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Sensitivity and specificity of HR HPV E6/E7 mRNA test in detecting cervical squamous intraepithelial lesion and cervical cancer

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ABSTRACT

Objectives: The paper assess the relevance of HR HPV E6/E7 mRNA test in women with abnormal Pap results.

Material and methods: Between 2013–2014, 125 women were subjects to the enhanced diagnostics due to abnormal Pap results. According to The Bethesda system, if ASC-US, AGC, LSIL, ASC-H, HSIL or cancer cells were present, the result was abnormal. The patients underwent the enhanced diagnostics which included the following procedures: Pap smear collection for molecular assessment of HR HPV E6/E7 mRNA test, the colposcopic examination and biopsy of clinically suspicious areas.

Results: High-grade squamous intraepithelial lesions constituted the most frequent cervical pathology in women with abnormal Pap test results, as well as with the positive results of HR HPV E6/E7 mRNA test. Test sensitivity in patients with the histopathological diagnosis of high-grade squamous intraepithelial lesion was estimated at 86.1%.

Conclusions: HR HPV E6/E7 mRNA test identifying neoplastic lesions and cervical cancer is characterised by a high relevance which is reflected by means of sensitivity and specificity. In fact, test sensitivity and specificity increased with the age in the group of patients up to 50 years old.

Key words: HPV E6/E7 mRNA; SIL; squamous intraepithelial lesion; HSIL

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INTRODUCTION

According to the World Health Organization (WHO), cervical cancer constitutes the 4th most frequent malignant cancer in women worldwide. In 2012, about 530000 new cases were recorded, and nearly 90% of 270000 deaths occurred due to this disease in mid and low socioeconomic status countries. Moreover, high mortality rate may be reduced only if a comprehensive approach is introduced including broadly defined prevention, that is education, effective and efficient screening, as well as early diagnosis and treatment [1].

The introduction of cervical cancer screening has largely decreased both the incidence and the mortality rate of women in Europe over the years, although the success rate is radically different in particular countries [2]. Nowadays, 34000 new cases of cervical cancer are found in Europe every year, with 13000 deaths due to this disease [3]. In Poland, since the 90's the tendency constantly decreases, reflecting the improvement in the epidemiological situation, although further steps need to be taken in order for the method to be fully successful. In Poland in 2014, the diagnosis of cervical cancer was made in 9 women a day, and nearly half of them died of it. [4, 5].

In 2005 a Polish national programme for cervical cancer prevention was implemented which aimed at an early detection of precancerous lesions classified as CIN (Cervical Intraepithelial Neoplasia) 1, CIN 2, CIN 3. According to the current recommendations, CIN 1 is referred to as LG SIL (Low Grade Squamous Intraepithelial Lesion), whereas CIN 2 and CIN 3 are both called HG SIL (High Grade Squamous Intraepithelial Lesion).

The basic factor in cervical cancer development is a persistent infection with HR HPV, where the most cancerogenic

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types are HPV 16, 18, 31, 33, 45. Cervical intraepithelial neoplasia lasts ca. 7–10 years, and following another 3–5 years may consequently lead to a pre-invasive and invasive cervical cancer. Moreover, current data indicate the presence of various HPV DNA types in 99.7% cervical cancer biopsies [6].

As early as 2003, the American College of Obstetricians and Gynecologists was the first to include HR HPV DNA test in the screening guidelines. Furthermore, since 2012 more and more recommendations have indicated and proved the HPV DNA test superiority over conventional cytology test in female patients aged 30–65 [7].

There is evidence suggesting that co-testing, i.e. combining Pap test with HR HPV DNA test, contributes to a decrease in the incidence of invasive cancer, as well as generates lower costs in comparison to the annual Pap test performed for 30 years [8, 9].

The current European guidelines recommend HR HPV DNA test as a screening method in women 35–60 years of age [10].

