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The compliance of cytology and cytology combined with HPV HR test with a histological biopsy result, indicating cervical pathology

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Summary

Aim: The compliance of cytology and cytology combined with HPV HR test with cervical biopsy result, indicating the presence of cervical pathology was studied. *Materials and Methods:* The study enrolled 1,300 patients of Laboratory of Cervical Pathophysiology of University of Medical Sciences, who have had an abnormal Pap test and / or suspected cervical pathology following the clinical picture. The largest group consisted of 496 patients with cytological diagnosis of low-grade intraepithelial lesions (LSIL) and 388 patients diagnosed with atypical squamous cells of undetermined significance (ASC-US). Women age ranged from 16 to 79. In each of the women, cytological smear was performed, together with the DNA HPV HR (high-risk oncogenic) test and colposcopy with biopsy of suspected cervical changes. *Results:* After histopathological verification, 461 (35.4%) of abnormal results were obtained. Compliance of cytological diagnosis of ASC-US with abnormal cervical biopsy reached 15.2%, for atypical squamous cells - cannot exclude HSIL (ASC-H) - 40.4%, for LSIL - 42.1%, for high grade intraepithelial lesions (HSIL) - 63.8%, for atypical glandular cells (AGC) - 25.0%, and for suspected cancer – 77.8%, respectively. Considering HPV test result, the results of cytology with abnormal histopathology was by 5.8–25% higher, depending on the diagnosis, then the compliance of the single Pap smear. *Conclusions:* Cytology combined with molecular test, results in a significant increase in compliance of the cytological diagnosis with histopathological result. This compliance is the lowest for the cytological diagnosis of ASC-US and gradually increases with advancing cytological diagnosis, reaching the highest values when the cancer is suspected. Both cytological smear and HPV DNA HR test, applied separately, have false negative rates.

Key words: Cytological smear; HPV test; Screening; Cervical cancer; ASC-US atypical squamous cells of undetermined significance.

Introduction

Cervical cancer is currently in fourth place among cancers in women worldwide and is becoming the fourth leading cause of death in women due to cancer incidence [1]. In Poland, cervical cancer incidence has decreased over the past three decades to 30% [2]. Still, compared to the average of the European Union countries, the incidence of cervical cancer in Poland is about 15% higher.

The aim of screening programs is to detect precancerous cervical lesions, determined in accordance with the current terminology as low-grade intraepithelial lesions (LSIL) and high grade intraepithelial lesions (HSIL) [3]. Despite many imperfections, cytodiagnostics remains an indispensable tool for screening programs.

The current The Bethesda System (TBS) classification allows to provide a detailed description of the changes, consistent with the pathological terminology. This allows to separate cytological diagnosis indicating the presence of neoplasia from diagnoses related to inflammation, infection, and atrophy. The proportion of abnormal Pap smears ranges from 1% to 8% of all rated smears. In Poland, in terms of the Cervical Cancer Prevention and Early Detection Programme in 2008, abnormal Pap smears accounted for 2.43%. [4] In countries using TBS classification, the most usual abnormal cytological result is atypical squamous cells of undetermined significance (ASC-US), which in the US is approximately 67% of all incorrect diagnoses. [5] However, it has been shown that only 20% of the results described as ASC-US coincides with the actual change in neoplastic cervix. [6] The low specificity of ASC-US and LSIL diagnoses, followed by a large number of false positives results, leads to unnecessary invasive diagnostic procedures, which is not indifferent to patients' health and psyche. Furthermore, this situation also causes unnecessary costs of in-depth diagnostics. As the relation between persistent HPV infection and the development of cervical intraepithelial neoplasia and cancer is indisputable, worldwide there are researches ongoing to determine the place of molecular diagnostics in screening programs. There are following issues to be resolved: if HPV test is safe enough to use it as a study of first-line screening, and should it be used in parallel with cytology (the idea of the so-called co-testing), or maybe it should be seen as complementary cytology, in case of incorrect result (the so-called idea of reflex-testing).

The aim of the study was to assess the compliance of the single Pap smear and the Pap smear combined with the

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HPV high risk test with the result of histological biopsy of the cervix indicating the presence of cervical pathology.

Materials and Methods

The study group comprised 1,300 patients enrolled in the in-depth cervical diagnosis in Cervical Pathophysiology Laboratory in Poznan in 2011-2014. The indications for colposcopy was abnormal cytology and / or suspected cervical pathology following the clinical picture. The incorrect results, based on the TBS classification included diagnoses of ASC-US, atypical squamous cells - cannot exclude HSIL (ASC-H), LSIL, HSIL, atypical glandular cells (AGC) and a suspicion of cancer. The study included 156 (12%) women with normal cytology followed by macroscopic picture which implied a cervical pathology. Age of patients ranged from 16 to 79 years.

