower presence of normal findings and slightly higher presence of abnormal and unsatisfactory cytology findings (17).

In our study population, patients varied widely in age, which reflects the real situation in the general population. The number of patients who were outside the range of years envisaged by a screening program (i.e., younger than 25 and older than 64 years) minimally contributed (4.16%) to the total number of patients.

It is noted that there was a slight asymmetry in the distribution of patients who participated in the screening, weighted towards a younger age. The distribution of mean values for different age groups of cytological findings in our study was related to the fifth decade of life, except for squamous cell carcinoma and adenocarcinoma in situ, which were detected cytologically in the sixth decade, in agreement with the field's knowledge of the pathophysiology of cervical cancer.

Results of a large meta-analysis, reported by Melnikova et al., have been drawn from data from 1966 to 1996; these data linked women with cervical atypia per cytology (ASC-US) and those with low-grade lesions to a low level of invasion of cervical cancer over a follow-up duration of 24 months, and they also showed that the high level of the lesion (HSIL) had a higher likelihood of progression to cancer than the probability of finding a regression to normal, which is consistent with the biological theory of the origin process for cervical dysplasia (13).

According to this study, the regression of ASC-US and LSIL without treatment within 24 months occurred approximately 53% of the time, whereas the progression for these results in the same period was less than 1%. The regression rate within 24 months was 68.19% for ASC-US and 47.39% for LSIL, whereas the rate of regression for HSIL over the same period was 35.03%. The rate of progression to invasive cancer within 24 months of follow-up was 0.25% for ASC-US, 0.15% for LSIL and 1.44% for HSIL. From these results, it can be observed that HSIL has the highest rate of progression to invasive carcinoma, whereas LSIL had the lowest rate of progression within 24 months (Table 8)(13). This finding is consistent with the pathogenesis of low-grade dysplasia, which is normally considered to be the consequence of transient infection of HPV. The cumulative rate of progression over 24 months resulting

Table 8 Rates of progression and regression of pre-invasive squamous lesions within 24 months (13,16)

	Regression (%)	Progression to HSIL (%)	Progression to invasive cancer (%)
ASC-US	68	7	0,25
LSIL	47	21	0,15
HSIL	35	/	1,4

from the ASC-US/LSIL Triage Study of ASC-US in CIN was 8-9%, whereas the cumulative rate of progression from LSIL to HSIL was 6.6% over 6 months and 20.8% over 24 months (11, 18).

Several studies have shown that the diagnosis of ASC-US cannot be ignored. The percentage of the participation of ASC-US cytology results in abnormal cytology is the largest, accounting for 90-95% of all results (11). The percentage of squamous intraepithelial lesions obtained after biopsies of these findings ranges from 36% to 63%. According to a study by Massad et al. (2001), on the basis of histological results of cytological findings, 30% of ASC-US findings really are histologically negative, 47% as associated with lumps, and 18% reflected CIN 1, 3% CIN 2 and 3% CIN 3 (19). Thus, the introduction of this category of Bethesda classification seems justified because eliminating this category would result in a portion of high-grade lesions being unidentified.

In a clinical study, Barcelos et al. found that after repeated cytology, 42.8% of the female patients with ASC-USA results are sent to biopsy, 16.6% of which have an HPV infection, and 30% have CIN (10% CIN1, 10% CIN2 and 10% CIN 3), whereas 53.3% have a normal biopsy. It is believed that 9-17% of women with ASC-US results have definitive CIN (11).

The frequency of atypical squamous cells of undetermined significance in abnormal cytological findings in our study is somewhat lower (41.8%) than expected based on the results of other studies (11). In our study, we used strict criteria for the interpretation of ASCUS cytology according to standardized instructions provided for the interpretation of cervical cytology smears (12). It should be noted that, in this study, the population consisted of women without any disease symptoms included in a screening program, where the expected presence of HPV infection was lower than in a population of women with gynaecological complaints and cervical changes.

In our study, after a colposcopy and/or an HPV typing or a repeated cytology, a biopsy was carried out on a certain percentage of cytological ASC-US results. The results of our study showed that a definite CIN was found for 8.5%, which is slightly lower compared to previous published data (19). In biopsies with a cytological ASC-US result, the LSIL category dominates in relation to the HSIL, which is in accordance with the results of other authors (19). For ASC-US, CIN1 and CIN2 alone have been confirmed following biopsy.

