EJGO European Journal of Gynaecological Oncology

Original Research

How safe is a three-year screening interval for cervical cancer with conventional cytology in southeastern Serbia?

R. Zivadinovic[1,5], D. Stankovic-Djordjevic[2,8], B. Zivadinovic[3,6], D. Krtinic[4,7], A. Petric[1,5], A. Zivadinovic[9], V. Radovanovic[9]

[1]Department for Gynecology and Obstetrics, [2]Department for Microbiology and Immunology, [3]Department for Neurology, [4]Department for Pharmacology and Toxicology, Faculty of Medicine, University of Nis
[5]Clinic for Gynecology and Obstetrics, [6]Clinic for Neurology, [7]Clinic for Oncology, Clinical center, Nis
[8]Institute for Public Health, Nis
[9]Faculty of Medicine, University of Nis, Nis (Serbia)

Summary

Aim: Purpose of this investigation was to determine the influence of cervical cytology on the reduction of high-grade cervical intraepithelial lesions (HSIL) and cervical cancer (CC) incidence. *Materials and Methods:* All the patients treated for HSIL and CC at the Clinic for Gynecology and Obstetrics Nis in the last three years. All filled in questionnaires. Before the surgery treatment, human papillomavirus (HPV) test samples were obtained from all the patients. *Results:* The results have shown that a three-year cervical cancer screening using conventional cytology is not reliable enough and it has not been shown to result in statistically significant reduction of HSIL and CC incidence. *Conclusion:* HPV test has increased the reliability of cytology in detecting pre-invasive lesions and it has statistically significantly reduced false-negative rate of cytology results in micro-invasive and invasive changes of the cervix.

Key words: Conventional cytology; Human papillomavirus testing; Cervical cancer screening.

Introduction

Cancer screening mostly aims at detecting early stages of invasive disease. On the other hand, cervical cancer screening is based on detecting early invasive, but also pre-invasive intraepithelial changes that have potential to become an invasive cancer. These changes, due to their progressive oncogenic potential, were termed high-grade squamous epithelial lesions (HSIL) or cervical intraepithelial neoplasia II and III (CIN II and III). However, these lesions are heterogeneous since they involve changes that are not morphologically distinct, but they have different oncogenic potential. Anyway, the studies examining the nature of high-grade lesions have shown that over 30% of undetected and untreated HSILs may progress to cervical cancer [1].

Early invasive and HSIL changes are not visible to the naked eye, therefore exfoliative cytology is used for detecting these changes. Exfoliative cytology is a diagnostic procedure that reveals the status of the entire tissue according to spontaneously or mechanically desquamated individual epithelial cells characteristics. This method fulfills all the criteria for screening tests due to its non-invasiveness, relatively high sensitivity, rapid application, possibility of dynamic monitoring without side effects, inexpensiveness, and cost-effectiveness. Cytology-based cervical cancer screening and early detection programme implemented decades ago has resulted in morbidity and mortality reduction of this disease. Especially successful screening results have been achieved in countries with organized screening programmes [2]. However, a growing number of scientific papers has reported limitations of cytology, with its sensitivity in the range 30–87 % [3-5].

Studies on cervical cancer etiology and nature have noted growing evidence that persistence of oncogenic human paillomavirus (HPV) types is a necessary cause of cervical cancer development [6]. More than 90% of invasive cancers are HPV positive, so HPV typing in detecting cervical cancer has high sensitivity. Such evidence and aforementioned facts about cytology limitations have inevitably led to vital changes in modern approach to cervical cancer screening and to introduction of HPV typing being incorporated into screening [7]. On the other hand, due to a relatively high rate of HPV-positive results in young women below 30 years of age within transitory infection, the specificity of HPV typing in the range 77-90% is slightly lower

Revised manuscript accepted for publication June 10, 2019

Eur. J. Gynaecol. Oncol. - ISSN: 0392-2936
XL, n. 6, 2019
doi: 10.12892/ejgo5194.2019

