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The Application of Human Papilloma Virus Genotyping for the Identification of Neoplasm Lesions in the Cervix of Women with Abnormal Cytology Smears

Zastosowanie genotypowania wirusów brodawczaka ludzkiego w identyfikacji zmian neoplazmatycznych w szyjce macicy u kobiet z nieprawidłowym rozmazem cytologicznym

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. A connection between infections with a highly oncogenic type of human papilloma virus and the development of cervical intraepithelial neoplasia and preinvasive cervical cancer has been proven both experimentally and clinically. The period after which persistent virus infection will lead to the development of precancerous and invasive lesions is dependent on, among others, the HPV genotype. The oncogenic types of human papilloma virus destabilize the genome of an infected cell and thus initiate the carcinogenesis process.
Objectives. The aim of this work was to analyze the frequency of occurrence of different oncogenic HPV genotypes among women with abnormal cytological smears and the correlation of this data with the degree of cervical intraepithelial neoplasia exacerbation.
Material and Methods. The sample consisted of 75 women of child-bearing age (16–43 years old) with an abnormal cytological smear and positive test identifying an infection with an oncogenic type of human papilloma virus. In every case histopathological verification, aimed at excluding pathologies in the endocervix, was conducted using a colposcopy with guided biopsy and cervix abrasion.
Results. The authors found that the frequency of occurrence of different HPV genotypes of the groups of cytological diagnoses ASC-US, LSIL and HSIL do not differ statistically ($p = 0.57$). However, what is noteworthy is the more common occurrence of HPV 16 in type LSIL lesions (45.45%) and HPV 18 of a more advanced type HSIL (37.50%) pathology. Through the verification of the cytology results with histopathological diagnosis of the above groups the authors obtained statistically significant differences ($p < 0.001$) of individual pathological states. When regarding cytological HSIL diagnosis, CIN 1 was never diagnosed, while in other cytological groups cervical intraepithelial neoplasia of a low degree constituted over 40%. Analogically about 40% of HSIL diagnoses after histopathological verification turned out to be cancer of a pre-invasive state (CIS/AIS), the presence of which was not revealed by ASC-US and LSIL. What is more, CIN2/3 diagnosis was less frequent in the ASC-US cytological group than in the other two groups. While analyzing a share of other than HPV 16 and HPV 18 oncogenic types of human papilloma virus, the authors found that the most common were HPV 31, 45 and 33. In CIN 1 and CIN 2 their share was over 60%. In CIS/AIS type pathologies, no other types of human papilloma virus than HPV 16 and HPV 18 were shown.
Conclusions. Positive results of DNA HR HPV testing of women with abnormal cytology results identified a risk group for the development of cervical cancer. No statistically significant differences of the frequency of HPV 16 and HPV 18 type occurrences were found in analyzed groups with cytological and histopathological diagnoses (**Adv Clin Exp Med 2012, 21, 6, 759–766**).

Key words: human papilloma virus, cervical intraepithelial neoplasm.