Numerous research indicate a higher diagnostic value of the HR HPV DNA test in comparison with the Pap test. In fact, on the basis of the analysis including over 10000 women in Canada, HR HPV DNA test sensitivity for HG SIL lesions was estimated at 94.6%, as compared to cytological test sensitivity which was estimated at 55.4% [11].

A perfect screening method should comprise a nearly 100% sensitivity and specificity, as well as a high positive predictive value which in practice, however, is extremely difficult to obtain.

Incorporating tests detecting HR HPV E6/E7 mRNA test constitutes one of the most recent discoveries, and allows for the identification of patients with permanent viral infection, where the process of DNA incorporation in the epithelial cells genetic material has already been initiated. In fact, the neoplastic transformation process starts once HPV DNA integrates with the proper epithelial cell genome. Moreover, it is possible when HPV DNA circular form is damaged and chromatin displacement occurs within the chromosomal DNA of host's cells. Oncoprotein E6 and E7 expression in epithelial cells infected with HR HPV types is associated with an increase in proliferation and abnormal differentiation of these cells, and may lead to the development of neoplastic and malignant lesions [12–14]. HR HPV E6 protein contributes to the degradation of p53 protein which protects the genome, an thus may inactivate the genetic mechanisms controlling the cellular cycle and apoptosis. In fact, the function of p53 in the cellular cycle is based on the movement control from G1 phase to the S phase of the cellular cycle by means of inducing expression of p16, p21 and p27 cyclin inhibitors. Due to this mechanism it is possible to stop the cellular cycle in G1/S phase [12].

According to the sources, the described diagnostic procedure is characterised by a high sensitivity and specificity equal to 98% and 85% respectively.

The indisputable advantage of the abovementioned diagnostic method is the objectivity and repeatability, although the screening test of a given patient would not have to be performed as frequently as a conventional cytology. The clinical observations show that the progression risk increases when one of the highly oncogenic types: 16, 18, 31, 33, 45 is responsible for the persistent infection, and its mRNA presence constitutes an even poorer prognostic factor. In fact, it indicates an ongoing carcinogenesis on the molecular level and additionally, in 98% of cases, it entails the continuation and progression of the disease [15]. Further observations may be vital in the future, and may result in the introduction of new guidelines in patients diagnosed with LG SIL who may undergo a spontaneous regression in certain cases. Moreover, observation of regression in women with a negative HR HPV E6/E7 mRNA test could prevent them from additional stress and the necessity of performing unnecessary invasive procedures.

Objectives

The aim of the paper is to assess the relevance of HR HPV E6/E7 mRNA test in women, in female patient population with abnormal Pap test.

MATERIAL AND METHODS

Between 2013–2014 in the Laboratory of Pathophysiology of Uterine Cervix at Poznań University of Medical Sciences, 125 women were subjects to the enhanced diagnostics due to abnormal cytology results. According to The Bethesda system, if ASC-US, AGC, LSIL, ASC-H, HSIL or cancer cells were present, the result was abnormal. All women who participated in the study were adults, not pregnant and not breast-feeding. The study was approved by the Bioethics Committee of the University No 548/18. The paper constitutes a retrospective analysis.

Firstly, all patients were subjects to a detailed medical interview which included the oncological past, earlier cytology and histopathological tests results, if they had been performed, family history, obstetric history, the age of the first menstruation and the date of the last menstrual period. Secondly, the patients underwent the enhanced diagnostics which included the following procedures:

— Pap smear collection for molecular assessment of HR HPV E6/E7 mRNA test;
— The colposcopic examination;
— Biopsy of clinically suspicious areas assessed by a gynaecologist.

Pap smear for molecular assessment — the sample was collected with an endocervical Cyto-Brush, and then it was

cial for the examination to be satisfactory. In all cases, a trial with 3% aqueous solution of acetic acid was performed, as well as the Schiller's test with Lugol's iodine. The colposcopic images were evaluated according to Reid's Colposcopic Index which assesses the colour, lesion borders and surface, blood vessels and iodine test.