7847050 Canada Inc.
www.irog.net

Published: 10 December 2019

Table 1. — *The most recent cytology finding and age.*

Age (years)	The most recent Pap testing (years)				*p*
	< 1	< 3	> 3	Total	
< 30	24	6	3	33	
	72.70%	18.20%	9.10%	100.00%	
31-50	36	30	30	96	
	37.50%	31.20%	31.20%	100.00%	**0.001**
> 51	18	12	24	54	
	33.30%	22.20%	44.40%	100.00%	
Total	78	48	57	183	

Table 2. — *Annual visit to gyneacologist and the Pap testing.*

Annual visit to gyneacologist	The most recent Pap testing (years)				*p*
	< 1	< 3	> 3	Total	
No	0	15	54	69	
	0.00%	21.70%	78.30%	100.00%	
Yes	78	33	3	114	**< 0.001**
	68.40%	28.90%	2.60%	100.00%	
Total	78	48	57	183	

Table 3. — *Current pathohistological finding compared to the most recent Pap test.*

Pathohistological finding	The most recent Pap test (years)				*p*
	< 1	< 3	> 3	Total	
CIN II	21	9	9	39	
	53.80%	23.10%	23.10%	100.00%	
CIN III	39	24	30	93	
	41.90%	25.80%	32.30%	100.00%	0.51
MIC and IC	18	15	18	51	
	35.30%	29.40%	35.30%	100.00%	
Total	78	48	57	183	

in comparison to cytology [8].

By incorporating HPV typing into cervical cancer screening, multiple options for cervical cancer screening are offered – from cytology-based screening alone, simultaneous adjunctive HPV typing, and cytology utilization, to primary HPV screening that has recently become common in many European countries.

The aims of the study were: to determine whether a regular three-year interval screening with conventional cytology reduces the incidence of high-grade cervical intraepithelial lesions and cervical cancer; to examine whether shortening of the cytology screening interval may increase the reliability of conventional cytology in the secondary prevention of cervical cancer and to determine compatibility of cytological and pathohistological findings. This study also investigated the association of false-negative cytological results with age and the stage of the disease, histological type, HPV status, and to determine to what extent HPV test may increase the reliability of cytology.

Materials and Methods

This is a prospective study carried out on all the patients who have been treated and operated for HSIL - CIN II and CIN III, and cervical cancer (CC) at the Clinic for Gynecology and Obstetrics, Clinical Center Nis, Serbia in the last three years. Considering the fact that there are no specialized oncology centers in Serbia, this Clinic is a referential institution in southeastern Serbia where surgeries on patients with premalignant and malignant cervical changes are performed. The study enrolled 183 patients who underwent conization, hysterectomy, and radical hysterectomy, depending on the stage of the disease. Patients with the disease being diagnosed in inoperable stage were excluded from the study due to unavailability of relevant medical records.

All the patients filled in questionnaires about epidemiological data, information on the last Pap test and cytology results at the time of diagnosis. Before the surgery treatment, HPV test samples were obtained from all the patients. HPV typing was carried out at the Institute for public health Nis by using HPV genotyping PCR method for oncogenic HPV types.

A software package SPSS version 16.0 was used for statistical data analysis. All the preliminary results were statistically analyzed and presented in tables and graphs. The Chi-square test of independence (χ^2) was used for statistical analysis of a relationship between categorical variables, and p coefficient was used to measure the strength of the relationship. Statistically significant relationship was present if the condition of $p < 0.05$ was fulfilled.

Table 4. — *Distribution of cytology test in comparison to pathohistological finding at the moment of abnormal changes diagnostics, the reason patients came for surgical treatment.*

Cytological findings	Pathohistological findings				Total
	CIN II	CIN III	AIS and AC	MIC and IC	
NILM	0	15	18	6	39
	0.00%	38.50%	46.20%	15.40%	100.00%
Pathological finding	39	57	3	45	144
	27.08%	39.58%	2.08%	31.25%	100.00%
Total	39	72	21	51	183

Table 5. — *Distribution of false-negative (NILM findings) and pathological results of cytology in comparison to patients' age.*

Age (years)	NILM *vs.* others		
	NILM	Pathological finding	Total
< 30	9	24	33
	27.30%	72.70%	100.00%
31-50	18	78	96
	18.75%	81.25%	100.00%
> 51	12	42	54
	22.20%	77.80%	100.00%
Total	39	144	183

Results

By analyzing obtained epidemiological data, the authors concluded that patients over 50 years of age checked for recommended three-year interval Pap test screening less commonly, which was statistically significant ($p < 0.05$). On the other hand, younger patients of 30 years of age or less had the most recent Pap testing performed more routinely than recommended, one year ago ($p < 0.001$). These data are shown in Table 1.