Streszczenie

Wprowadzenie. Związek między zakażeniem wysoko onkogennym typem wirusa brodawczaka ludzkiego a rozwojem śródnabłonkowej neoplazji szyjki macicy i raka przedinwazyjnego szyjki macicy jest eksperymentalnie i klinicznie udowodniony. Okres, po jakim przetrwała infekcja wirusowa doprowadzi do powstania zmian przednowotworowych i inwazyjnych jest uzależniony między innymi od genotypu HPV. Onkogenne typy wirusa brodawczaka ludzkiego destabilizują genom zakażonej komórki, a tym samym inicjują proces karcynogenezy.
Cel pracy. Analiza częstości występowania różnych onkogennych genotypów HPV wśród kobiet z nieprawidłowym wymazem cytologicznym oraz korelacja powyższych danych ze stopniem nasilenia śródnabłonkowej neoplazji szyjki macicy.
Materiał i metody. Grupę badaną stanowiło 75 pacjentek w wieku rozrodczym (wiek: 16–43 lat) z nieprawidłowym wynikiem badania cytologicznego i dodatnim testem identyfikującym zakażenie onkogennym typem wirusa brodawczaka ludzkiego. W każdym przypadku w celu weryfikacji histopatologicznej wykonano kolposkopię z biopsją celowaną oraz abrazją kanału szyjki w celu wykluczenia patologii w *endocervix*.
Wyniki. Stwierdzono, że częstość występowania poszczególnych genotypów wirusa brodawczaka ludzkiego, w grupach rozpoznań cytologicznych ASC-US, LSIL i HSIL nie różni się statystycznie ($p = 0,57$). Zwraca uwagę jednak częstsze występowanie HPV 16 w zmianach o typie LSIL (45,45%), a HPV 18 w bardziej zaawansowanej patologii typu HSIL (37,50%). Weryfikując wyniki badania cytologicznego z rozpoznaniem histopatologicznym w powyższych grupach, uzyskano istotnie statystyczne różnice ($p < 0,001$) w poszczególnych patologicznych stanach. W cytologicznych rozpoznaniach HSIL ani razu nie stwierdzono CIN 1, podczas gdy w pozostałych grupach cytologicznych śródnabłonkowa neoplazja niskiego stopnia stanowiła ponad 40%. Analogicznie ok. 40% rozpoznań HSIL po histopatologicznej weryfikacji okazało się nowotworem w stadium przedinwazyjnym (CIS/AIS), którego obecności nie wykazano ASC-US i LSIL. Dodatkowo rozpoznanie CIN2/3 rzadziej stawiano w grupie cytologicznej ASC-US niż w dwóch pozostałych. Analizując udział innych niż HPV 16 i HPV 18 onkogennych typów wirusa brodawczaka ludzkiego, najczęściej stwierdzano HPV 31, 45 i 33. W CIN 1 i CIN 2 ich udział wynosił ponad 60%. W patologii o typie CIS/AIS nie wykazano obecności innych niż HPV 16 i HPV 18 typów wirusa brodawczaka ludzkiego.
Wnioski. Dodatni wynik testu DNA HR HPV u kobiet z nieprawidłowym wynikiem badania cytologicznego identyfikuje grupę ryzyka rozwoju nowotworu szyjki macicy. Nie stwierdzono statystycznie istotnych różnic w częstości występowania typów HPV 16 i HPV 18 w analizowanych grupach rozpoznań cytologicznych i histopatologicznych (**Adv Clin Exp Med 2012, 21, 6, 759–766**).

Słowa kluczowe: wirus brodawczaka ludzkiego, śródnabłonkowa neoplazja szyjki macicy.

A link between infections with high risk human papilloma virus (*HR HPV*) and the development of cervical intraepithelial neoplasia (*CIN*) and preinvasive cervical cancer *(CIS carcinoma intraepitheliale /AIS adenocarcinoma in situ)* has been proven both experimentally and clinically [1]. Protracted oncogenic human papilloma virus infection increases the risk of the development of cervical intraepithelial neoplasia by up to 500 times [2]. The period after which persistent virus infection will lead to the development of precancerous and invasive lesions is dependent on, among others, the HPV genotype. Only oncogenic types of human papilloma virus destabilize the genome of an infected cell and by this initiate the carcinogenesis process [3]. Cytology is the routine examination in the prevention of cervical cancer but because of its limited sensitivity, low predictive value of a negative result and the need of frequent screening as well as the inability of direct HPV identification, it requires complement and in some cases replacement with molecular diagnostics which is developing very fast and is becoming increasingly available.

Knowledge about human papilloma virus biology and the molecular mechanisms leading to the development of intraepithelial neoplasia used in the diagnosis of this pathology has influenced in a significant way the management algorithms of women with abnormal cytology smears.

The aim of this work was an analysis of the frequency of occurrence of different oncogenic HPV genotypes among women with an abnormal cytological smear and the correlation of the above data with the degree of cervical intraepithelial neoplasia exacerbation.