The ASC-H result comprises 5-10% of all ASC results. In our study, 34.6% of CIN results were confirmed within the ASC-H cytology results, 15.79% of which were CIN2 and CIN3. The results of our study are consistent with the results of other studies, which in 30-40% of the ASC-H cases, CIN is diagnosed, 25-50% of which is CIN2 or CIN3 (8, 11, 20).

It is noted that the colposcopic results after the ASC cytology were significantly more abnormal in the ASC-H group than in ASC-US group (21). This is explained by the fact that the ASC-H category possesses a higher predictive value for HSIL compared to the ASC-US category and a lower one than the predictive value of the HSIL category (8).

In women with a LSIL result, 17% had CIN2 and 12% had CIN3 (11). In our study, female patients with LSIL cytology results had a dominant histological CIN1 (LSIL) result, whereas the HSIL result was confirmed in only 3.4%. The results of our study have shown that the percentage of benign HP findings in the LSIL category corresponded to approximately one-third of the sample, which is in accordance with the data from previous studies, which show that approximately 30% of LSIL findings are over-diagnosed (15).

Studies show that, with a cytological HSIL result, more than 50% of the female patients will have CIN2 or a more severe finding, and 2% will have invasive carcinomas (11). Data also show that between 20% and 30% of HSIL results are not diagnosed by cervical cytology (15).

Our study showed that, for 68.57% of the biopsies performed, there was a CIN result and that the prevalence of invasive cancer within the HSIL cytology results in this study fits with previous published data (11).

In 44% of women with atypical glandular cells, squamous lesions are found after further histological procedures (8). In our study, the cytological result of AIS with clear cytological elements of this cancer in the biopsy was confirmed as HSIL, which confirms the results of previous research (8)—i.e., squamous and glandular lesions coexist at high rates in the pathology of the cervix.

In this study, there were no cases of a negative cytology after which the biopsy showed an invasive carcinoma. This coincides with the conclusions made by other studies—that is, the Pap test detects all malignant lesions, but there are very frequent false-positive results (15). Of the total number of Pap tests performed in this study, 2.65% were false positives, i.e., of the total number of biopsies performed, 38.9% had benign results.

We see from the study results that, in the cytological categories of ASC-H and HSIL, there is a percentage shift towards a category of dysplasia, lower than would be expected based on other studies. This can be explained by the possible coexistence of large LSIL and small focal HSIL lesions. It is possible that, in some cases, these small lesions are not revealed by a targeted biopsy. If one takes into consideration the quantity of cells that can be found in the Pap test with an ASC-H result (a small number of cells with given characteristics), we can hypothesize that the number of cells in the cytological smear corresponds to the size of the lesions in the conizate.

In this study, HSIL was confirmed for small percentages in the ASC-US and LSIL results. Based on data from the ASC-US LSIL Triage study, Sherman et al. concluded that CIN3 lesions found after LEEP excision were smaller (<10 mm) than those expected for micro invasive carcinoma (63.5 mm). The researchers in this study drew the conclusion that the CIN3 lesions detected after a less severe cytological result than HSIL tend to be small. The small CIN3 result also has a slightly increased risk of being related to a false negative HPV test (22).

The bias that is present in this study exists as an inevitability, given that histological diagnoses were not performed for all participants in the study, because in cases of normal cytological and colposcopic results, it would represent over-treatment.

A certain percentage of patients with cytological HSIL results, where a histological examination would be expected, were lost in this study, and we had no follow-up on further diagnosis. According to this study, approximately 1% of the total number of patients were women diagnosed with squamous lesions of high-grade cytology, for which data on further histological examination was missing. Because the screening was carried out on a voluntary basis, which for now is a reality in our society, it is necessary to educate patients and physicians about the need to comply with all clinical pathways, protocols and guidelines.

