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The development of screening, diagnosis and management with abnormal Pap test results

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SUMMARY

Cervical cancer is a significant oncological problem from the point of view of primary and secondary prevention as well as extended diagnosis of abnormal Pap test results. This disease is the fourth most common cancer and the fourth cause of death from cancer among women, with the worldwide prevalence of 600,000 and death rate of 300,000 in 2018. In Poland, there were 2,622 new cases of cervical cancer and 1,550 deaths from this disease in 2018.

Cervical cancer incidence and mortality rates are gradually declining, particularly in highly developed countries, owing to elimination of risk factors, improvement of socioeconomic and hygienic conditions, and introduction of screening programs. Cervical cancer prevention involves primary (avoidance of HPV infection, HPV vaccination) and secondary techniques (Pap smears and other modern ways of detecting precancerous conditions). The aim of screening in healthy women is early detection of the disease, enabling early initiation of effective treatment. At present, the Pap test is commonly used in cervical cancer prevention. However, due to its low sensitivity and specificity, new methods of extended diagnosis are being developed in order to detect precancerous conditions of the cervix accurately, rapidly and in a relatively non-expensive way.

Apart from the Pap test, other promising techniques of screening and extended diagnosis are: folate receptor-mediated staining, transition zone scanning with ZedScan, automated visual evaluation of the cervix with 3–5% acetic acid staining, and the use of silver nanoparticles in the management of chronic inflammation of the cervix and abnormal Pap smear results. The new methods, tested in clinical practice at the 2nd Department of Obstetrics and Gynecology of Wroclaw Medical University in Poland, markedly improve the sensitivity and specificity of CIN2+ detection. This paper presents new techniques of screening and extended diagnosis on the background of results offered by Pap smears and own experience. The plethora of currently tested methods of extended screening for cervical cancer is promising and gives hopes that the Pap test will soon be supplemented with more accurate examinations. The presented methods share common advantages: they are non-invasive, simple to implement and yield real-time results. Works on new techniques may contribute to the development of screening methods and make effective screening for cervical cancer more widespread in Poland, where the prevalence of this disease is still high compared with other European countries.

Key words: abnormal Pap smear; inflammation; electrical resistance of the cervical epithelium; automated visual evaluation of the cervix; silver nanoparticles; folate receptors

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INTRODUCTION

Cervical cancer is still a significant oncological problem from the point of view of primary and secondary prevention as well as extended diagnosis of abnormal Pap test results. This disease is the fourth most common cancer and the fourth cause of death from cancer among women, with the worldwide prevalence of 600,000 and death rate of 300,000 in 2018 [1]. The data from the Polish National Cancer Registry from 2018 state that, at that time, there were 2,622 new cases of cervical cancer and 1,550 deaths from this disease in Poland [2].

Cervical cancer prevalence and mortality rates are gradually declining, particularly in highly developed countries, owing to elimination of risk factors, mainly due to the introduction of HPV vaccination [1]. Improvement of socioeconomic and hygienic conditions, low parity in the population and a decrease in the incidence of sexually transmitted diseases have also contributed to a reduction of cervical cancer prevalence [1,3]. Screening programs based on evidence-based medicine involve classical Pap smears, liquid-based cytology (LBC) and detection of oncogenic HPV types to identify precancerous conditions [4].

Cervical cancer preventions involves primary (avoidance of HPV infection, HPV vaccination) and secondary techniques (Pap smears and other modern ways of detecting precancerous conditions) [5]. The aim of screening in healthy women is early detection of the disease, enabling treatment at an early stage to reduce mortality [6]. Screening should be characterized by appropriate sensitivity, indicating the capability of the test to detect the disease accurately, and specificity, meaning that a test is capa-

ble of ruling out the disease with certainty. Screening for cervical cancer is relatively simple owing to the access to the organ for examination and availability of the classification of precancerous conditions. At present, the Pap test is common in cervical cancer prevention. However, due to its low sensitivity and specificity, new methods of extended diagnosis are being developed for prompt and accurate detection of precancerous conditions of the cervix. Apart from superior accuracy, these methods should be characterized by lower costs and reduction of patient engagement and stress linked with the diagnostic process.