Material and Methods

The sample consisted of 75 women of childbearing age (16–43 years old) with an abnormal cytological smear and a positive test identifying an infection with an oncogenic type of human papilloma virus.

Genetic material in the form of a smear was sent to the accredited Molecular Diagnostics Laboratory in Wrocław where the presence of the DNA of 19 oncogenic HPV genotypes was detected through the amplification of the sought DNA fragments by PCR. The cytology results were classified according to the Bethesda 2001 system in the Cytology and Histopathology Diagnostics Laboratory in Wrocław.

Further diagnosis was necessary by way of cytological examination for identifying atypi-

cal squamous cells of undetermined significance (ASC-US); atypical squamous cells where the presence of high grade lesions could not be excluded (ASC – H atypical squamous cell of undetermined significance with high grade squamous intraepithelial lesion); low/high grade squamous intraepithelial lesions (ASC – H); low/high grade squamous intraepithelial lesion (LSIL/HSIL) and atypical glandular squamous (AGC). The next step in diagnosis was the performance of a colposcopy with directed biopsy and cervical canal abrasion in order to exclude pathologies in the endocervix.

Frequency analysis was conducted by way of a chi square test. Variance analysis was conducted in order to compare means between more than two groups.

Results were considered statistically significant if the predictive value p was lower than 0.05.

Results

Confirmation of persistent infection with human papilloma virus together with abnormal cytology results provided a unique chance to increase the detectability of cervix pathologies in their early stage and to select the so-called „actual risk group for developing pathology." Table 1 presents the diagnostic effect of combining screening cytology with HPV genotyping.

Analysis of the above data revealed that the frequency of the occurrence of individual genotypes of human papilloma virus in the cytological diagnostic groups ASC-US, LSIL and HSIL do not differ statistically ($p = 0.57$).

However, what is noteworthy is the more common occurrence of HPV 16 of type LSIL lesions (45.45%) and HPV 18 of a more advanced type HSIL (37.50%) pathology.

By verifying the cytology results with a histopathological diagnosis of the above groups the authors obtained statistically significant differences ($p < 0.001$) in individual pathological states. When it comes to cytological HSIL diagnosis, CIN 1 was never diagnosed, while in other cytological groups, cervical intraepithelial neoplasia of a low degree constituted over 40%. Analogically about 40% of HSIL diagnoses after histopathological verification turned out to be cancer of a pre-invasive state (CIS/AIS), the presence of which was not revealed by ASC-US and LSIL. What is more, CIN2/3 diagnosis was less frequent in the ASC-US cytological group than in the two other groups.

In the sample of 75 patients, the most common cytological diagnosis was a low-grade squamous epithelial lesion (LSIL), detected in 33 patients, i.e. in 44% of all the diagnoses. HPV 16 and HPV 18 infections were identified by way of molecular testing of 15 and 7 women respectively and a mixed HV 16/18 infection was identified in one case. Smears of the other 10 patients revealed the presence of other oncogenic types of human papilloma virus.

Histopathological verification of tissue specimens revealed low-grade intraepithelial lesions CIN1 in 14 biopsy specimens (43%) and medium-

Table 1. Number and frequency of occurrence of individual cytological diagnoses in correlation with the type of human papilloma virus and histopathological diagnosis

Tabela 1. Liczba i częstość występowania poszczególnych cytologicznych rozpoznań w korelacji z typem wirusa brodawczaka ludzkiego i histopatologicznego rozpoznania