Patients who more commonly had a routinely annual gyneacological exam also had a Pap test, which was statistically significant ($p < 0.001$), while those who did not have a routine gyneacological visit, more commonly reported to have the most recent Pap test done more than three years ago, which is statistically significant ($p < 0.001$), as shown in Table 2.

Table 3 shows that statistical significance was not found in the frequency of pathohistological finding of different stage and severity in comparison to the timing of the most recent Pap testing. Recommended three-year interval screening, as well as shortened and prolonged screenings, were not statistically significant in detecting intraepithelial neoplasia, and, above all, they do not significantly reduce the incidence of invasive diseases – microinvasive cancer (MIC) and invasive cancer (IC). Only 31.14% of patients had the Pap test done more than three years ago.

False-negative NILM cytological findings were statistically significantly more commonly ($p < 0.001$) reported in patients with diagnosed premalignant and malignant changes of endocervical glandular epithelium (adenocancer-in-situ (AIS) and adenocancer (AC)), 46.20% of them. Cytology showed pathological Pap tests and greater statistical significance ($p < 0.05$) in detecting squamous premalignant intraepithelial changes in CIN II, as shown in Table 4.

Table 4 demonstrates that statistical significance of cytology in detecting micro-invasive and invasive disease stages of squamous epithelium has not been proved, since 15.40% of patients in this group had normal Pap test results. It is well-known that the Pap test is not recommended in the presence of visible carcinoma, because of bleeding, cytolysis, necrotic detritus, small-cell types similar in size to formed blood elements rather than to epithelial cells.

Table 5 demonstrates that statistical significance of the relationship between false-negative NILM cytological findings, and patients' years of age has not been found.

Table 6 shows that lower false results or results of lower-grade for CIN II and CIN III changes included NILM, atypical squamous cell of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), while for adenocancer (AC), microinvasive cancer (MIC) and invasive cancer (IC) findings HSIL was also a lower false result. Statistically significant pathohistological findings of CIN II, adenocancer in situ (AIS) and AC and MIC and IC were more commonly registered among cytological lower false results ($p < 0.05$). Among cytological correct findings, pathological finding CIN III was recorded more commonly, with statistical significance $p < 0{,}001$.

Comprehensively, Table 7 demonstrates that all age groups had correct cytological finding HSIL and IC, with statistical significance $p < 0.001$. In all age groups cytological findings NILM, ASCUS, and LSIL were falsely lower in comparison to pathohistological finding, with statistical significance of $p < 0.05$. Total statistically significant differences according to groups and according to the total sample were $p < 0.001$.

Table 8 shows no statistically significant difference in positive HPV testing results compared to patients' age. HPV test as a diagnostic method is reliable across all years of age.

Positive HPV testing result in women over 50 years of age shows statistically significant correlation with the presence of the invasive disease highest-grade (MIC and IC) (p

Table 6. — *Distribution of correct and cytological findings of lower grade in comparison to biopsy-obtained pathohistological grade.*

Cytological finding	Pathohistological finding				Total
	CIN II	CIN III	AIS and AC	MIC and IC	
Correct finding	6	42	0	9	57
	10.50%	73.70%	0.00%	15.80%	100.00%
Lower false result	33	30	21	42	126
	26.19%	23.81%	16.67%	33.33%	100.00%
Total	39	72	21	51	183

Table 7. — *Distribution of lower-grade cytological results in comparison to patients' age.*

