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The role of cervical smear in cervical cancer diagnosing

Profilaktyka cytologiczna raka szyjki macicy

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S u m m a r y

Cervical cancer is the second most common malignant cancer in females worldwide. It is well-established that Human Papillomavirus (HPV) infections play a critical role in the development of cervical cancer. However, a large number of women infected with oncogenic HPV types will never develop cervical cancer. About 90% of adenocarcinoma and 75% squamous cervical cancers are causally related to persistent cervical infections by oncogenic human papillomavirus genotypes 16, 18. It is estimated that oncogenic papillomavirus genotypes 16 and 18 is the most frequent sexually transmitted virus. The HPV vaccination does not protect patients from all oncogenic HPV types, so it is essential to continue cytological screening. In this review article we describe the role of cervical smear in precancerous lesion and cervical cancer diagnosing. According to the recommendation the population-screening of cervical lesions should be performed every three years for women at age 21-59 years. This test can be completed but it can be replaced by a molecular test DNA HPV. However, the most effective way to avoid the cervical cancer is health education about the indicating factors.

Key words: cervical smear, cervical cancer, cervical intraepithelial neoplasia

S t r e s z c z e n i e

Rak szyjki macicy jest drugim pod względem częstości nowotworem złośliwym u kobiet na świecie. Zakażenie wirusem brodawczaka ludzkiego HPV stanowi najistotniejszy czynnik zachorowania na raka szyjki macicy. Przetrwałe zakażenie wywołane wirusem brodawczaka ludzkiego typu 16 i 18 przyczynia się do powstania ponad 90% płaskonabłonkowych i 75% gruczołowych raków szyjki macicy. Obecnie szacuje się, że HPV typu 16 i 18 jest najbardziej rozpowszechnionym wirusem przenoszonym drogą płciową. Szczepionka przeciwko wirusowi brodawczaka ludzkiego nie chroni przed wszystkimi onkogennymi jego typami, dlatego niezbędne jest kontynuowanie badań przesiewowych. W poniższym artykule przedstawiamy rolę badania cytologicznego w diagnostyce zmian przedrakowych i raka szyjki macicy. Według światowych rekomendacji populacyjny skrining zmian szyjki macicy powinien odbywać się w oparciu o cytologię wykonywaną co 3 lata u kobiet w wieku 21-59 lat. Badanie to może być uzupełnione, ale nie może zostać zastąpione przez test molekularny DNA HPV. Jednak najskuteczniejszą metodą unikania czynników rozwoju raka szyjki macicy jest edukacja zdrowotna informująca o czynnikach zwiększonego ryzyka zachorowania i kształtująca zachowania prozdrowotne.

Słowa kluczowe: cytologia, rak szyjki macicy, śródnabłonkowa neoplazja

Cervical cancer is the second most common malignant cancer in females worldwide. More than 470 000 new cases and 230 000 deceases are diagnosed every year. The average age of women in whom cervical cancer develops fluctuates between 45 and 50. An infection with Human Papillomavirus (HPV) constitutes the most essential factor of cervical cancer incidence. In 2008 the Nobel Prize Committee recognised the discovery of HPV in the process of cervical cancer carcinogenesis as the most important happening in the field of physiology and medicine. The main oncogenic type of the virus is HPV genotype 16, the second being genotype 18. HPV is transmitted mostly sexually, but also vertically and through a direct contact with an infected person. Serological typing of the virus allowed to differentiate non-oncogenic genotypes (HPV 6, 11, 40, 42, 43, 44, 54, 61), which cause *condylomata acuminata* on genital organs, and oncogenic genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58) connected with a high risk of pre-neoplasm and neoplasm lesions of genital organs and anus. In women under 25 most infections with HPV regress spontaneously within 12 to 18 months. **An infection lasting longer than 24 months is the main factor predisposing to the development of cervical cancer.**

Cervical cancer prevention involves initial and secondary prophylaxis:

- initial prophylaxis – preventive vaccines, avoiding HPV infection,
- secondary prophylaxis – cytological screening, HPV testing or a combination of both techniques.

According to the WHO definition a screening test is a test, which conducted on healthy people allows for an early detection and treatment of a disease, the result of whose is decreased mortality in a population. A screening test does not diagnose a disease but only indicates its presence.

The key aim of population screening tests is to detect direct harbinger cancer lesions – that is cervical intraepithelial neoplasia (CIN) lesions or cancer. Detecting cervical pathology is possible due to the use of numerous standard and non-standard methods. The most common standard diagnostic methods are: conventional cytological smear, liquid based cytology (LBC), immunocytodiagnostics and colposcopy.