HPV type (Typ HPV)	TBS result (Wynik cytologii wg systemu Bethesda)				
	ASC-US 22 (29%)	ASC-H 3 (4%)	LSIL 33 (44%)	HSIL 16 (21%)	AGC 1 (2%)
HPV 16	7 (31.82%)	0 (0.00%)	15 (45.45%)	4 (25%)	1 (100%)
HPV 18	6 (27.27%)	3 (100%)	7 (21.21%)	6 (37.50%)	0 (0.00%)
Other types (Inne typy)	6 (27.27%)	0 (0.00%)	10 (30.30%)	4 (25%)	0 (0.00%)
HPV 16/18	3 (13.64%)	0 (0.00%)	1 (3.03%)	2 (12.50%)	0 (0.00%)
Biopsy result (Wynik biopsji)					
CIN 1	10 (45.45%)	0 (0.00%)	14 (42.42%)	0 (0.00%)	0 (0.00%)
CIN 2	9 (40.91%)	0 (0.00%)	9 (27.27%)	4 (25%)	0 (0.00%)
CIN 2/3	3 (13.64%)	0 (0.00%)	10 (30.30%)	6 (37.50%)	0 (0.00%)
CIS/AIS	0 (0.00%)	3 (100%)	0 (0.00%)	6 (37.50%)	1 (100%)

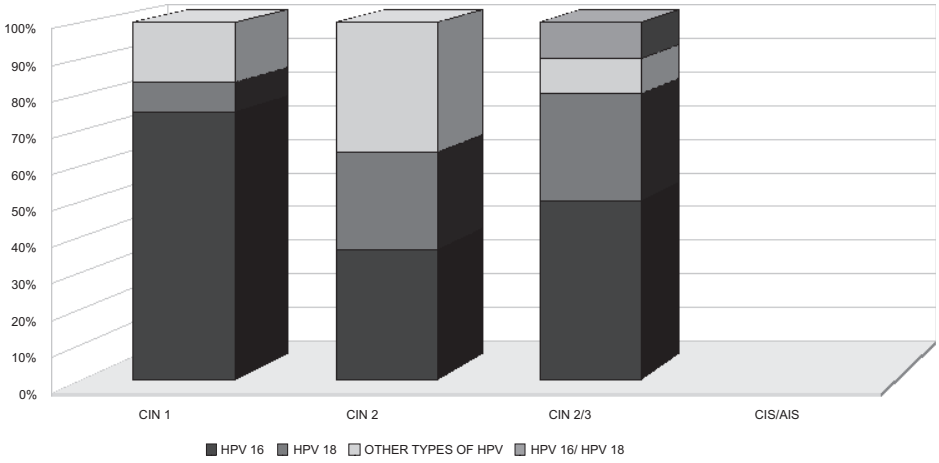

Fig. 1. The percentage of HPV genotypes in different histopathological diagnoses in the group of patients with LSIL

Ryc. 1. Odsetek genotypów HPV w różnych rozpoznaniach histopatologicznych w grupie pacjentek z LSIL

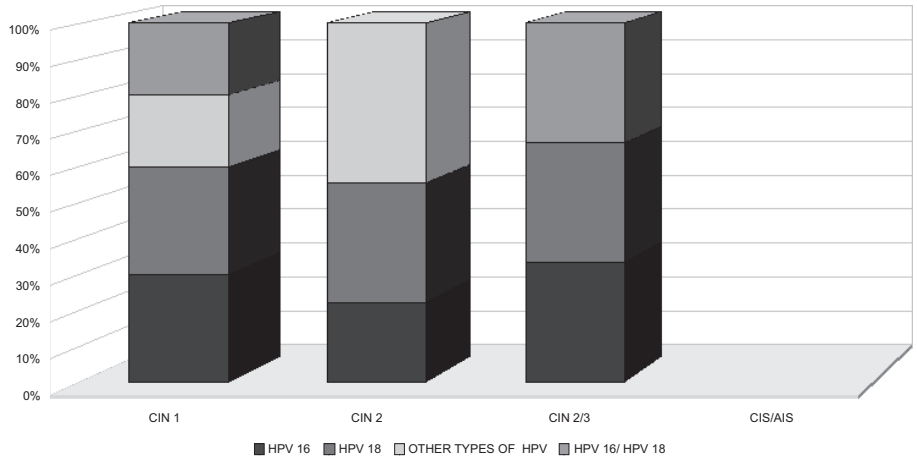

Fig. 2. The percentage of HPV genotypes in different histopathological diagnoses in the group of patients with ASC-US

Ryc. 2. Odsetek genotypów HPV w różnych rozpoznaniach histopatologicznych w grupie pacjentek z ASC-US

grade intraepithelial lesions in 9 cases (27%). Pathological CIN2/3 tissue was found in the specimens of 10 women (30%). In the LSIL diagnosis group, histopathological examination did not reveal lesions of the pre-invasive cancer type. Figure 1 presents the above data.

