

## CORRELATION BETWEEN CYTOLOGICAL ANOMALIES, HPV INFECTION AND HISTOPATHOLOGICAL RESULTS -THE EXPERIENCE OF DR CONSTANTIN PAPILIAN EMERGENCY MILITARY CLINICAL HOSPITAL, CLUJ-NAPOCA, ROMANIA

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### ABSTRACT

Cervical cancer is the third most common type of cancer that affects the female population worldwide. Conventional cytology or the Pap test represented and still represents the gold standard screening method for cervical cancer, being used on a large scale since the middle of the 20th century. Human papilloma virus which has a high tropism for squamous and metaplastic cells has been demonstrated to have a central role in the development of these lesions. This study aims is to validate the contribution of HPV infection in the occurrence of high-grade dysplastic lesions by establishing some correlations between the strains of HPV present, cervical cytology and the histopathological examination of the cervical biopsy. The access to screening methods and health education it's the first step for early diagnosis of cervical dysplasia and cervical cancer.

**Keywords:** Cervical cancer, HPV infection, cervical dysplasia.

DOI: 10.19193/0393-6384\_2023\_2\_51

Received October 15, 2022; Accepted January 10, 2023

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### Introduction

Cervical cancer is the third most common type of cancer that affects the female population worldwide<sup>(1)</sup>. In 1950 when the Pap smear test was introduced as a screening method, an important milestone in understanding the occurrence and the process of development of this neoplasia was reached. Nearly 7% of screened women will have an abnormal result such as L-SIL or H-SIL<sup>(2)</sup>. Conventional cytology or the Pap test represented and still represents the gold standard screening method for cervical cancer, being used on a large scale since the middle of the 20th century. In the more modern era, the advancement

of technology allowed the introduction of liquid-based cytology in the 90s. Of course, liquid-based cytology is a more efficient method of analysis and has a number of advantages, including the analysis for human papilloma virus (HPV) infection from the same specimen, but the comparison between the 2 methods in terms of sensitivity and specificity did not demonstrate significant differences in the detection rate of cervical neoplasia<sup>(2)</sup>. Human papilloma virus which has a high tropism for squamous and metaplastic cells has been demonstrated to have a central role in the development of these lesions. Currently, about 200 types of HPV have been discovered, out of which 30-40 types have tropism

for the lower anogenital tract<sup>(4)</sup>. Regarding cervical modifications, HPV is responsible for about 95-99% of dysplastic or neoplastic lesions occurring at this level, and depending on the influence in the neoplastic process they were divided into low risk (LR) and high risk (HR) strains<sup>(4)</sup>. The most frequently encountered strain is 16, which is found to be responsible for about 45% of CIN III lesions and 55% of cervical neoplasia<sup>(4)</sup>. The main phases in the process of carcinogenesis include HPV infection, HPV persistence, the occurrence of dysplasia and progression to invasion. Of course, these phases are not common to all types of HPV, spontaneous or after undergoing drug treatment clearance is possible, especially in cases of infection with low risk strains of HPV. Data from current literature reports a spontaneous clearance of HPV in approximately 67% of cases within 12 months and 90% within 2 years<sup>(5,8)</sup>. Persistent HPV infection leads over time to the occurrence of low grade (CIN I), moderate grade (CIN II) or high grade (CIN III/CIS) dysplasia, and finally to the invasive form of neoplasm.

However, recent data has demonstrated that CIN I does not always precede severe dysplasia, and CIN III may appear directly from normal epithelium infected with HPV HR, depending on the degree of methylation of some genes<sup>(6-9)</sup>. Methods used for prevention of cervical cancer include primary prophylaxis - anti-HPV vaccination and screening for the early detection of pre-neoplastic or neoplastic lesions. Treatment of dysplastic lesions includes monitoring, anti-inflammatory treatment (for low-grade lesions), cryotherapy, thermoablation, conization with an electric loop or scalpel (for high-grade lesions)<sup>(7-10)</sup>. The incidence rate of cervical cancer is unevenly distributed worldwide as well as in Europe, which is why a regional and local analysis is necessary. Romania takes a leading place next to Lithuania in terms of the annual mortality rate due to cervical cancer. At the other end is Finland with the lowest mortality rate due to cervical cancer<sup>(11)</sup>.