Age (yeras)	Cytological finding	Cytological finding			*p*
		Correct finding	Lower false finding	Total	
< 30	NILM	0	9	9	**< 0.001**
		0.00%	100.00%	100.00%	
	ASCUS, LSIL	0	15	15	
		0.00%	100.00%	100.00%	
	HSIL, IC	9	0	9	
		100.00%	0.00%	100.00%	
	Total	9	24	33	
31-50	NILM	0	18	18	**< 0.001**
		0.00%	100.00%	100.00%	
	ASCUS, LSIL	6	39	45	
		13.33%	86.67%	100.00%	
	HSIL, IC	27	6	33	
		81.80%	18.20%	100.00%	
	Total	33	60	96	
> 51	NILM	0	12	12	**< 0.001**
		0.00%	100.00%	100.00%	
	ASCUS, LSIL	0	15	15	
		0.00%	100.00%	100.00%	
	HSIL, IC	15	12	27	
		55.60%	44.40%	100.00%	
	Total	15	39	54	
Total	NILM	0	39	39	**< 0.001**

Table 8. — *HPV typing result in relation to the patients' age.*

Age		HPV-	HPV+	Total
Years	< 30	3	30	33
		9.1%	90.9%	
	31-50	9	87	96
		9.38%	90.62%	
	> 51	6	48	54
		11.11%	88.88%	
Total	18	165	183	

< 0.05), as shown in Table 9.

HPV-16 positive finding at a much younger age, beginning even from 30 years of age, shows statistically significant association with the presence of the highest-grade invasive disease (MIC and IC) ($p < 0.05$), as clearly seen from Table 10.

Table 11 illustrates no statistically significant difference in positive HPV test in patients with correct and false-negative Pap test. HPV test is highly sensitive and it detects 91.60% of previously detected cytological changes, but also 84.60 % of undetected cytological changes.

All the patients with microinvasive and invasive cancer who had false-negative cytological results, or cytological results of lower value than final pathological result, were HPV+. HPV testing statistically significantly ($p < 0.05$) reduced the percentage of low-grade or undetected most severe microinvasive or invasive cytological changes of the cervix. On the other hand, adenohistological changes were more commonly HPV negative (28.60%), what is statistically significant. Such a finding related to adenohistological types requires future investigation regarding the risk factors and possible histological proximity to adenocancer of the body of the uterus. All of this can be seen in Table 12.

Table 9. — *Findings of HPV typing in relation to the age and invasive disease onset.*

Age (years)	HPV status	MIC and IC	Other	Total	*p*
< 30	HPV-	0	3	3	
		0.00%	100.00%	100.00%	
	HPV+	6	24	30	
		20.00%	80.00%	100.00%	
	Total	6	27	33	0.361
31-50	HPV-	0	9	9	
		0.00%	100.00%	100.00%	
	HPV+	24	63	87	
		27.60%	72.40%	100.00%	
	Total	24	72	96	0.057
> 51	HPV-	0	6	6	
		0.00%	100.00%	100.00%	
	HPV+	21	27	48	
		43.75%	56.25%	100.00%	
	Total	21	33	54	**0.043**
Total	HPV-	0	18	18	
		0.00%	100.00%	100.00%	
	HPV+	51	114	165	
		30.90%	69.10%	100.00%	
	Total	51	132	183	0.005

Discussion

In spite of a great number of organized opportunistic cytology screenings and projects of the Ministry of Health and European Union, the incidence of cervical cancer in Serbia has only slightly decreased since 2002 when the incidence rate of 27 per 100,000 women was the highest in Europe [9]. Based on the latest official Globocan estimates, Serbia ranks fifth in Europe and the incidence rate is twice as high as the average incidence rate in European countries (ten patients per 100,000 women) [10].

In Serbia there is no organized national cervical cancer screening programme, only opportunistic screening recommended by the National Guidelines as a three-year interval screening based on conventional cytology. As for HPV typing, since few years ago it has been recommended as a method to further guide the management of ASCUS cytology results (reflex HPV testing), as well as a follow-up method for women who had undergone surgical treatment for HSIL premalignant changes of the cervix, rather than primary screening method [11].

Epidemiological data obtained by a questionnaire and illustrated in Tables 1 and 2 showed statistically significant association of age, cultural practices, and annual gyneacological visit with followed recommendations on regular cervical cancer three-year screening interval. As seen in Table 1, patients from the youngest age group (below 30 years of age) did not attend recommended screening and only in 9% of cases. This difference was statistically significant ($p < 0.001$). The reason for this requires understanding of generational changes regarding cultural beliefs and attitudes and the impact of modern information technology, Internet, and social networks as well.

