Analysis of cervicovaginal smears in terms of their technical quality

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ABSTRACT

Introduction: The aim of this study was to analyze the technical evaluation of cervicovaginal smears as well as the correlation of the technical quality of the smear with the patient's age.
Materials and methods: The study included a group of 43,696 women who underwent exfoliative cytology. The smears were stained according to the Papanicolaou method and then underwent cytological evaluation based on the Bethesda system.
Results: The technical quality of the cytology smears was found to be satisfactory (AI) in 80.6% of cases, acceptable for cytological evaluation (AII) in 19% mainly as a result of poor readability of the smear due to numerous inflammatory cells (35.5%), whereas not suitable for evaluation (AIII) was in 0.4% of cases. Moreover, there was a high proportion of smears without cells from the cervical canal in women under 50 years of age. In women over 50, the most common cause of conditional evaluation of the smear was its excessive dryness
Conclusions: In all age groups, an abundance of inflammatory cells was a factor that hindered cytologic evaluation of the smear. Only representative material containing cells from the cervical canal should be collected. It is of utmost importance to pay special attention to the correct and quick fixation of smears taken from women over 50 years of age.
Keywords: Bethesda system, cervical cancer, cervical cytology, cervical smear, technical evaluation

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INTRODUCTION

The aspect of high incidence of and the rate of mortality from cervical cancer, especially in young women, is a significant public health problem in Poland and worldwide. The incidence of invasive cervical cancer is preceded by many years of development of precancerous conditions occurring as a result of a sexually transmitted infection with oncogenic types of human papillomavirus in the area of cervical epithelial cells. Pre-cancerous conditions called cervical intraepithelial neoplasia (or cervical dysplasia) can be detected by cytology [1-3].

Cervical cancer is a malignancy that meets the conditions for effective population-based screening. Its progression includes well-described precancerous lesions that precede the development of invasive cancer by many years. These conditions can be treated successfully even via outpatient care. Importantly, the implementation of treatment of precancerous conditions in almost all cases prevents the development of the invasive form [1,4]. In member states of the European Union, screening began in the 1970s and has led to a decrease in morbidity and mortality of up to 80%. In developing countries, where screening is not performed, the rate of mortality from cervical cancer is very high. Poland ranks among countries with high incidence of and mortality from cervical cancer. In 2019, there were 2407 new cases of the disease and 1569 deaths caused by it [2,4].

Secondary prevention aims to locate precancerous lesions and early forms of cancer when no subjective or physical symptoms are yet present. Population-based screening is used for this purpose. In Poland there is the National Program for Active Prevention of Cervical Cancer. One of its components consists in sending named invitations to every woman aged 25–59 who has not had a cytology test performed within three previous years. Cytological smears are evaluated according to the Bethesda system and Papanicolaou system [5,6]. A Central Coordination Center (COK) and Provincial (Voivodeship) Coordination Centers (WOKs) were established in Poland, which, according to the authors of this project, will optimize the implementation of the cytology invitation system.

Cytodiagnosis of the cervix is based on the evaluation of smear preparations of cells exfoliated and taken from the surface of the epithelium that lines the lower part of a woman's reproductive organ. The smear should contain cells of the stratified squamous epithelium covering the ectocervix and the simple glandular epithelium lining the cervical canal. Gynecological cytodiagnosis is of tremendous importance in the prevention of cervical cancer as well as in the diagnosis of actual pathologies of the cervix. It has proven to be the optimal method for detecting asymptomatic precancerous conditions [5,6].

In 1988, the National Cancer Institute in the USA proposed a new method for interpreting cytological smears instead of the Papanicolaou classification – the so-called Bethesda system. Nowadays, after modifications introduced in 2001 and 2014, this method is accepted as the only appropriate and effective one to assess a gynecological cytodiagnosis. The Bethesda system consists in a several-step evaluation of the smear. First, it is determined whether the smear contains representative material for examination and whether it is suitable for evaluation, based on by the number of cells and the presence of epithelial cells of the cervical canal. Then, a general decision is made on whether the smear is normal or not. In the next step, a detailed description of the lesions found is presented in accordance with the current terminology [5-7]. The Bethesda system is widely accepted by its potential users who emphasize its numerous advantages resulting from the detailed analysis of cytological images.