So-called ASC-US, classified in the Bethesda system as atypical squamous cells of undetermined significance, were found in 22 patients, i.e. 29% of all the abnormal cytological results, which is the second most numerous group of examined women. HPV 16 was identified in 7 patients while HPV 16 and HPV 18 were found in 3 patients. Half of the remaining 12 women had positive HPV DNA test results, while the remaining 6 had other types of human papilloma virus.

Histopathological examination carried out on the group of women diagnosed with ASC-US revealed CIN1 in 10 cases (45%) and CIN2 in 9 cases (41%). CIN2/3 pathology was identified in the tissue specimens of the 3 remaining patients (14%). In this cytological diagnosis group, like in LSIL, CIS/AIS cell types were not found in the biopsy specimens.

Figure 2 presents the above data.

In the analyzed sample, high grade intraepithelial lesions, classified as HSIL, were found in 16 cases, which constitute 21% of all the abnormal cytology results. In the complementary molecular examination DNA HPV 18, HPV 16 and coinfection with HPV 16 and HPV 18 were identified in 6, 4 and 2 women, respectively. Histopathological verification of biopsy specimens from 16 patients revealed advanced lesions of CIN 2 type in 4 cases (25%) and cervical intraepithelial neoplasia of high grade in 6 cases (38%). Six other women diagnosed with HSIL were informed about histopathological confirmation of pre-invasive cervical cancer. Cells typical of CIS *carcinoma intraepitheliale* were found in 4 cases, of which two patients had HPV 16 infection and two others HPV 16/18 co-infection. Lesions of AIS *adenocarcinoma in situ* type were found in two women with confirmed HPV 18 infection. Figure 3 presents the range of the above data in a schematic way.

Out of three women diagnosed with ASC-H, that is 4% of the sample, one was histopathologically diagnosed with CIS type lesions while the other two biopsy specimens contained cells characteristic of AIS. In every case, the test for DNA

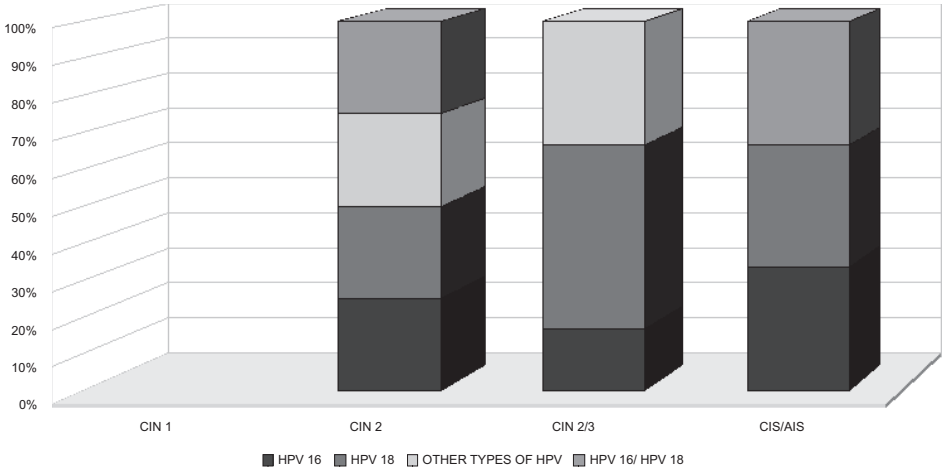

Fig. 3. The percentage of HPV genotypes in different histopathological diagnoses in the group of patients with HSIL

Ryc. 3. Odsetek genotypów HPV w różnych rozpoznaniach histopatologicznych w grupie pacjentek z HSIL