Cytological examination consists in collecting cervical specimens from the vaginal portion of the cervix and the endocervical canal with the use of a cytobrush on a microscope slide, and then fixing cells with a cytofix preparation. During the oncological assessment of smears a five-level Papanicolau classification (tab. 1) and the Bethesda system are used (tab. 2).

Table 1. The classification of cytological smears s according to Papanicolau.

Cytological result	Cytological image
I°	Present correct cells of stratum superficiale and intermedium, single leukocytes
II°	Present correct cells of stratum superficiale and intermedium, existing glandular cells, metaplastic, existing bacterial flora, leukocytes, histiocytes
III°	Present dysplastic cells
IV°	Present dysplastic cells and single neoplasm cells
V°	Numerous neoplasm cells, leukocytes and erythrocytes

Table 2. The assessment classification of cytological smears according to the Bethesda system. Brak odwołania do tabeli w tekście.

Cytological diagnosis according to TBS	Cytological image
ASC-US	Atypical squamous cell undetermined significance
ASC-H	Atypical squamous cell cannot excluded HSIL
LSIL	Low-grade squamous intraepithelial lesion
HSIL	High-grade squamous intraepithelial lesion
AGC	Atypical glandular cells

The classification by Papanicolau has played an invaluable role in the detection of pre-neoplasm states and cervical cancer. Nevertheless, it is currently being superseded by the Bethesda system (TBC), as the classification did not take into consideration the present knowledge about the carcinogenesis process in the area of cervix. The Bethesda system accounts for information about the quality of the specimen. It conditionally allows a smear for cytological assessment (it describes the presence or the lack of cells of the endocervical canal, the presence of inflammatory cells, the presence of erythrocytes) or disqualifies a smear from the assessment due to an incorrect technical procedure, too few cells in the specimen or an unreadable image because of numerous inflammatory cells, erythrocytes. Moreover, in case of an anomaly in the smear a cytologist uses a similar terminology to the one applied in histopathological diagnoses. **According to the cytological classification which is in line with the Bethesda system and its modification from 2001 abnormalities of squamous cells which correspond to pre-cancer states were divided as follows:**

1) ASC – atypical squamous cell:
   a) ASC-US – undetermined significance,
   b) ASC-H – cannot excluded HSIL,
2) LSIL – low-grade squamous intraepithelial lesion,
3) HSIL – high-grade squamous intraepithelial lesion.

The classification according to the Bethesda system separates a group of atypical squamous cells, in which lesions do not allow a cytologist to define them as dysplastic cells, but which are abnormal, and patients with such a diagnosis should undergo a close observation and further diagnostics. In the classification by Papanicolau the above-mentioned phenomenon was classified as group II, at the same time lowering the alertness of clinicians, and releasing them from the duty to conduct further diagnostics.

The contemporary supplementation of a cytological smear is molecular diagnostics, which identifies in a DNA or mRNA material highly oncogenic genotypes of HPV. These tests do not detect neither cervical intraepithelial neoplasia (CIN), not cancer, but determine the risk of lesions development. The HPV HR test has the highest prognostic value while selecting women with an incorrect cytological result. A negative DNA test of HPV excludes the presence of high-degree dysplasia and cervical cancer and indicates that in a tested woman cancer will not develop within the next 6 years. A negative test result does not exclude CIN 1 and CIN 2, because part of these lesions may be caused by the presence of a virus of low oncogenic potential. A single positive DNA HPV HR test result only shows the fact that the virus is present, and does not differentiate women with accidental and persistent infections. Therefore, a molecular test should not be repeated more frequently than every 12 months. A DNA HPV test is rarely used as a single examination due to low sensitivity, especially in women under 35, because the prevalence of HPV infections in this population is very high.

The mRNA test allows to differentiate between accidental and persistent infections. A positive result indicates a persistent infection with HPV and the beginning

of the carcinogenesis process. A woman with a positive result of a RNA HPV test is in the group of a very high risk of cervical intraepithelial neoplasia (CIN) development and cervical cancer.

Non-standard methods for detection of cervix pathology include: photodynamics, optoelectronics and spectroscopy.