HISTORY OF SCREENING IN POLAND

The history of screening for cervical cancer (Fig. 1) dates back to the 1920s and to studies on cells obtained from cervical smears conducted in Weill Medical College in New York by George Papanicolaou [7]. This test is commonly known as the Pap test or Pap smear. As this was the first test, its efficacy was never evaluated in randomized clinical trials, but epidemiological studies performed in countries that had implemented this form of screening revealed a significant reduction of mortality and morbidity due to cervical cancer. The introduction of the Pap test in the USA in the 1950s and its widespread use resulted in improved detectability of precancerous conditions and reduced mortality due to cervical and endometrial cancers from 20.9 deaths per 100,000 women in the 1930s to 10.1 deaths in the 1960s and 4.3 deaths in the 1990s [8].

In Poland, the cervical cancer screening program was initiated by the Act of 1 July 2005 on adopting the multiannual program "The National Program for Combating Cancer" (Dz.U. [Polish Journal of Laws] No 143, item 1200 as amended) [9]. By virtue of this act, the Population Program of Prevention and Early Detection of Cervical Cancer was started, but was initially of a very limited range. When the program had been implemented, the incidence of cervical cancer reduced from 16.6 in 2005 to 15.5 in 2010, while mortality decreased from 10.0 in 2005 to 8.7 in 2010 (raw values per 100,000 women) [10]. However, screening rates were still low. In 2007–2009, after sending invitations to a target group of almost 10 million women, the Pap test was performed in 24.14% of them [11].

A milestone in screening for cervical cancer was the introduction of the classification of cytological images. The classification system was named *Bethesda*, after a city that hosted a conference of experts who developed it. According to this classification, cytological images were divided into normal, low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL) [12–14].

Subsequent discoveries contributed to the deeper understanding of the natural history and risk factors of cervical cancer. In 1984, works supervised by Professor Harald zur Hausen led to the isolation of type 16 and 18 human papilloma virus (HPV) from cervical carcinoma tissue, and the disease was linked with infection with these highly oncogenic types of the virus [15]. Only in the year 2000 did the FDA approve an HPV test, i.e. Hybrid Capture 2 (Qiagen, Maryland, USA), for routine use in combination with the Pap test. The discovery of the role of HPV in the pathogenesis of cervical cancer was awarded a Nobel Prize in physiology and medicine in 2008.

METHODS OF EXTENDED DIAGNOSIS

The Pap test is recommended as the basic tool for cervical cancer prevention even though it is characterized by a high rate of false negative and false positive results, which is mainly linked with the subjectivity of the staff evaluating a smear and the specificity of collecting the material [16]. The study conducted in a group of 687 Polish women with histologically confirmed cervical intraepithelial neoplasia (CIN) verified the sensitivity of the test at the level of 58.02% and specificity of 63.28% [17]. The is why a number of ongoing studies are investigating new tools for screening and extended diagnosis that would enable rapid, inexpensive and objective identification of cases requiring medical intervention. The promising techniques include: folate receptor-mediated staining, transition zone scanning with ZedScan, automated visual evaluation of the cervix with 3–5% acetic acid staining, and the use of silver nanoparticles in the management of chronic inflammation of the cervix and abnormal Pap smear results.

FRD staining solution

The FRD staining solution (*Shaanxi Gaoyuan Medical Equipment Service Co*., Ltd., *China*)

consists predominately of a reduced folate–methylene blue complex, dimethyl sulfoxide, ascorbic acid and trace amounts of folic acid. Owing to folate receptor á overexpression in the epithelium with high-grade lesions, the FRD complex binds with the folate receptor and activates endocytosis. Methylene blue undergoes the process of oxidation inside cells, thus producing blue, blue–black or black staining indicating CIN2+ lesions.