The largest group consisted of 496 (38.1%) patients with LSIL result and 388 (29.8%) patients with ASC-US result. Other diagnoses included HSIL (191 patients - 14.6%), ASC-H (52 patients - 4.0%) and AGC (8 patients - 0.6%) (Figure 1). In nine patients, based on cytologic smear, cervical cancer was suspected. In all 1,300 patients following tests were carried out: Pap smear test, molecular test, colposcopy, and guided biopsy of the vaginal part of the cervix with histopathological evaluation of the material.

The material from ectocervix and endocervix was collected with the use of cytobrush and transferred to a glass slide. The preparation was then fixed, washed and stained with hematoxylin solution, Orange dye and EA. After staining and fixation, the smear was covered with xylene and Canada balsam. Smears were evaluated by a senior cytotechnician and classified according to the current TBS classification. All preparations cyto-oncologically incorrect were re-evaluated by a physician pathologist in the Laboratory of Cervical Pathophysiology in University of Medical Sciences.

The material was collected with the cytobrush from ectocervix and endocervix and put in the liquid buffer - ThinPrep PreservCyt Solution. To evaluate HPV, a diagnostics test was performed. This test method based on real-time PCR, identifies 13 most common highly oncogenic HPV types in terms of in vitro. Cell control is provided by simultaneous performance of PCR amplification of DNA β-globin.

Colposcopy in the Laboratory of Cervical Pathophysiology was performed with the use of stereoscopic colposcope. Visualization of the entire transformation zone was the basis for the classification of video colposcopy as satisfactory. Each time an attempt was made with a 3%-solution of acetic acid and Shiller's test with Lugol liquid. Evaluation of colposcopy images was based on the Reid's scale, considering the margin and whitening changes after application of acetic acid, negative iodine, and the nature of the vessels.

In each of the patients enrolled in the study, guided biopsy of suspicious lesions of the cervix and endocervical curettage were performed. The choice of performing biopsy was indicated by colposcopic evaluation. The material was fixed in buffered formalin, after dehydration, embedded in paraffin blocks, and stained by H&E scheme. All preparations were prepared and subjected to independent blinded assessment in the Laboratory of Cervical Pathophysiology of Gynecology and Obstetrics Clinical Hospital in Poznan.

Results

In the entire group of 1,300 women following pathological verification, in 461 (35.4%) patients abnormal results were obtained. LSIL were found in 211 (16.2%) women, HSIL in 235 (18.0%) patients and 15 (11.5%) of patients were diagnosed with cervical cancer; there were 13 cases of squamous cell carcinoma and two cases of adenocarcinoma.

In the group of 156 patients with a baseline normal cytological results, in 12 (7.7%) LSIL was diagnosed and in 29 (18.5%) HSIL was indicated. The remaining 115 (73.7%) women received the correct histology result.

ASC-US result was the indication for in-depth diagnosis in 388 patients, while 46 (11.8%) were diagnosed with LSIL and in 13 (3.3%) HSIL were observed.

In the most numerous group with cytologic diagnosis of LSIL (496 women), there were 121 (24.3%) cases of LSIL, and 88 (17.7%) cases of HSIL.

In patients with HSIL cytological result (191 women): 24 (12.5%) cases of LSIL were diagnosed, 90 (47.1%) cases of HSIL were observed, and eight cases of cervical cancer were detected.

In eight patients with confirmed AGC, the presence of squamous cell carcinoma was diagnosed in two cases (25.0%); in the remaining six cases correct result was obtained.

In nine patients with suspected malignant carcinoma, three cases of squamous cell carcinoma were diagnosed, one case of adenocarcinoma and one case of high-grade changes were observed, while in two patients low-grade changes were indicated. The results are detailed in Table 1.

When analysing the compliance of each diagnosis with histological result, it was seen that it was the highest (77.8%) for cytological suspicion of cancer. In case of HSIL it amounted to 63.8%, for LSIL – 42.1% and for ASC-H – 40.4% respectively. The lowest compliance with biopsy diagnoses were obtained for AGC and ASC-US - 25.0% and 15.2% respectively. The results are shown in Table 2 and Figure 2.