INCORRECT CYTOLOGICAL RESULTS – PROCEDURE

ASC-US (atypical squamous cells of undetermined significance) – is the most often formulated, incorrect cytological diagnosis worldwide. Approximately 40% of women with such a diagnosis is at the same time infected with HPV, whereas 60% of women does not show the presence of DNA HPV HR. According to the Polish Gynaecological Society it is permissible to repeat a cytological test in 4-6 months or colposcopy or supplement a cytological test with a molecular DNA HPV HR test. In case when doubled cytological tests conducted with an interval of 4-6 months are correct, then the patient should undergo a cytological test in line with routine screening. Colposcopy is necessary only when a consecutive cytological test is incorrect or when the result of a molecular test for minimum 14 genotypes of HPV is positive. However, if there is no DNA HPV in a specimen from the vaginal portion of the cervix or the endocervical canal, a repeated cytological test should be carried out in 12 months. In case when both colposcopy and guided biopsy results do not show lesions, then there is a need to explain the status of the infection with the virus – whether it is an accidental or persistent infection. To this end an mRNA HPV test should be conducted. In case of women after menopause and an incorrect cytological diagnosis ASC-US, the cytological test should be repeated after a 7-day transvaginal oestrogen therapy. When in the above-mentioned women coexist a positive test result for DNA HPV, then colposcopy should be conducted.

ASC-H (atypical squamous cells, cannot exclude HSIL) – the result indicates a high probability of the presence of cervical neoplasia with no adequate lesions in cells collected in the smear. A correct procedure is colposcopy and biopsy from the suspicious places. However, it is not recommended to leave out colposcopy for the sake of a molecular test.

The algorithm in all diagnoses classified as ASC – atypical squamous cells requires thorough diagnostics, i.e. colposcopy. A satisfactory colposcopy examination requires a revealed area of lesions, that is the border between stratified squamous epithelium and glandular epithelium of cervix. In case when the area of lesions is not revealed, colposcopy should be supplemented with biopsy of the endocervical canal.

LSIL (low-grade squamous intraepithelial lesion) – in approx. 80% of sexually active women under 25, features of an active or endured infection with HPV were found, that is why it is recommended that these women repeat a cytological test every 6 months. It is a time when a regression of the viral infection might occur and abnormalities might recede in the morphological image of a cell of stratified squamous epithelium. Nevertheless, the algorithm does not exclude a colposcopic examination and the possibility to carry out a molecular test. In case of women after menopause with a negative diagnosis for cytological abnormalities the LSIL result should be supplemented with a repeated cytology test after a 7-day transvaginal oestrogen therapy.

HSIL (high-grade squamous intraepithelial lesion) – is an indication to conduct a colposcopic examination with a guided biopsy of lesions suspected of cervical intraepithelial neoplasia (CIN), as well as a biopsy of the endocervical canal, or electroresection of lesions with a biopsy of the endocervical canal.

AGC (atypical glandular cell) – this diagnosis includes abnormalities of glandular epithelium of the endocervical canal. The above-mentioned result cannot be verified only with repeated cytological examinations or a molecular test, though it is an absolute indication for colposcopy and a diagnostic abrasion of the endocervical canal.

In 1960 in Finland a national scheme for active prevention was implemented, it was based on sending personal invitations for a cytological examination. In 1970 a similar scheme was introduced in Iceland, Sweden, Denmark, the Netherlands, and slightly later in the UK and Italy. In Poland in March 2007 active screening was launched for women over 25 which lasts until 59 years of age. According to the recommendations of the American Cancer Society – ACS from 2013, this examination should be started in women aged between 21-29 and conducted every third year. While in women aged between 30-65 a cytological examination should be accompanied by a molecular test "co-test" every 5 years. Cytological prevention should be finished in women at the age of 65 who have at least three consecutive correct cytological results or 2 negative results of the "co-test" conducted during the last 10 years (which results from the fact that the time of carcinogenesis of cervical cancer amounts up to 10 years). An exception from these recommendations constitute women with immunosuppression, infected with HIV or treated with dietylostilbestrol, who require more frequent cytological examinations.

In the Polish population scheme a cytological examination is conducted every 3 years in women aged between 25 and 59. The recommendations of the Polish Gynaecological Society from 2006 show that controlled should be women infected with HIV, taking immunosuppressive medicines, infected with HPV of high oncogenic risk, treated in the past for cervical intraepithelial neoplasia (CIN 2, CIN 3) or cervical cancer. The screening test should be carried out earlier in people HIV positive and in teenagers who were sexually harassed in their childhood and puberty. These examinations should be implemented at the moment of determining the above-mentioned facts. In case of women whose uterus was removed together with the cervix because of CIN lesions, a cytological examination

should be continued through the period of 10 years from the surgery, whereas in case of an operative treatment due to cervical cancer, cytology should be conducted until the end of a patient's life.

To sum up, it should be stressed that according to the worldwide recommendations a population screening test of cervical lesions is conducted on the basis of cytology carried out every 3 years in women aged 21-59. This examination may be supplemented but it cannot be replaced by a molecular DNA HPV test. However, the most efficient method for avoiding factors which develop cervical cancer is health education about the indicating factors and the shaping of pro-health behaviours.

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