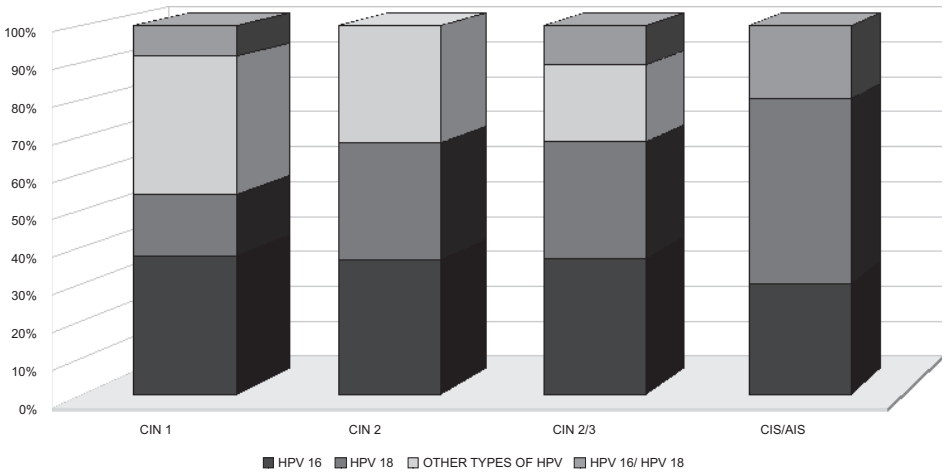

Fig. 4. The frequency of occurrence of different HPV genotypes in the individual stages of CIN advancement

Ryc. 4. Częstość występowania różnych genotypów HPV w poszczególnych stadiach zaawansowania CIN

HPV indicated persistent genotype 18 infection. Moreover, atypical lesions in gland cells, AGC, were found in one of the analyzed cytology smears. Unfortunately, in this case histopathological examination revealed pathology of the *adenocarcinoma in situ* type with coexisting HPV 16 infection.

As the share of individual HPV type in the development and intensification of individual cervical intraepithelial lesions is still debatable, the material was analyzed in order to establish the distribution of HPV types frequency of occurrence in terms of the stage of CIN advancement.

Figure 4 presents the examined problem in a schematic way.

Analysis of the above data does not reveal any statistically significant differences in the frequency of occurrence of individual types of human papilloma virus in different stages of intraepithelial lesions advancement. Therefore, the distribution of individual HPV genotypes does not depend on the stage of CIN advancement ($p = 0.20$).

However, it is worthy of attention that the share of HPV 18 in CIN 1 type lesions is relatively small whereas it is prevalent (50%) in pre-invasive cancer in comparison with other HPV types.

Moreover, in CIS/AIS pathology no human papilloma virus types other than HPV 16 and HPV 18 were found. What also seems to be interesting is the fact that co-infection with HPV 16 and HPV 18 occurs twice as often in CIS/AIS as in other histopathological diagnoses.

What also draws attention is the characteristic distribution of HPV 16 and HPV 18 infections in the small group of patients diagnosed with CIS/AIS.

The presence of specific HPV genotypes differs significantly depending on the histopathological pattern of the lesions. HPV 16 definitely occurred more often in *carcinoma intraepitheliale*. The same relationship was true for co-infection with HPV 16 and HPV 18.

The opposite phenomena were observed in HPV 18 cases, which was dominant in the *adenocarcinoma in situ* type of lesions.

While analyzing a share of other than HPV 16 and HPV 18 oncogenic types of human papilloma virus, the authors found that the most common were HPV 31, 45 and 33. In cervical intraepithelial neoplasia of low degree and high degree, their share was over 60%.