Molecular test for the detection of HPV (HPV HR - high risk) was performed in all 1300 patients. A positive result, thus confirming the presence of any of 13 highly oncogenic types was found in 752 patients (57.8%). Among HPV HR positive women, there were 418 (55.5%) abnormal cervical biopsy results confirmed and in the remaining 334 (44.4%) no changes were observed. In the group of 548 HPV HR - negative women, in 30 (5.5%) LSIL were detected, in 12 (2.2%) HSIL were observed, in one patient cervical adenocarcinoma was diagnosed. Compliance of positive HPV test result with abnormal biopsy was calculated at 55.5%. The results are presented in Table 3.

Table 4 presents the histopathological results for each diagnosis, considering molecular test. Table 5 presents the compliance of abnormal cytology results and positive HPV

Table 1. — *Abnormal histological outcomes for each cytological result.*

Cytological result	ASC-US number (%)	ASC-H number (%)	LSIL number (%)	HSIL number (%)	AGC number (%)	Suspicion of cancer number (%)
LSIL (low grade intraepithelial neoplasia)	46 (11.8%)	6 (11.5%)	121 (24.3%)	24 (12.5%)	-	2 (22.2%)
HSIL (high grade intraepithelial neoplasia)	13 (3.3%)	14 (26.9%)	88 (17.7%)	90 (47.1%)	-	1 (11.1%)
Squamous cell carcinoma	-	1 (1.9%)	-	7 (3.6%)	2 (25.0%)	3 (33.3%)
Adenocarcinoma	-	-	-	1 (0.5%)	-	1 (11.1%)

Table 2. — *Histopathological outcomes in patients with abnormal Pap smear results.*

Cytological result	ASC-US number (%)	ASC-H number (%)	LSIL number (%)	HSIL number (%)	AGC number (%)	Suspicion of cancer number (%)
Abnormal results	**59 (15.2%)**	**21 (40.4%)**	**209 (42.1%)**	**122 (63.8%)**	**2 (25.0%)**	**7 (77.8%)**
Normal results – no pathology	329 (84.8%)	31 (59.6%)	287 (57.9%)	69 (36.2%)	6 (75.0%)	2 (22.2%)

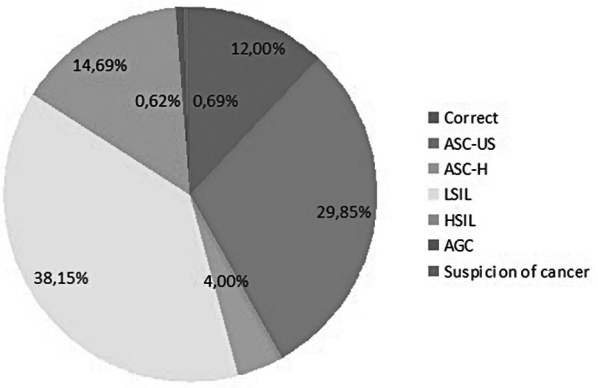

Figure 1. — Cytological diagnosis in the tested material.

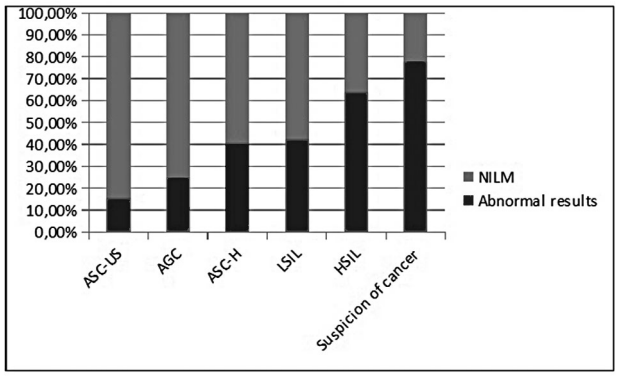

Figure 2. — Histopathological outcomes in patients with abnormal Pap smear results.

Table 3. — *Histopathological outcomes in HPV-positive and HPV-negative patients.*

HPV test result	HPV HR positive number (%)	HPV HR negative number (%)
Abnormal results	**418 (55.5%)**	43 (7.8%)
Normal results – no pathology	334 (44.4%)	505 (92.1%)
Altogether	752	548

test result with the result of the biopsy.

After combining the cytology with molecular test, a significant increase of compliance of incorrect results with abnormal histopathological results was indicated (Figure 3). In case of women with ASC-US diagnosis, HPV-positive, this compliance reached 35.0%, indicating 19.8% more than in the case of relying on a single cytology.