Table 3 shows the percentage data for women who regularly participated in screening, as well as those who have skipped regular screening. Statistical analysis revealed that attending a regular three-year interval cytological screening was not statistically significant in reducing the incidence of invasive cancer. Further analysis of obtained data, regarding the current cytology results and the diagnosis of HSIL and invasive cancer for which the patients were referred to the Clinic, clearly suggests the limitations and relatively low sensitivity of this method. Thus, Table 4 clearly shows false-negative Pap tests in 21.31% of patients.

Cytology as a screening method has significantly contributed to the cervical cancer incidence and mortality rate reduction in the countries with organized screening programmes. This type of screening has especially been successful in Scandinavian countries [2].

A growing number of papers discuss a limited sensitivity of cytology-based screening. The reason for such limited sensitivity is probably attributable to the subjective interpretation, problems in the differential diagnosis, inadequate collection of samples and interpretation errors. The sensitivity of cytology described in papers is within broad range from 30% to 87%, with an average sensitivity of about 51% [12].

Screening programmes basically aim at detecting HSIL and invasive cancer. Large randomizes studies have demonstrated cytology limitation itself in detecting the most severe pathological changes of the cervix [13].

This problem in cytology-based detection was present in the present study too, as can also be seen in Table 4. In 15.40% of clinically manifested cancers, cytological finding was normal NILM. A possible reason for such a

Table 10. — *Positive HPV-16 type finding in relation to age and the invasive disease onset.*

Age (years)	HPV type 16	MIC and IC	Other	Total	*p*
< 30	No	3	6	9	
		33.30%	66.70%	100.00%	
	Yes	3	21	24	
		12.50%	87.50%	100.00%	
	Total	6	27	33	0.486
31-50	No	3	36	39	
		7.70%	92.30%	100.00%	
	Yes	21	36	57	
		36.84%	63.16%	100.00%	
	Total	24	72	96	0.012
> 51	No	3	18	21	
		14.30%	85.70%	100.00%	
	Yes	18	15	33	
		54.50%	45.50%	100.00%	
	Total	21	33	54	0.035
Total	No	9	60	69	
		13.05%	86.95%	100.00%	
	Yes	42	72	114	
		36.84%	63.16%	100.00%	
	Total	51	132	183	0.003

Table 11. — *HPV test finding in patients with false-negative NILM cytological finding and pathological ASCUS + finding.*

Cytological findings	HPV-	HPV+	Total
NILM	6	33	39
	15.40%	84.60%	100.00%
Pathological finding	12	132	144
	8.30%	91.60%	100.00%
Total	18	165	183
	9.80%	90.20%	100.00%

Table 12. — *HPV test finding in patients with lower cytological finding in comparison to pathohistological finding.*

Pathohistological finding	HPV-	HPV+	Total
CIN II	9	30	39
	23.1%	76.9%	
CIN III	3	69	72
	4.20%	95.80%	
AIS, AC	6	15	21
	28.60%	71.40%	
MIC, IC	0	51	51
	0%	100%	
Total	18	165	183

distribution of cytology findings may be due to the fact that it is conventional cytology, smears are unpurified and with the presence of large quantities of cell detritus, blood, and leukocytes in cases of invasive cancer. One of the possible explanation involve small cell cervical cancer forms whose malignant cells on the specimens are more like formed blood elements rather than epithelial cells [14].

A special problem in cytological detection involves specific histological types, particularly adenocancers, as also illustrated in Table 4. Normal cytological findings were found in 46% changes belonging to this histological type. The reason for such a poor sensitivity is due to a single layer epithelium with unclearly defined precancerous stages. Adenoepithelial cells are fragile and their cytoplasm is easily lost during sampling, thus making the evaluation of nucleoproptoplasmatic ratio more difficult. Cellular pleomorphism is also highly present, and the degree of their atypical features is not identified according the well-known parameters, but according to aggregation, three-dimensional correlation, formation of pseudoacini, and nuclear protrusion. A special problem in detecting adenocancer in the present study was also because of non-homogeneous cytological smear sampling, and the fact that endocervical cytological sampling was performed with cervical brush instead of cotton-tipped swabs in a great number of cases [15].