The aim of this study was to analyze the technical evaluation of cervicovaginal smears as well as the correlation of the technical quality of the smear with the patient's age.

MATERIALS AND METHODS

The study included a group of 43,696 women who underwent exfoliative cytology, diagnosed at the Academic Centre for Pathomorphological and Genetic-Molecular Diagnostics in the years 2011-2012. Consent to the study was obtained from the Bioethics Committee of the Medical University of Bialystok. The material analyzed was divided into 8 age groups: 1 (0-20 y.o.), 2 (21-30 y.o.), 3 (31-40 y.o.), 4 (41-50 y.o.), 5 951-60 y.o.), 6 (61-70 y.o.), 7 (71-80 y.o.) and 8 (81-100 y.o). The smears were stained according to the Papanicolaou method and then underwent cytological evaluation based on the Bethesda system.

RESULTS

Technical evaluation of the examined material according to the Bethesda system

The technical quality of the preparation was found to be satisfactory for evaluation (AI) in 80.6% of cases. The smear conditionally acceptable for cytological evaluation (AII) was obtained from 19% of the patients, whereas smear not suitable for evaluation (AIII) was found in 0.4% of cases (Table 1).

In the study population, there were air-dried smears – 18.2%, hypocellular smears – 6.8%, smears not very clear due to numerous inflammatory cells – 35.5% and smears not very clear due to

numerous erythrocytes – 15.3%. Absence of cells from the cervical canal was found in 31.8% of the smears, significant cytolysis or autolysis – in 4.5% and 0.1% of the smear samples contained exogenous substances. It was determined that 0.4% of the smears were not suitable for evaluation, due to the following reasons: too hypocellular – 63.1%, dried before fixation – 30.0%, poorly fixed –21.8%, unreadable due to numerous inflammatory cells 4.4%, unreadable due to numerous erythrocytes – 20.1% and unreadable due to other reasons – 1.2% (Table 1).

Table 1. Technical evaluation of the evaluated material according to the Bethesda system

Technical evaluation of the evaluated material according to the Bethesda system		**Number of tests n = 43696 (%)**
AI	smear adequate for evaluation	35220 (80.6%)
AII	smear conditionally adequate for evaluation	8316 (19%) *
AIIa	air-dried smear	1516 (18.2%)
AIIb	hypocellular smear	569 (6.8%)
AIIc	smear not very clear due to numerous inflammatory cells	2955 (35.5%)
AIId	smear not very clear due to numerous erythrocytes	1276 (15.3%)
AIIe	no cells from the cervical canal	2649 (31.8%)
AIIf	significant cytolysis or autolysis	376 (4.5%)
AIIg	smear contains exogenous substances	10 (0.1%)
AIII	smear inadequate for evaluation	160 (0.4%)
AIIIa	too hypocellular smear	101 (63.1%)
AIIIb	smear air-dried before fixation	48 (30.0%)
AIIIc	smear poorly fixed	35 (21.8%)
AIIId	smear unreadable due to numerous inflammatory cells	7 (4.4%)
AIIIe	smear unreadable due to numerous erythrocytes	33 (20.1%)
AIIIf	smear unreadable due to other reasons	2 (1.2%)

* The higher number of AII, AIII cases is caused by the fact that there may have been 2 or more factors in a single patient that hindered the evaluation of the smear.

Technical evaluation of the smear versus the patient's age

In all age groups, numerous inflammatory cells were a factor that hindered cytologic evaluation of the smear. Moreover, there was a high proportion of smears without cells from the cervical canal in women under 50 years of age. In women over 50, the most common cause of conditional evaluation of the smear was its excessive dryness (Table 2).