Biopsy of the clinically suspicious area visible in colposcopy was performed in each patient classified for the examination. Cervical samples were fixed in buffered 10% formalin solution.

Calculations were performed using the statistical package Statistica (data analysis software system), ver. 13.1 and graphs — using Excel. It was estimated whether increasing age resulted in higher rates of sensitivity, specificity, PPV and NPV by Chi-square test for the trend. Statistical hypotheses were verified at the level of significance of = 0.05.

RESULTS

120 patients participated in the study who were classified into 4 age groups:

— 18–29 years of age → n = 50,
— 30–39 years of age → n = 42,
— 40-49 years of age → n = 15,
— over 50 years of age → n = 13.

The number of participants in particular age groups is shown in Figure 1. In the course of the histopathological analysis of the ectocervix and/or endocervix biopsies, 49.17% of samples were associated with SIL lesions, with the following results:

— 23 patients presented CIN 1 — LG SIL,
— 20 patients showed CIN 2 — HG SIL,
— 13 patients had CIN 3 — HG SIL,
— 2 patients presented squamous cell cancer,
— 1 patient showed adenocarcinoma,
— 61 patients had no SIL.

The incidence of individual histopathological diagnoses with reference to particular age groups is shown in Figure 2.

The average age of patients was 28, with 19 years of age as the youngest, median: 28, and 66 years of age as the oldest.

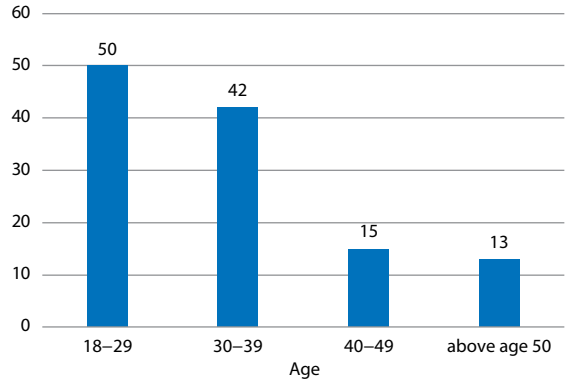

Figure 1. Number of patients

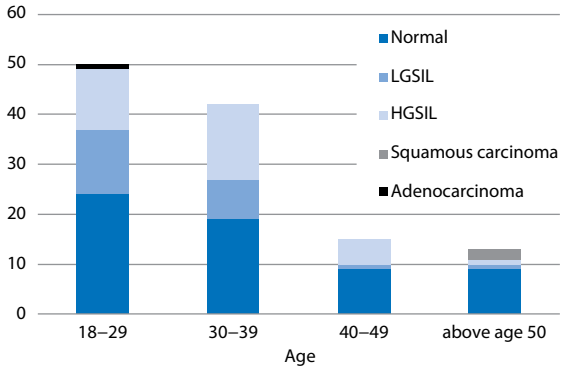

Figure 2. Histopathological diagnose

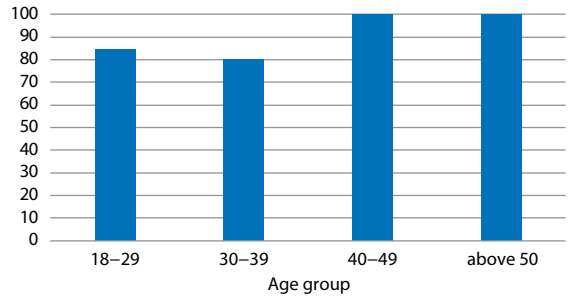

Figure 3. mRNA test sensitivity

Results of molecular HR HPV E6/E7 mRNA test

Test sensitivity in patients with the histopathological diagnosis of low-grade squamous intraepithelial lesion was estimated at 82.6%.

Test sensitivity in patients with the histopathological diagnosis of high-grade squamous intraepithelial lesion was estimated at 86.1% which is shown in Figure 3 with reference to particular age groups.