From Table 5 it can be concluded that limited sensitivity of cytology is not influenced by patients' age. Cytology is equally reliable across all age groups. From the data in Tables 3, 4, and 5 as well, it can be concluded that the reliability of conventional cytology screening is not influenced by shortening the screening intervals to one or two years and time distance since last screening, but it is caused by the limited sensitivity of conventional cytology as a diagnostic method.

The primary task of cytology is to move closer its correlation with histopathology regarding the diagnostics

and terminology. This was exactly one of the reasons for the change in terminology and classification by introducing the Bethesda terminology. According to this classification, a cytological finding of lower grade in comparison to HSIL biopsy finding is: NILM, ASCUS, and LSIL, while in invasive cancer a lower-grade cytological result is NILM, ASCUS, LSIL, and HSIL. Table 4 shows the greatest reliability of cytology and histopathology in HSIL, and the lowest in invasive and adenochanges. Cytological differential diagnosis between HSIL and invasive cancer is a difficult and subjective task, with unclearly defined criteria depending on the histological type of cancer, the amount of cytoplasm, and pleomorphism in size and shape of malignant cells. For these reasons a great number of cytologists more readily choose HSIL cytological diagnosis than that of a cancer. The reliability of cytology in comparison with histopathology is not affected by the age of the patients, as seen in Table 7. Regardless the fact that cervical epithelium is hormone-sensitive and the fact that patients from older age group present atrophy and deeper cell layers, these physiological changes did not significantly affect the sensitivity and its reliability with pathology. In the light of these data, conventional cytology will not probably fulfill the needs for higher reliability and prediction of further pathogenesis of premalignant changes in future. Thus, even now there is a great need for additional markers to improve the accuracy of screening and triage of unreliable and mildly abnormal Pap smears [16].

Detection of high-risk HPV types has been shown to be far more sensitive in detecting HSIL changes. Literature data reveal that HPV typing has a 31.4% higher sensitivity, but about 3% lower specificity than cytology [4]. Distribution of age-related HPV test results is presented in Table 9 and it shows that 10% of patients with pathological pathohistological finding of HSIL and invasive cancer had normal HPV test results. There is no difference in HPV testing regarding the age of patients, indicating that it is equally sensitive for all age groups, which is about 90%. The only statistically significant difference has been proved in relation to positive HPV test in patients with invasive cancer and HSIL. Table 9 shows that HPV positive patients over 50 years of age had a diagnosis of invasive cancer, which is statistically significant. Persistent HPV infection and chronic genotoxicity caused by HPV integration with multipotent cells in the basal cell layer of the epithelium are responsible for such a distribution. Only long-term chronic and persistent HPV infection can result in the development of cancer. However, from Table 10 it can be seen that HPV type 16 infection has the greatest oncogenic potential at much earlier age, it statistically significantly more commonly leads to the development of invasive disease [17].

HPV type 16 is not only the most common type identified in the etiopathogenesis of cervical cancer, but it also has a pronounced oncogenic potential of E6 and E7 fragments that may lead to faster disease progression after a short-time persistence [18, 19].

HPV test result should be interpreted in relation to age. The positivity for the virus even after 30 years of age suggests that there is a problem in its elimination caused either by the aggressivity of oncogenic viral potential, or by poor immune reaction of the host. In such circumstances, the prognosis of the HPV infection indicates accelerated carcinogenesis. Many papers report that testing positive for HPV after 30 years of age should be treated as a pathological cytological finding (ASCUS), because the risk of CIN II + changes is 1:10. If HPV positivity is accompanied by borderline cytological abnormalities of ASCUS smears, the risk of CIN II + changes is over 25% [20].

Age and HPV positivity are important factors of sensitivity and specificity of HPV typing method in cervical cancer screening. Due to its high positivity, this test is not recommended for women under 30 years of age, since the infections at this age are only transient. Recent studies have been trying to investigate the difference in specificity for women aged 29-33 years and for women over 34 years of age. The results are still contradictory, but it has been shown that HPV typing in the group of patients aged 29-33 years does not decrease the specificity and does not detect a greater number of regressive lesions [5, 21].