Developing countries face real difficulties in terms of primary and secondary prophylaxis. Current Romanian legislation reintroduced free anti-HPV vaccination for the 11-18 age group in 2020, but results regarding vaccination rate are still modest. Regarding secondary prophylaxis, in 2012 a national screening program was implemented through conventional cervical cytology based on the European quality assurance guide for cervical cancer screening published in 2008<sup>(11)</sup>. Taking into account HPV infection, the reports are even more alarming

due to the fact that there is no data, on the general prevalence among the asymptomatic population or with a normal cervical cytology<sup>(13-15)</sup>. In this regard, at the moment there is no free national screening program for the detection of HPV infection. The November 2021 WHO report shows that only 3 out of 10 women in Romania underwent a screening method in the last 5 years<sup>(12)</sup>.

## Material and method

### *Study design and sample design*

The current study design is cross-sectional prospective observational with the role of validating the contribution of HPV infection in the occurrence of high-grade dysplastic lesions. The study sample is represented by patients who presented in the Obstetrics and Gynecology outpatient clinic of the Military Emergency Hospital "Dr. Constantin Papilian" Cluj Napoca between 07/01/2021-12/31/2021 and who had abnormal cervical cytology results. The current study was approved by the ethics committee of the hospital unit and the ethics committee of the "Iuliu Hațieganu" University of Medicine and Pharmacy in Cluj-Napoca with no. 239/30.06.2021. All subjects included in the study expressed their favorable consent for processing their medical results.

The inclusion criteria were patients with no history of cervical dysplasia or known HPV infection, who presented for cervical cytology during the mentioned period, patients who gave their consent for HPV testing and cervical biopsy, where it was necessary. The exclusion criteria were represented by the refusal to process medical data, histopathological examination revealing invasive neoplasia or patients with a history of abnormal results who presented for repeating cervical cytology.

The number of necessary appointments was established thereas: first one to perform cervical cytology, the next in order to perform HPV screening for patients with abnormal cytology results, and the final visit in order to repeat cervical cytology or to perform cervical biopsy. As a result of the first visit, the patients were allocated according to the results into two different groups: the first group with normal cervical cytology and the second with an abnormal cytological result. Patients in the first group were advised to perform routine screening by repeating the cytology after 1-2 years. For all patients who presented an abnormal cytological result, the presence of HPV infection was studied.

Treatment in this group was based on the grade of dysplasia, as follows:

- LSIL – local treatment with a preparation containing carboxymethyl beta-glucane and polycarbophil for 3 months, followed by the repetition of cervical cytology;
- ASCUS (atypical squamous cells of unspecified significance) – local treatment with a local antiseptic and anti-infective substance that contains the following active substances: neomycin, polymyxin B and nystatin, followed by repeating the cervical cytology;
- ASCH (atypical squamous cells that cannot exclude HSIL) – colposcopy accompanied by cervical biopsy;
- HSIL–colposcopy and cervical biopsy;
- AGC-NOS (atypical endocervical cells of undetermined significance) – colposcopy with cervical biopsy and endocervical curettage.

The collected data was entered into a database and processed with the help of the Microsoft Office Professional Plus - Office program, the SPSS Inc. program. and Evolu.

**Results**

In the above-mentioned period, a number of 536 conventional cytologies were performed, through the national free screening program. Among the 536 investigated patients, 48 presented an abnormal result (cervical dysplasia), therefore resulting in a cervical dysplasia incidence rate of 8.96% in the investigated patients group. The average age of patients with abnormal cytology was 39.92 years. The abnormal results discovered during the cervical cytology identified different degrees of dysplasia according to Figure 1.