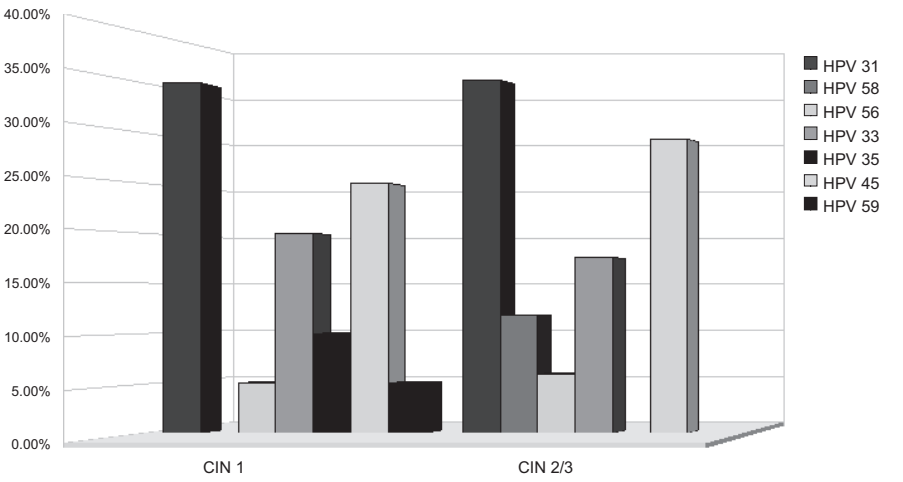

Fig. 5. The percentage share of other highly oncogenic HPV types in correlation with CIN

Ryc. 5. Procentowy udział innych wysoko onkogennych typów HPV w korelacji z CIN

Figure 5 presents the percentage share of individual HPV types.

Discussion

Despite the unquestionable benefits of cytological examination for cervical cancer prevention, over time the authors have observed the imperfections of this type of screening. Even in certified Cytology Diagnostic Units the sensitivity of a cytodiagnostic does not exceed 75% [4, 5]. An additional diagnostic problem is adenocarcinoma in situ (AIS), in the prevention of which, early precursory lesions have not been identified. Yet adenocarcinomas are most common in women who regularly undergo screening [6, 7].

The fact that in as many as 15% of women testing positive for HR HPV DNA with morphologically healthy cervix of uterus, an abnormal cytology result will only appear after about 5 years [8, 9] is an argument for the necessity of HPV genotyping.

Wherever cytological smears are classified according to the TBS system, the most common abnormal cytology diagnosis is ASC-US. Numerous recommendations in the case of atypical epithelial cell identification prescribe testing for DNA HPV HR, which is the basis for further diagnosis [10].

The argument for this type of testing is the fact that about 60% of women diagnosed with ASC-US are positive for DNA HPV HR and the extended diagnosis in this group of patients reveals about 180,000 lesions of HSIL and CIN 2/3 types a year in the world [11,12]. In our research ASC-US constituted 29% of all the cytological smears, while the most numerous group was low-grade squamous epithelial lesions, so-called LSIL, which constituted 44% of all the examinations. Advanced intraepithelial lesions of CIN 2 and CIN 2/3 types were found in 58% of all the original LSIL diagnoses and 55% of ASC-US. While analyzing the above information, one can say that a positive result of a DNA HR HPV test for women with a cytological diagnosis of ASC-US and LSIL is proof of the existence in this group of patients of intraepithelial neoplasia in the so-called molecular sense. Such a diagnosis should result in the maximum shortening of the diagnostic process and a quick decision as to the course of treatment.

Persistent HPV 16 and HPV 18 infection can be a strong indicator for the potential development of cervix pathologies. Over several years of observation of about 40% of women positive for DNA HPV 16 and 18 with original CIN1 diagnosis, lesions of CIN 2 and CIN 2/3 types were found [12, 13]. Infection with other human papilloma virus types with oncogenic potential involves the risk of progression with lower dynamics of intraepithelial lesions. Over several years of observation, advanced cervical intraepithelial neoplasia was found only in about 20% of patients with HPV 31, HPV 33 and HPV 45 infection [14, 15]. In reference to the data from literature and the documented small, at 2–3%, share of other than HPV 16 and HPV 18 types of human papilloma virus in the development of pre-cancerous lesions and cervical cancer, these infections could be treated less aggressively in individual cases, especially as they coexist mainly with morphological lesions of a low grade [16]. Present own research confirms the above information. The share of other than HPV 16 and HPV 18 genotypes was observed most often in the CIN 1 and CIN2 lesions. However, one should not ignore genotypes that are so-called average and low risk. In a few cases of cervical cancer, only HPV 6 or HPV 11 were identified [16]. Cytological HSIL diagnosis indicates a high probability of the presence of advanced intraepithelial CIN2/3 lesions. According to some authors, confirmed persistent infection with HR HPV in patients with cytological HSIL diagnosis because