Table 2. Technical evaluation of AII smears vs. the patient's age

	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**	**Group 8**
AIIa	51 (12.6%)	356 (13.7%)	279 (13.0%)	181 (10.6%)	324 (21.2%)	229 (33.2%)	78 (35.6%)	13 (33.3%)
AIIb	22 (5.4%)	153 (5.8%)	115 (5.4%)	73 (4.3%)	97 (6.3%)	65 (9.4%)	39 (17.8%)	4 (10.4%)
AIIc	143 (35.5%)	944 (36.1%)	676 (31,.7%)	418 (24.5%)	474 (31%)	215 (31.2%)	65 (29.7%)	13 (33.3%)
AIId	37 (9.3%)	327 (12.6%)	369 (17.3%)	348 (20.4%)	133 (8.7%)	46 (6.7%)	14 (6.4%)	1 (2.6%)
AIIe	123 (30.5%)	695 (26.7%)	606 (28.3%)	617 (36.1%)	455 (29.7%)	121 (17.5%)	22 (10.1%)	7 (17.8%)
AIIf	27 (6.7%)	129 (4.9%)	90 (4.2%)	69 (4.0%)	46 (3.0%)	14 (2.0%)	0 (0%)	1 (2.6%)
AIIg	0 (0%)	4 (0.2%)	2 (0.1%)	2 (0.1%)	1 (0.1%)	0 (0%)	1 (0.4%)	0 (0%)
Total	403 (100%)	2608 (100%)	2137 (100%)	1708 (100%)	1530 (100%)	690 (100%)	219 (100%)	39 (100%)

* The higher number of patients in this analysis results from the fact that in some cases 2 or more features contributing to poorer quality of the smear were found in one patient.

Insufficient number of cells in the examined smear was the factor preventing cytologic evaluation of the smear in each age group. In women over 50 years of age, an additional factor excluding smear evaluation was overdrying of the sample before fixation (Table 3).

Table 3. Technical evaluation of AIII smears vs. the patient's age

	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**	**Group 8**
AIIIa	3 (42.8%)	23 (41.8%)	20 (50%)	13 (52%)	16 (41%)	11 (50%)	10 (50%)	3 (42.8%)
AIIIb	2 (28.6%)	8 (14.5%)	4 (10%)	6 (24%)	12 (30.7%)	8 (36.5%)	5 (25%)	2 (28.6%)
AIIIc	1 (14.3%)	9 (16.4%)	3 (7,5%)	2 (8%)	10 (25.7%)	3 (13,5%)	4 (20%)	2 (28.6%)
AIIId	0 (0%)	3 (5.5%)	13 (32.5%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
AIIIe	0 (0%)	12 (21.8%)	0 (0%)	4 (16%)	1 (2.6%)	0 (0%)	0 (0%)	0 (0%)
AIIIf	1 (14.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	7 (100%)	55 (100%)	40 (100%)	25 (100%)	39 (100%)	22 (100%)	20 (100%)	7 (100%)

***** The higher number of patients in this analysis results from the fact that in some cases 2 or more features contributing to poorer quality of the smear were found in one patient.

DISCUSSION

An important element of diagnosis in the Bethesda system is information about the quality of the smear. We analyzed the percentage of smears suitable for evaluation as well as the reasons for classifying smears as conditionally suitable or unsuitable for evaluation. A statistical analysis of the cytological smears that were delivered to the Academic Centre for Pathomorphological and Genetic-Molecular Diagnostics in the years 2011–2012 revealed that 80.6% of smears were suitable for evaluation (AI), 19% of cytology specimens were only conditionally accepted for evaluation (AII), mainly as a result of poor readability of the smear due to numerous inflammatory cells (35.5%). The second most common reason for conditional acceptance of the smears for evaluation was the absence of cells from the cervical canal (31.8%). A similar analysis had been conducted earlier: in 2001, by Chosia et al. [8], in a group of 71,444 patients diagnosed in the Department of Pathomorphology at the Pomeranian Medical University in Szczecin. In their study group, 60.3% of smears were conditionally evaluable, and the primary cause of smear imperfection was the absence of cells from the cervical canal (68.1%). Excessive dryness of the smear (36.7%) was described as the second cause of classifying preparations as AII. Smears that were barely readable due to numerous inflammatory cells, which ranked first in our own study, took the fourth place in the study conducted in the Cytology Laboratory of the Department of Pathomorphology at the Pomeranian Medical University in Szczecin. The high differences in the percentage of smears suitable for conditional evaluation in two different diagnostic centers may result from the subjective assessment of the diagnostician. It is also worth noting that the study by Chosia et al. [8] dates back to 2001, i.e., the time when the Bethesda system was still being introduced to the diagnosis of cervical cytology in Poland. However, it is the only detailed analysis of smear quality in gynecologic cytodiagnosis in both domestic and foreign literature. On the other hand, the lower percentage of preparations suitable for conditional evaluation due to the absence of cells from the cervical canal in our study compared to the study by Chosia et al. [8] may be caused by increased awareness of material collection among gynecologists. Furthermore, Chosia et al. [9] compared the quality of smears in two groups of active (GSA) and inactive (GSN) screening. Smears not fully suitable for evaluation constituted 30.6% of the GSA group, while in the GSN group there were twice as many samples of that kind. The lack of cervical cells was also cited as the main reason for the said situation. An analysis of the evaluation of the cytological preparation quality was also conducted in 2007 at the Department of Gynecology of the Clinical Hospital of the Medical University of Bialystok [10] in a group of 5106 patients. However, the researchers classified AI and AII smears as a single group of preparations suitable for evaluation, which amounted to 99.1% (in our study, it is 99.6%). Attention was paid to smears unsuitable for evaluation (AIII; 0.9%), the main reason for which was an excessively thick layer of erythrocytes covering the entire field of view, and the second