Among the 48 abnormal cytologies identified, the majority were ASCUS (n=22), followed by ASCH (n=15) and LSIL (n=8). Only one case of HSIL was identified. In all these cases, HPV typing was performed, which identified the presence of the infection in 54% of cases according to Figure 2. The abnormal cervical cytology results correlated with the identified HPV strain required either local anti-inflammatory treatment followed by another test through cervical cytology, or performing colposcopy and cervical biopsy to obtain a definitive diagnosis followed by appropriate treatment. Figure 3 shows the distribution of the type of dysplasia in cases with positive HPV. LSIL type lesions dominate this distribution, followed by ASCH and ASCUS.

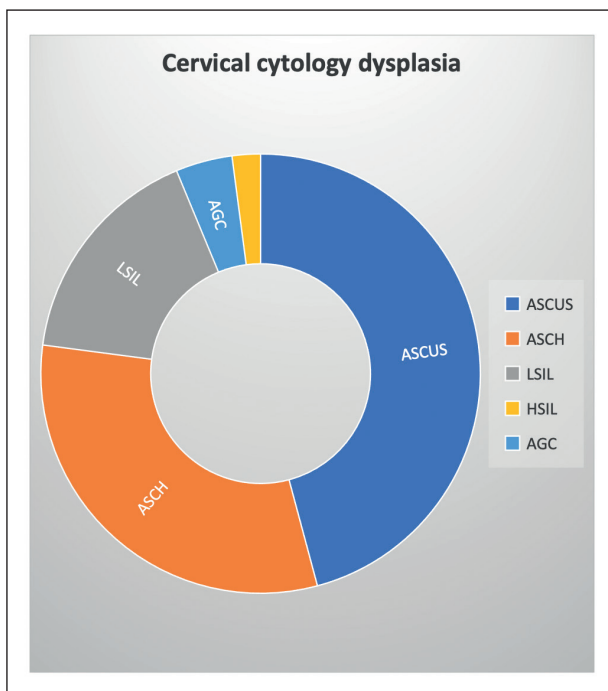


Figure 1: Cervical cytology dysplasia.

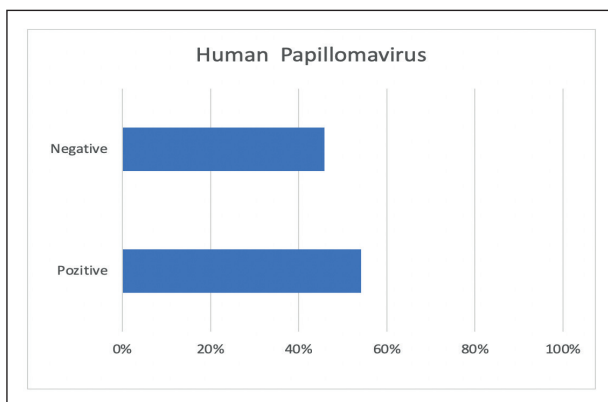


Figure 2: Human Papillomavirus infection.

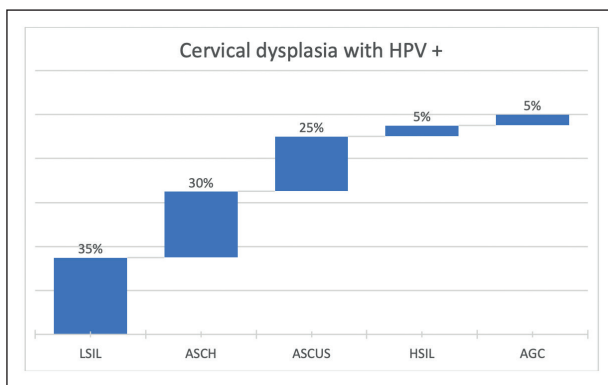


Figure 3: Type of dysplasia with HPV positive.

Figure 3 – Type of dysplasia with HPV positive  
 Regarding the HPV strains, it is important to identify the presence of 16 and 18 HPV subtypes, strains responsible for more than 45% of cervical neoplasias<sup>(2)</sup>. Subtype 16 was found in a percentage

of 20% of HPV infections, subtype 18 occurring in a much smaller percentage, respectively 5%.