Studies on over 14 thousand women have demonstrated that the sensitivity of this method in detecting CIN2+ lesions amounts to 85.7%, specificity to 76.4%, positive predictive value to 61.3% and negative predictive value to 92.5% [18]. Other studies have confirmed the efficacy of this method and underlined the simplicity of staining and immediate availability of results [19, 20]. The Second Department of Gynecology and Obstetrics of Wroclaw Medical University in Poland is conducting the first European clinical trial to evaluate the sensitivity of this method in cervical cancer screening.

ZedScan device

The ZedScan device (*Zilico Limited,* Manchester, UK) is used to measure electrical resistance of the cervical epithelium, the change of which enables differentiation between normal epithelium and epithelium with ongoing neoplastic transformation. Transition zone scanning with ZedScan is used as a supplementation of traditional colposcopy with acetic acid and Lugol's solution staining.

Brawn et al. have demonstrated that both sensitivity and specificity of electrical impedance measurement in differentiating patients with normal epithelium from those with CIN lesions reach 92% [21]. To date, several studies have confirmed an increase by a dozen or so per cent in specificity and sensitivity of colposcopy when combined with ZedScan impedance measurement [22–24]. The availability of results at real time is also an advantage of this examination.

The ZedScan device has been used at the Second Department of Obstetrics and Gynecology of Wroclaw Medical University since 2018. Since then, we have examined 100 patients in accordance with the protocol that assumes visual cervix evaluation, staining with 3–5% acetic acid and videocolposcopic documentation as well as scanning transition zone resistance with the ZedScan device (score 10–12 depending on the size of the ectocervix) and Schiller's test. Patients with high grade-lesions detected on colposcopy and/or red staining in the ZedScan test, underwent a biopsy. The ZedScan test combined with colposcopy helped detect additional cases with a positive histopathological result compared with colposcopy alone.

Automated visual evaluation

Automated visual evaluation (AVE) uses a so-called *deep learning algorithm* to detect CIN2+ lesions of the cervix with a colposcope. The evaluation is conducted after cervical staining with 3–5% acetic acid, and the image recorded during colposcopy is processed by an application whose algorithm recognizes and identifies pathological images of the cervix using a self-learning technology by recognizing patterns with the use of multiple processing layers. The success of the application is confirmed by a 7-year-long study of 9,406 patients and cervical images, conducted by Professor Mark Schiffman from the National Cancer Institute. The results are promising and indicate higher accuracy of this technology in cervical cancer screening when compared with traditional evaluation of cervical images and Pap smears (area under the curve [AUC] = 0.91; 95% confidence interval [CI] = 0.89–0.93 compared with AUC = 0.69; 95% CI = 0.63–0.74; $p<0.001$ and AUC = 0.71; 95% CI = 0.65–0.77; $p < 0.001$, respectively) [25].

A trial to evaluate the sensitivity and specificity of this method with the use of a mobile colposcope has been carried out at the Second Department of Obstetrics and Gynecology, Wroclaw Medical University since May 2019.

THE MANAGEMENT WITH ABNORMAL PAP SMEARS – SILVER NANOPARTICLES

Patients with abnormal Pap smear results showing a low-grade type of lesions, i.e. atypical squamous cells of undetermined significance (ASC-US) and low-grade squamous intraepithelial lesions (LSIL), carry the lowest risk of CIN2+ lesions, ranging from 6 to 12%. In this group of patients, the proposed algorithm involves a repeated Pap test, HPV test and, in selected cases, colposcopy [26]. In the Second Department of Gynecology and Obstetrics of Wroclaw Medical University, patients with low-grade lesions identified on colposcopy and with no need to collect samples/biopsy from the canal are recommended a 10-day treatment with a vaginal product containing titanium dioxide