Test sensitivity of patients with the histopathological diagnosis of both high- and low-grade squamous intraepi-

preserved in PreservCyt® (Hologic Corp.) and SurePath® (BD Diagnostics-TriPath) reserved for the biological material. NucliSENS EasyQ® HPV v1.1 test by bioMérieux was employed for the detection and nucleic acid amplification in real-time, allowing for qualitative identification of E6/E7 messenger RNA (mRNA) for five cancerogenic HPV virus types: 16, 18, 31, 33, 45 in epithelial cells.

Colposcopic examination — the examination was performed in the Laboratory of Pathophysiology of Uterine Cervix by means of the stereoscopic colposcope Olympus OSC-500. In fact, the visualization of the affected area is cru-

Test specificity in patients with the histopathological diagnosis of both high- and low-grade squamous intraepithelial lesion was estimated at 54.1%, which is shown in Figure 5 with reference to particular age groups.

The sensitivity value of the HR HPV E6/E7 mRNA test increases with the patients' age up to 50 years of age, and then decreases.

Sensitivity of detecting squamous intraepithelial lesions by means of this test was the highest in the age group of 40–49 years and above 50 years of age.

Among Pap-test diagnoses listed below: ASC-H, LSIL, HSIL and cervical squamous cell carcinoma, a correlation was found between the diagnosis of pathology and the presence of HR HPV mRNA test. Only in the case of ASC-US diagnosis, in most cases the presence of HR HPV E6/E7 mRNA was not confirmed. Among the Pap-tests in which no pathology was found (NILM), in most cases the presence of HR HPV mRNAs was not confirmed. The results are presented in Figure 6.

The histopathological diagnoses were also taken into account — in the case of confirmed pathology, i.e. LGSIL, HGSIL and cervical squamous cell carcinoma , the presence of HR HPV E6/E7 mRNA was confirmed in the majority of cases. On the other hand, tests for the presence of HR HPV E6/E7 mRNA are still not proper to detect glandular dysplasia (Adenocarcinoma). The results are presented in Figure 7.

There were statistically significant differences in the presence of HR HPV E6/E7mRNA and the occurrence of pathology found in cervical biopsy ($p = 0.00001$). The dependencies in all age groups were also tracked. Only in the group of the youngest patients no statistically significant differences were found ($p > 0.05$). In contrast, in the other age groups, statistically significant differences were found-in the group of women aged 30–39 ($p = 0.01491$) and in the group of women over 50 ($p = 0.01086$). The strongest relationship was observed in the group of patients aged 40–49 ($p = 0.00082$).

DISCUSSION

According to the paper by Sørbye et al. published in 2014, diagnostic tests detecting HR HPV E6/E7 mRNA are characterized by a higher specificity than tests identifying HR HPV DNA. Comparative studies were conducted in Norway in a group of over 300 patients with abnormal Pap test, diagnosed with ASC-US or LSIL according to TBS. Positive predictive value for HSIL histopathological diagnosis in terms of HR HPV DNA molecular test was 21.5%, whereas for HR HPV E6/E7 mRNA test it was 34.6%. What is more, HR HPV DNA test was characterised by a higher sensitivity than the HR HPV E6/E7 mRNA test and detected more cases of histopathologically confirmed high-grade squamous intraepithelial lesion of uterine cervix [16].