most important cause was cell cytolysis. In our study, 0.4% of smears were classified as AIII. In contrast, the primary factor for unsuitability of smears for evaluation was hypocellularity of the smears (63.1%), followed by drying of the smear before fixation (30.0%), poor fixation (21.8%) and too numerous erythrocytes, preventing evaluation. These differences are related to the medical personnel's knowledge of how to collect and fix the material.

The analysis of smear quality in every age group appears to be particularly important. The representativeness of the cellular composition as well as technical quality of the smear largely affect the detection, or lack thereof, of pathological cells and, consequently, the number of false-negative cases. About 90% of lesions that may lead to the development of invasive cervical cancer arise in the so-called transformation zone [11]. Therefore, it is very important to collect material from the vaginal part of the cervix, the transformation zone and the cervical canal. In our study, we found that the percentage of cervical cell absence increases gradually with age, particularly in age group IV (36.1%). This may be connected with the fact that women at this age are often pregnant, take oral contraceptives or have undergone medical procedures regarding the cervix. In contrast, in women over the age of 50, the most common cause of conditional evaluation of the smear was excessive dryness of the preparation, found in 35.6% of age group VII. The air-drying of smears in these age groups is a natural phenomenon associated with the menopause of the examined women. Care should be taken to fix the smear immediately, otherwise the smear dries out and becomes unreadable. Epithelial cells with features of significant atrophy undergo rapid drying. Therefore, these smears often cause diagnostic difficulties, and in order to resolve them conclusively, short-term estrogen administration and repeated smear collection are recommended. In all age groups, an abundance of inflammatory cells was a factor hindering cytologic evaluation of the smear. Inflammatory cells are a common component of cervical smears and occur even in women without clinical symptoms. Their considerable accumulation in the smear may be diagnostically challenging. Inflammatory cells in smears are usually not accompanied by dysplastic or cancerous cells. In contrast, *Trichomonas vaginalis* was found 4 times more often in smears with dysplastic or cancerous cells [11]. The proper collection of the smear, which is inseparably related to the quality of the smear, is crucial to the effectiveness of screening. Failure to comply with this principle is the cause of false-negative diagnoses. The only study that has been conducted on smear quality in correlation with detection of pathological changes was conducted by Chosia et al. [8] in 2001. However, the researchers proved a close relationship between smear quality and the number of lesions detected. The percentage of lesions found in case of smears appropriate for evaluation (AI) was markedly higher, amounting to 0.84%, compared to not fully adequate (AII) smears – 0.46% [8]. It should also be mentioned that in the group of smears qualified as AII, the most frequently found feature was the lack of cervical cells. Thus, taking care of the correct quality of the smear is reasonable and brings tangible benefits in the diagnostic process.

CONCLUSIONS

1. In all age groups, an abundance of inflammatory cells was a factor that hindered cytologic evaluation of the smear.
2. Only representative material containing cells from the cervical canal should be collected.
3. It is of utmost importance to pay special attention to the correct and quick fixation of smears taken from women over 50 years of age.

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