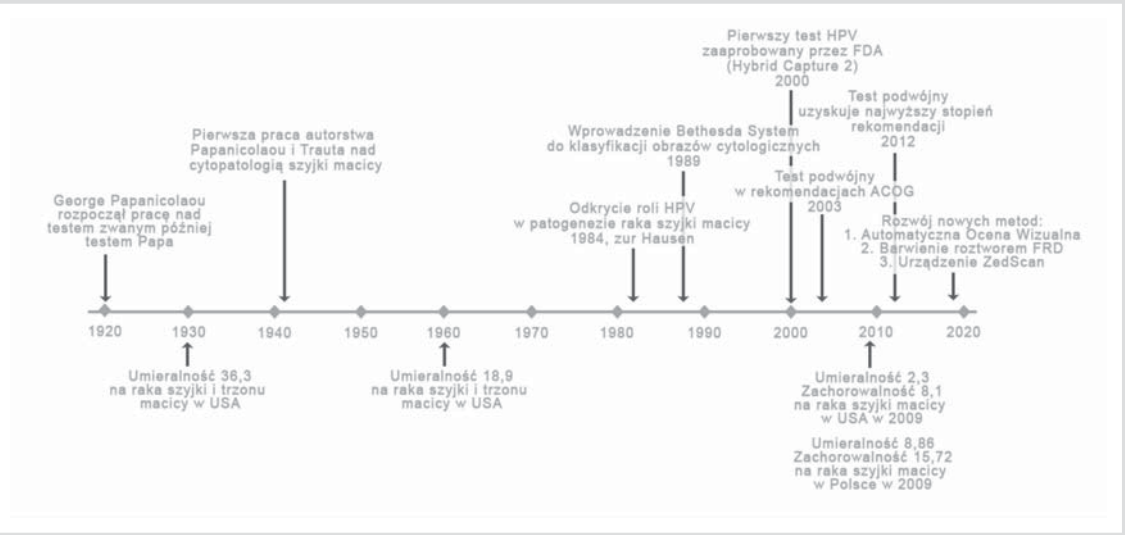

Fig. 1. History of screening for cervical cancer

microcrystals with covalently linked silver nanoparticles (HEXATIAB®, NTC, Milan, Italy) before a repeated Pap test. This product has protective properties. It prevents development and recurrences of bacterial, fungal and viral infections, including HSV-2 [27,28]. In this Department, 60 patients were enrolled in a study aiming to evaluate the efficacy of the silver nanoparticle complex. Patients with abnormal Pap smear results used the product with silver nanoparticles. Next, a repeated Pap test and colposcopy were performed. ASC-US lesions regressed in 16 patients, chronic inflammation or LSIL regressed to NILM in 6 patients, LSIL regressed to ASC-US in 6 patients and a repeated ASC-US results were noted in 12 patients. It was demonstrated that the product is effective in treatment of inflammation and abnormal Pap smear results (ASC-US, LSIL), with improvement observed in 76% of cases. Normal results enable a return to standard screening, which lowers costs and decreases the load with further examinations.

The literature reports are in line with our findings. They indicate efficacy of the complex with silver nanoparticles in the treatment of vaginal and vulvar inflammation, underlining its local antibacterial, antifungal and antiviral effects [28]. The mechanism of action of silver nanoparticles differs from antibiotic therapy. It may therefore supplement it without increasing the risk of drug-resistance [29,30]. The benefits of silver nanoparticles shown in studies in the fields of dermatology and gynecologic oncology corroborate its efficacy in treating dermatoses [31, 32]. Our own observations indicate that the silver nanoparticle product is effective in the treatment of inflammation and abnormal Pap smear results.

CONCLUSIONS

The plethora of currently tested methods of extended screening for cervical cancer is promising and gives hopes that the Pap test will soon be supplemented with more accurate examinations. The presented methods share common advantages: they are non-invasive, simple to implement and yield real-time results. This is significant as it may make effective screening for cervical cancer more widespread in Poland, where the prevalence of this disease is still high compared to other European countries.

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