The importance of HPV typing in detecting false-negative NILM cytological findings and low-grade cytological findings in comparison to pathohistological findings is shown in Tables 11 and 12. Statistical analysis of the obtained results revealed that HPV testing was equally positive both in NILM and in pathological cytology finding. Such a distribution shows that HPV testing is equally sensitive in detecting false-negative and accurate pathological results. As seen in Table 12, the most important thing is that HPV typing statistically significantly detected false-negative cytological results in patients with microinvasive and invasive cancer. Such a high sensitivity in detecting false-negative results has been confirmed by many recent studies [22]. Just for these reasons, numerous modifications of cervical cancer screening have been made, beginning from primary cytology-based screening, then simultaneous adjunctive cytology screening and HPV testing, to primary HPV testing. The data obtained in the present study pose the need to revise the existing screening using conventional cytology, as well as to consider the use of HPV testing as a method that not only increases the sensitivity in detecting cervical cancer, but also has a higher predictive value for cervical cancer.

Conclusions

Three-year cervical cancer screening is not reliable enough and does not statistically significantly decrease the incidence of HSIL and invasive changes of the cervix. The efficacy of screening is not increased by shortening screening interval using cytology to a period of one to two years due to relatively low cytology sensitivity. In 22% of patients cytological finding was normal at the time of diagnosis, while in 68% of patients cytological finding was of lower grade in comparison to the final pathological finding.

Conventional cytology had the lowest sensitivity in detecting pre-cancerous and cancerous changes of glandular adenoepithelium. In 46% of patients with adenocarcinoma the cytological finding was normal. The highest validity of cytology was in detecting pathological changes of high-grade HSIL. On the other hand, invasive changes displayed normal cytological finding in 12% of cases.

HPV typing showed significantly higher sensitivity in detecting intraepithelial and invasive changes of the cervix. In 10% of cases only HPV test was negative, particularly in HSIL intraepithelial changes, with possible spontaneous regression of some of them. HPV test increases the reliability of cytology in detecting preinvasive cytological changes, and statistically significantly decreases the percentage of false-negative cytological findings in microinvasive and invasive changes of the cervix. All the patients with diagnosed invasive cancer were HPV positive.

Special diagnostic focus is required in detecting HPV-16 which, unlike the other oncogenic types, statistically significantly results in invasive cancer at an earlier age, even in early 30s. HPV positivity in women over 50 years of age, or HPV-16 positivity after 30 years of age, statistically significantly more commonly result in cervical cancer.

Acknowledgements

This study was funded by Ministry of Education, Science and Technological Development of the Republic of Serbia grant number III 41018 and internal scientific research project of the Faculty of Medicine University of Nis No. 34.

References

[1] McCredie M.R., Sharples K.J., Paul C., Baranyai J., Medley G., Jones R.W., Skegg D.C.: "Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study". *Lancet Oncol*., 2008, *9*, 425.

[2] Arbyn M., Raifu A.O., Weiderpass E., Bray F., Anttila A. "Trends of cervical cancer mortality in the member states of the European Union". *Eur. J. Cancer.,* 2009, *45*, 2640.

[3] Meerding W.J., van Ballegooijen M., Burger M.P., van den Akker-van Marle M.E., Quint W.G., Habbema J.D. "Human papillomavirus testing for triage of women referred because of abnormal smears. A decision analysis considering outcomes and costs". *J. Clin. Epidemiol.,* 2002, *55*, 1025.

[4] Rijkaart D.C., Berkhof J., van Kemenade F.J., Coupe V.M., Rozendaal L., Heideman D.A., et al. "HPV DNA testing in population-based cervical screening (VUSA-Screen study): results and implications". *Br. J. Cancer.,* 2012, 106, 975.

[5] Rijkaart D.C., Berkhof J., van Kemenade F.J., Coupe V.M., Hesselink A.T., Rozendaal L., et al. "Evaluation of 14 triage strategies for HPV DNA-positive women in population-based cervical screening". *Int. J. Cancer.,* 2012, 130, 602.

[6] Schiffman M., Castle P.E., Jeronimo J., Rodriguez A.C., Wacholder S. "Human papillomavirus and cervical cancer". *Lancet*, 2007, 370, 890.

[7] Lowy D.R., Schiller J.T. "Reducing HPV-associated cancer globally". *Cancer. Prev. Res. (Phila),* 2012, 5, 18.