of the high oncogenic risk is a recommendation for immediate diagnostic-therapeutic conization [9]. In present own research this shortened diagnostic procedure resulted in the identification of four cases of carcinoma intraepitheliale and two of adenocarcinoma in situ. Numerous meta-analyses have revealed a significantly higher percentage of HPV 16 infection in advanced neoplasmatic lesions, pre-invasive cancer and invasive cervical cancer in comparison with other oncogenic HPV genotypes [17, 18].

In authors' own material, the authors determined a slightly different distribution of the frequency of occurrence of the individual HPV types. In the HSIL diagnoses, HPV 18 infections were prevalent. Interesting is also the fact that this type of virus was identified in all the ASC-H cases. Maybe the distribution of the frequency of occurrence of individual HPV genotypes in the Polish population is changing?

ASC-H diagnosis means the presence of atypical squamous cells of which some may have high-grade intraepithelial lesions [9]. Data from the literature suggests that ASC-H lesions constitute about 5–15% of all the ASC results but are characterized by a significantly higher predictive value of diagnosing CIN 2/3 in comparison with ASC-US [18]. Histopathological evaluation of the segments from the vaginal portion of the cervix in one case revealed the presence of cells characteristic of CIS and, in the material taken from the other patients, pre-invasive gland cervical cancer (adenocarcinoma in situ AIS).

Despite the fact that the most frequently diagnosed type of a human papilloma virus in the world is HPV 16, the above data suggests the need to pay more attention to persistent HPV 18 infection. Not only in the literature, but also in present own research this human papilloma virus genotype indicates distinctive tropism towards cancer with a gland pattern which is more and more frequently found in the Polish population. The most recent publications write about AIS presence in 40% of all the histopathological samples from patients referred to because of cytological diagnosis of squamous epithelium lesions, mainly HSIL and ASC-H [18]. Similar statistical results were obtained in present own research.

The pathology of the gland epithelium of the cervical canal is a source of the prognostically adverse diagnostic mistakes in the process of CIN detection. The literature informs us of about 30% of false negative cytological smears from women with neoplasia in the cervical canal, out of which 12% concerns *adenocarcinoma in situ* [18].

The detection of atypical lesions in the gland cells, so-called AGC, in the cytological smear requires, similarly to HSIL and ASC-H, further diagnostics, including, besides colposcopy, directed biopsy of the cervical canal and uterine cavity abrasion [9]. According to different authors, the frequency of the detection of clinically significant pathologies in patients with AGC oscillates between 10–40%, with up to 20% in the case of the invasive cancer, and is related to the patients' age [19, 20]. The oncologist's vigilance in one individual case of such a diagnosis resulted in the detection of adenocarcinoma in situ with persisting HPV 16 infection.

Programs of preventive screening, though updated over the years, have not achieved the anticipated effects, i.e. a decrease in cancer incidence and mortality. Unfortunately, many years of observation confirm the fact that population cytological screening programs are insufficient and have an upper limit of detectable pathological lesions. A vast store of knowledge about human papilloma virus biology and the molecular mechanisms of some of its genotypes leading to the development of cervical intraepithelial neoplasia and pre-invasive cancer may and should be practically used in order to improve the regrettably invariant statistics.

The authors concluded that a positive result of the DNA HR HPV test in women with abnormal cytology results identifies the risk group for developing cervical cancer. No statistically significant differences of the frequency of HPV 16 and HPV 18 types occurrence have been shown in the analyzed groups with cytological and histopathological diagnoses.

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