In case of women with ASC-H, HPV-positive, the compliance with the biopsy result was higher by 24.9% for LSIL - 14.6% for HSIL - 5.8%, and for the AGC up to 25%, after considering molecular test. In case of women with suspected cancer cytology and positive HPV test, compliance with the histological result was 9.7% higher than without considering the molecular test.

Considering all abnormal cytological diagnoses, after combining diagnostics with HPV test, an average of 16.6% more compliance with the final diagnosis was achieved.

Table 4. — *Abnormal histopathological outcome for individual Pap smear and HPV test result.*

Cytological result	ASC-US number (%)		ASC-H number (%)		LSIL number (%)		HSIL number (%)		AGC number (%)		Suspicion of cancer number (%)	
HPV test result	HPV (+) 140 (36.0%)	HPV (-) 248 (63.9%)	HPV (+) 26 (50.0%)	HPV (-) 26 (50.0%)	HPV (+) 333 (67.1%)	HPV (-) 163 (32.8%)	HPV (+) 165 (86.3%)	HPV (-) 26 (13.6%)	HPV (+) 4 (50.0%)	HPV (-) 4 (50.0%)	HPV (+) 8 (88.9%)	HPV (-) 1 (11.1%)
LSIL (low grade intra-epithelial neoplasia)	36 (9.2%)	10 (2.5%)	4 (7.6%)	2 (3.8%)	106 (21.3%)	15 (3.0%)	23 (12.0%)	1 (0.5%)	-	-	2 (22.2%)	-
HSIL (high grade intra-epithelial neoplasia)	13 (3.3%)	-	12 (23.0%)	2 (3.8%)	83 (16.7%)	5 (1.0%)	85 (44.5%)	5 (2.6%)	-	-	1 (11.1%)	-
Squamous cell carcinoma	-	-	1 (1.9%)	-	-	-	7 (3.6%)	-	2 (25.0%)	-	3 (33.3%)	-
Adeno-carcinoma	-	-	-	-	-	-	-	1 (0.5%)	-	-	1 (11.1%)	-

Table 5. — *Histopathological outcomes in HPV-positive women with abnormal Pap smear results.*

Cytological diagnosis with HPV test positive result	ASC-US HPV(+) number (%)	ASC-H HPV(+) number (%)	LSIL HPV(+) number (%)	HSIL HPV(+) number (%)	AGC HPV(+) number (%)	Suspicion of cancer HPV(+) number (%)
Abnormal results	**49 (35.0%)**	**17 (65.3%)**	**189 (56.7%)**	**115 (69.6%)**	**2 (50%)**	**7 (87.5%)**
Normal results –no pathology	91 (65.0%	9 (34.6%)	144 (43.2%)	50 (30.3%)	2 (50%)	1 (12.5%)

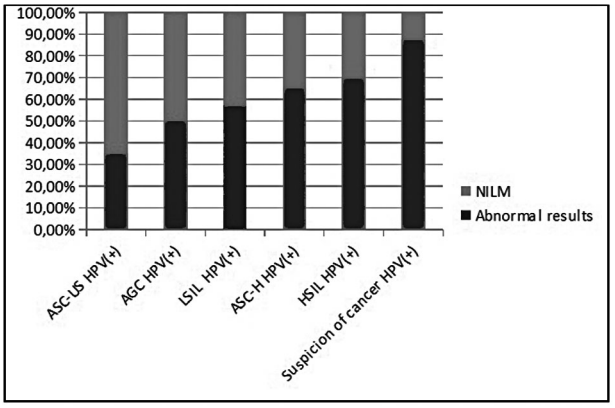

Figure 3. — Histopathological outcomes in women with abnormal cytology and HPV positive test result.

Discussion

Introducing cytodiagnostics in twentieth century, which became for the next decades the basis of screening programs, has contributed to a large decline in the incidence and mortality of cervical cancer worldwide. In countries pursuing organized prevention programs, the mortality decreased by 70% over the last 40-50 years. [7] In recent years, it has been observed that cytodiagnostics has had difficulties to meet the requirements of modern test screening, such as high sensitivity and specificity, objectification and test automation, lengthening intervals between researches and the ability to isolate a group of patients at high risk. Optimal diagnostic tool is expected above all to present precise identification of patients with precancerous stage. The results obtained in the present study confirm that screening based on a single cytodiagnostics is imperfect. In the group of women with normal cytology analyzed in this work, following verification of histopathologic changes, there were 7.7% cases of LSIL, indicated and 18.5% of HSIL observed. It is commonly indicated in many works that the high number of false negative results is a major drawback of cytological smears. Some studies show that from 20% to 40% of new cases of cervical cancer are diagnosed in women undergoing regular Pap smear screen [8, 9]. This may be due to human error at all stages of the diagnostic process - collection, dyeing, recording and evaluation by cytotechnician. Depending on the center, from 1% to 8% of Pap smears are determined as unsuitable for evaluation. In the study of Islam *et al.*, the smears unsuitable for evaluation were re-analyzed and the presence of atypical cells and even cancer were indicated in 7% of cases. [10] Another important problem presented in this work resulted to be a significant number of false-positive results. This particularly concerned ASC-US and AGC diagnoses. The results of cervical biopsy confirmed the existence of cervical pathology only in 15.2% of cases of ASC-US and 25.0% of AGC. Although the AGC diagnosis involved only eight women in this study, a group with a cytological diagnosis