[8] Mayrand M.H., Duarte-Franco E., Rodrigues I., Walter S.D., Hanley J., Ferenczy A., et al; Canadian Cervical Cancer Screening Trial Study Group. "Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer". *N. Engl. J. Med.,* 2007, 357, 1579.

[9] Ferlay J., Soerjomataram I., Dikshit R., Eser S., Mathers C., Rebelo M., et al. "Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012". *Int. J. Cancer.,* 2015, 136, E359.

[10] Ferlay J., Shin H.R., Bray F., Forman D., Mathers C., Parkin D.M. "Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008". *Int. J. Cancer.,* 2010, 127, 2893.

[11] Službeni glasnik Republike Srbije. No. 73/2013 and 83/2013: "Uredba o nacionalnom programu ranog otkrivanja karcinoma grlića materice".

[12] Cuzick J., Clavel C., Petry K.U., Meijer C.J., Hoyer H., Ratnam S., *et al.*: "Overview of the European and North American studies on HPV testing in primary cervical cancer screening". *Int. J. Cancer,* 2006, *119*, 1095.

[13] Ronco G., Giorgi-Rossi P., Carozzi F., Confortini M., Dalla Palma P., Del Mistro A., et al; New Technologies for Cervical Cancer Screening Working Group. "Results at recruitment from a randomized controlled trial comparing human papillomavirus testing alone with conventional cytology as the primary cervical cancer screening test". *J. Natl. Cancer. Inst.,* 2008, *100*, 492.

[14] Paraskevaidis E., Kitchener H.C., Miller I.D., Mann E., Jandial L., Fisher P.M.: "A population-based study of microinvasive disease of the cervix—a colposcopic and cytologic analysis". *Gynecol. Oncol.,* 1992, *45*, 9.

[15] Castanon A., Landy R., Sasieni P.D. "Is cervical screening preventing adenocarcinoma and adenosquamous carcinoma of the cervix"? *Int. J. Cancer,* 2016, *139*, 1040.

[16] Nanda K., McCrory D.C., Myers E.R., Bastian L.A., Hasselblad V., Hickey J.D., Matchar D.B.: "Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review". *Ann. Intern. Med.,* 2000, *132*, 810.

[17] Ronco G., Giorgi-Rossi P., Carozzi F., Confortini M., Dalla Palma P., Del Mistro A., et al; New Technologies for Cervical Cancer screening (NTCC) Working Group. "Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial". *Lancet Oncol.,* 2010, *11*, 249.

[18] Herdman M.T., Pett M.R., Roberts I., Alazawi W.O., Teschendorff A.E., Zhang X.Y., *et al.*: "Interferon-beta treatment of cervical keratinocytes naturally infected with human papillomavirus 16 episomes promotes rapid reduction in episome numbers and emergence of latent integrants". *Carcinogenesis*, 2006, 27, 2341.

[19] Tsai T.C., Chen S.L.: "The biochemical and biological functions of human papillomavirus type 16 E5 protein". *Arch. Virol.,* 2003, *148*, 1445.

[20] Stoler M.H., Wright T.C. Jr., Sharma A., Apple R., Gutekunst K., Wright T.L.; ATHENA (Addressing THE Need for Advanced HPV Diagnostics) HPV Study Group: "High-risk human papillomavirus

testing in women with ASC-US cytology: results from the ATHENA HPV study". *Am. J. Clin. Pathol.,* 2011, *135*, 468.
[21] Rodríguez A.C., Schiffman M., Herrero R., Hildesheim A., Bratti C., Sherman M.E., *et al.*: "Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection". *J. Natl. Cancer. Inst.,* 2010, *102*, 315.
[22] Veijalainen O., Kares S., Kujala P., Vuento R., Osuala V., Tirkkonen M., *et al.*: "Implementation of HPV-based cervical cancer screening in an organised regional screening programme: 3 years of experience". *Cytopathology*, 2019, *30*, 150.

Corresponding Author:
R.ZIVADINOVIC, M.D., PhD
Bul. Dr Zorana Djindjica No.81
18000 Nis (Serbia)
e-mail: radomir.zivadinovic@medfak.ni.ac.rs