When interpreting the results of this study, it should be noted that the works of M. H. Stoler and Schiffman show that, between different clinicians, there is moderate consistency in the identification of monolayer cytology smears, as well as a moderate consistency in the identification of histopathological biopsy interpretation. The major sources of disagreement in monolayer cytology are the ASC-US results. Another significant source of disagreement between cytologists is the interpretation of the HSIL results that are sometimes referred to as LSIL or even as ASC-US. The explanation for the down-grading of HSIL results to LSIL is noted in literature as the often-difficult distinction between mild and moderate dysplasia, whereas in the case of down-grading to ASC-US, controversy may arise in connection with small atypical squamous cells of the immature metaplasia type, when it cannot be determined with certainty whether the entity is distinct from HSIL. With the biopsy results, histological variability results in the highest percentage of the disagreement over stage CIN1 (23). There are also cases that record errors in the assessment of HP and when CIN that is present is not properly diagnosed (15). The data indicate the possibility of "false-negative" histological results in which very small CIN2 or CIN3 lesions are not diagnosed, despite cytology and virology records. According to a study by Castle et al., cases of "false-positive HSIL cytology" in fact are not false positives but instead are CIN3 lesions that were not detected upon initial colposcopy but were then discovered during intensive monitoring of the female patient. It is important to emphasize that a colposcopy diagnosis has limitations in the detection of very small CIN3 lesions, which on the other hand, can be detected as minor cytological changes in a Pap test (24).

A positive characteristic of this study compared to similar performed studies is the involvement of a large number of female patients in both the reproductive and the postmenopausal period, comprising a representative sample that reflects the true state of the prevalence of cervical lesions and the actual representation of HPV infection in the general population. Different degrees of interpretation of cytological smears in organized screening have been reduced by the two-way analysis of smears, with the secondary smear interpretation given by a clinical supervisor with many years of experience in cervical cytology, according to the standards and in compliance with the uniformity of the Bethesda system for reporting cervical cytology.

This study demonstrated that cervical cytology and cervical biopsy are dependent features, between which there is a connection of medium strength, as established by other researchers using a similar methodology (15).

As in similar studies, this study has confirmed the high sensitivity, specificity, positive and negative predictive value of the procedure . However, this study also shows that cervical cytology does not represent an absolutely valid method. The overall recorded accuracy of this method in our study was greater than 90%. In relation to cytology, the HPV test has a higher sensitivity in the detection of cervical intraepithelial lesions of a high grade. The data obtained from organized tests, the subject of which was a comparison of primary screening organized by HPV testing and a screening organized on a basis of cervical cytology, have shown that the HPV-based screening test has a high sensitivity but a low specificity in comparison with screening-based cytology (25). Based on these results, the cytological cervical smear (Pap smear) is legally provided as a primary screening test in this country (26). The HPV test, due to its high specificity, has proved to be useful in determining the level of oncogenic potential of the cervical lesions present, which is directly related to the presence/absence of HPV with high oncogenic potential. The data show that 32.6-44.7% of women with ASC-US results and the majority of women with LSIL results are positive for HPV of high oncogenic potential (27,28).

INSTEAD OF A CONCLUSION— CLINICAL RECOMMENDATIONS

In this study, it was shown that none of the clinical methods used in the diagnosis of precancerous and cancerous changes in the cervix is absolutely accurate, whether it be cytology, colposcopy or histopathological analysis. Accordingly, a decision on further procedure and treatment of patients with present changes in the cervix should be made in a multi-disciplinary way, with respect to clinical recommendations from the 2011 National clinical guidelines for the diagnosis and treatment of cervical cancer in the Republic of Serbia and on the basis of European and American guidelines for the management of women with abnormal cytology (11, 29, 30). An individualize approach to treatment of the disease based on the patient's medical history is also necessary.

In this study, we have shown that cervical cytology is not only a consultative result but also a diagnostic tool that plays an active role in the decision about further treatment and the treatment of cervical disease.

In the case of positive cytology accompanied by negative colposcopy and/or histopathology, it is necessary to

actively monitor and determine the cytological HPV status of the female patient.

After obtaining a histopathological result, it is also necessary to respect the recommendations made in the guidelines of good clinical practice for the treatment of this disease.

In order to determine the type of treatment, it is of special importance to determine oncogenic potential through HPV testing. Our clinical recommendation is that, in the case of an LSIL result in combination with an HPV virus of high oncogenic potential, further active treatment of these lesions should be applied, regardless of age.

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