of ASC-US consisted of 388 patients, thus it can be considered as representative. Worldwide, it is emphasized that even in the best centers considering fulfillment of smears quality conditions in terms of being suitable for evaluation, the scope of ASC-US is very wide. [11] The resulting compliance of ASC-US with a histopathological score of 15% is very close to the data obtained in many other studies that have shown that in 50-80% of women with this diagnosis, histological verification does not confirm the existence of pathology of squamous epithelium. [6] Recommended by the American Society for Colposcopy and Cervical Pathology, diagnostic algorithm for the diagnosis of ASC-US cytology includes performing HPV test and further evaluation (colposcopy and cervical biopsy) only in case of HPV positive test result. Women with ASC-US and a HPV negative test result should return to the screening used in the general population, considering the recommendations for a particular age group. The premise behind this procedure is the risk of developing HSIL in HPV-negative women with ASC-US diagnosis which is less than 2%. [12]

Applying this recommendation, in practice allows as an exception not performing the colposcopy in 1.5 million women in the US each year. [13] In this study cytology combined with HPV HR test for the diagnosis of ASC-US resulted in an increase of compliance with abnormal biopsy by 19.8%. In the group of 248 HR HPV-negative women diagnosed with ASC-US, there was no single case of HSIL changes or cancer, while ten cases of low-grade changes were indicated, which accounted for 2.5% of all patients with ASC-US. The results confirm overdiagnosing of changes known as ASC-US and the usefulness of molecular test to a much more precise identification of patients with neoplasia, maintaining safety of screening procedure (no HSIL change and cancer in ASC-US HPV-negative women).

In the most numerous in the present study group of 496 women diagnosed with LSIL, cervical biopsy confirmed epithelial abnormalities in 42.1% of cases. After combining cytology with HPV test, the percentage of compliance with the abnormal results increased to 56.7%. It should be noted that five cases of HSIL were diagnosed in the group of women with LSIL HR HPV-negative, which in this group accounted for 1% of the results. Similarly, in the group of HPV-negative patients with HSIL cytology results, five cases (2.6%) of high grade changes were detected and one case (0.5%) of adenocarcinoma was found. Therefore, it was confirmed that both cytodiagnostics and molecular test performed separately are not free from false negative results. However, in case of HPV test, the amount of these results was twice as less than in case of cytology. Both methods are also burdened with false positive results, for cytology particularly concerning ASC-US and AGC diagnoses. In the present study, cytology and HPV test were performed in all women, where the age range was from 16 to 79. Due to the fact that HPV test specificity increases together with patient's age, it can be stated that if it had been performed in accordance with worldwide recommendations, only in women over 30 years, the percentage of false positive results would be much lower. The factor which might increase the specificity of molecular test can be the use of 16/18 HPV genotyping - i.e. HPV types that are significantly more often associated with the cervical pathology [13].

It has been observed that more advanced stages of abnormal cytology diagnoses correlated with a higher proportion of HPV-positive patients in each group. Thus, the group of patients diagnosed with ASC-US HPV-positive was 36.0%, the group with ASC-H diagnosis was 50.0%, LSIL concerned 67.1% of patients and the group with cytological diagnosis of cancer was 88.9%. Therefore, combining molecular test with Pap screening seems to be particularly useful for less advanced diagnosis, such as ASC-US and LSIL.

Conclusions

Compliance of abnormal cytology with biopsy result indicates that the presence of cervical pathology is the lowest for ASC-US diagnosis and gradually increases reaching the highest values in cases of suspected cancer. Cytology combined with HPV HR test results in a significant increase in compliance of cytological diagnosis with histopathological results. Cytological smear and HPV HR test used separately are not free from false negative results.

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