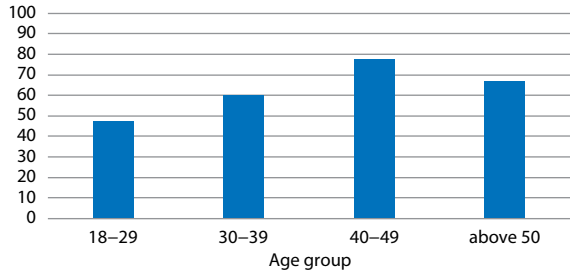

Figure 4. mRNA test sensitivity for LGSIL nd HGSIL

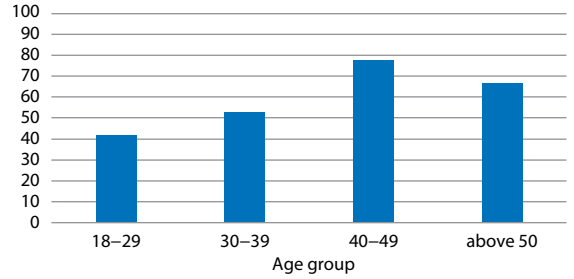

Figure 5. mRNA test specifity

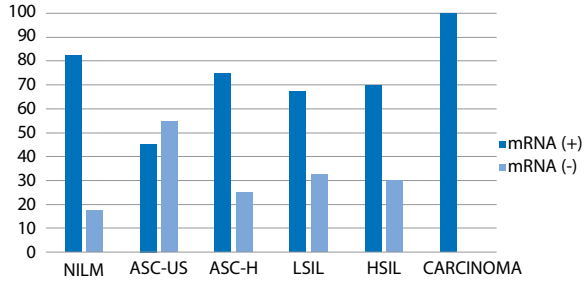

Figure 6. The incidence of mRNA positive and negative results according to PAP tests

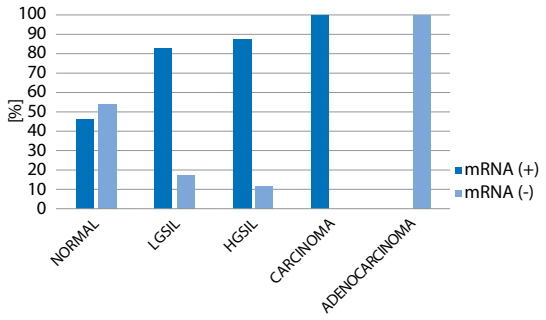

Figure 7. The incidence of mRNA positive and negative results according to histopathological diagnoses

thelial lesion a was estimated at 84.7% with reference to particular age groups is presented in Figure 4.

Yao Yl et al. in 2017 confirmed the relevance of HR HPV E6/E7 mRNA test in monitoring HR HPV positive patients. In the abovementioned paper no statistically relevant difference was shown between the sensitivity and specificity of the Pap test and HR HPV E6/E7 mRNA test in detecting HSIL lesions among HPV positive patients. Moreover, the sensitivity and specificity of the abovementioned test was estimated at 89.52% and 48.96% respectively in the diagnosis of high-grade squamous intraepithelial lesion of uterine cervix. Additionally, the percentage of positive HR HPV E6/E7 mRNA test results was significantly higher in the histopathological HSIL diagnoses than LSIL [17].

According to a 2013 analysis by Perez Castro et al., HR HPV DNA tests are characterized by a high sensitivity, but a relatively low specificity in identifying uterine cervix oncological pathologies. Due to this fact, new and more precise enhanced diagnostic methods are anticipated which could be employed in patients with abnormal cytology results, namely ASCUS or LSIL. It is vital to notice that test detecting HR HPV E6/E7 mRNA test may significantly increase the molecular tests specificity in identifying HSIL lesions, while retaining high sensitivity and negative predictive value. In the already mentioned paper by Perez Castro et al., the HR HPV E6/E7 mRNA test sensitivity for low-grade lesions, i.e. LSIL, was estimated at 81.3%, whereas for high-grade lesions, that is HSIL, at 84.1%. Additionally, positive predictive value (PPV) was estimated at 97.4% for HSIL lesions. In the summary, the authors confirm the relevance of HR HPV E6/E7 mRNA test in the diagnosis of HR HPV DNA positive population [18].

Fontecha et al. in their paper confirmed the high specificity of HR HPV E6/E7 mRNA test in HPV positive patient population, where progression of squamous intraepithelial lesions occurred in a 2-year observation period. In this paper, the molecular test was characterised by 100% sensitivity in HSIL lesions detection [19].

Combining the aforementioned methods, i.e. PAP test and molecular diagnostics detecting HR HPV E6/E7 mRNA, may significantly contribute to the earlier and more precise detection of cervical neoplasia pathology in high-risk patients groups [20]. Furthermore, the aforesaid management algorithm may also considerably influence the number of surgical procedures which is particularly crucial in pregnant patients. In addition, the future identification of patients with the HSIL and cervical cancer risk development on the basis of a negative molecular test result will allow for a decrease in the numbers of invasive cervical biopsy procedures. What is more, the conducted analysis substantiates the diagnostic value of molecular tests enabling the detection of uterine cervix precancerous and cancerous lesions in pregnant patients.

Verification diagnostics of abnormal cytology results in pregnant patients constitutes a difficult task, lacking particular algorithms and guidelines. Furthermore, colposcopic examination in pregnancy is extremely difficult to interpret, and thus involves human error risk due to the examination high subjectivity level. A gynaecologist has to frequently consider the validity of a comprehensive surgical procedure, that is a cervical biopsy, and the risk of complications in normally developing pregnancy in patients with questionable cytology results, according to The Bethesda System. In fact, ASCUS and LSIL cytological diagnosis constitutes the most frequent abnormal result in pregnant patients [21].

In the 2017 paper, Cobas and Aptima tests were compared. The analysis included over 1800 patients with the histopathological HSIL diagnosis. Both tests were characterized by high sensitivity. However, the Aptima test possessed a statistically higher specificity in detection of high-grade lesions, i.e. HSIL, in comparison to the Cobas test which was estimated at 41% and 13% respectively. Positive predictive value of the Aptima and Cobas tests amounted to 41% and 13% respectively, whereas test accuracy was equal to 50% and 25% respectively. High specificity of the Aptima test, combined with its sensitivity, significantly influences cost reduction of verification diagnostics in abnormal cytology results and positive results of HR HPV DNA tests. It is crucial to bear in mind the fact that the Aptima test detects 14 types of HR HPV E6/E7 mRNA [22].

In the paper by Duvlis et al., 413 patients were analysed with both normal and abnormal cytology results. In all patients, the DNA and mRNA tests detecting HR HPV virus types were conducted. The test identifying E6/E7 mRNA transcripts of HPV 16, 18, 31, 33 and 45 was characterized by 50% specificity and 62% positive predictive value in the HSIL detection. In comparison, the specificity of HR HPV DNA test was equal to 18%. What is more, the authors emphasise the fact that the introduction of modern molecular diagnostics may significantly decrease the number of surgical procedures, and thus lower the costs associated with colposcopic examinations and cervical biopsies [23].

In 2017 Granados et al. confirmed the relevance of HR HPV E6/E7 mRNA test in patients under 35 years of age in detection of HSIL lesions. The Aptima test was characterised by a slightly higher sensitivity comparing to a liquid-based cytology in the diagnosis of CIN 2+ in 5000 patients aged 25–65. Furthermore, Aptima test presented 100% sensitivity in HSIL lesion detection. On the other hand, the sensitivity of cytological examination in the group of patients with the positive Aptima HPV molecular test result was estimated at 60.6% [24].

Cadagrande et al. in a 2016 paper confirmed high specificity and negative predictive value of HR HPV

E6/E7 mRNA test in patients with LSIL lesions, or without cervical pathologies. In addition, in patients diagnosed with ASCUS and LSIL, HR HPV E6/E7 mRNA positive test was more frequent. Moreover, in all subjects with negative molecular test result, i.e. with no expression of the genetic material, the Pap test result was also within the normal range — NILM (negative for intraepithelial lesion and malignancy) [25].

CONCLUSIONS

High-grade squamous intraepithelial lesion constituted the most frequent lesion in women with abnormal cytological test results, as well as with the positive results of HR HPV E6/E7 mRNA test.

Furthermore, HR HPV E6/E7 mRNA test identifying neoplastic lesions and cervical cancer is characterised by a high relevance which is reflected by means of sensitivity and specificity. In fact, test sensitivity and specificity increased with the age in the group of patients up to 50 years old.

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