The most frequent strain encountered was 31, found in 25% of cases. Following the result of cytology and HPV typing, biopsy was necessary in 52% of cases (n=25), 56% of which had abnormal cytology results and high risk HPV infection, 12% had abnormal cytology results and HPV LR infection, and 32% had only an abnormal cytology result without HPV infection.

Regarding the abnormal cytology results without the presence of HPV infection which required biopsy, 6 out of the 8 patients had ASCH, one lesion was defined as LSIL and one lesion was AGCNOS. Abnormal cytology results associated with HPV LR infection for which biopsy was performed, were represented by 2 ASCH results and one LSIL result. Figure 4 shows the type of dysplasia encountered in the cases of abnormal cytology and HPV HR infection that required biopsies.

The results of performed biopsies show a high incidence rate of LSIL type lesions (44%), followed by HSIL type lesions (28%), normal results in 16% of cases and cervicitis in 8% of cases, data also presented in Figure 5.

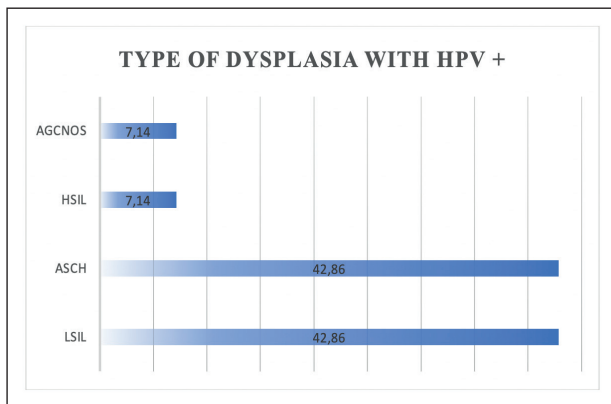


Figure 4: Type of dysplasia with HPV HR which required biopsy.

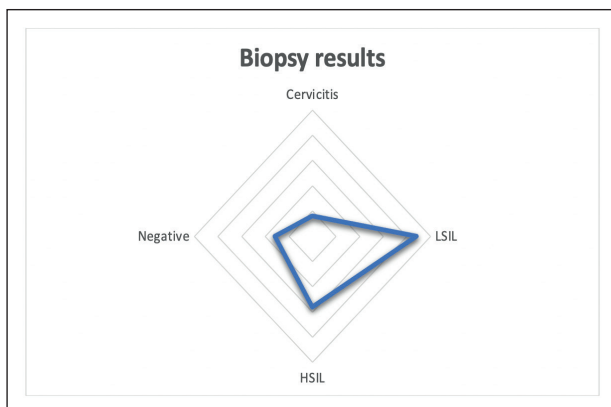


Figure 5: Biopsy results in selected cases.

High-grade lesions received colposcopy and cervical biopsy in all cases. HSIL was identified in a single case that also associated the presence of HPV HR infection, and the biopsy result revealed LSIL. A significantly higher number was represented by ASCH lesions (32.25%). Of these ASCH lesions, 40% were associated with HPV HR infection, and the rest showed either HPV LR infection or the absence of infection. The biopsy results of these cases were summarized in Table 1.

HPV HR	6	HSIL	83.33%
		LIL	16.67%
HPV negative or LR	9	HASIL	22.22%
		LIL	44.44%
		Cervicitis	22.22%
		Negatives	11.11%

Table 1: Biopsy results for abnormal ASCH cytology.

### Discussions

Within the population sample included in the current study, the incidence rate of cervical dysplasia was 8.96%, compared to 7% described in the literature worldwide<sup>(2)</sup>. The higher rate in our country is to be expected considering that Romania is the European leader in mortality rate from cervical cancer<sup>(11)</sup>. Taking into account the WHO report from November 2021 which shows that only 3 out of 10 women in Romania have been screened for cervical cancer in the last 5 years, we consider this rate lower than the real one for the population of our country<sup>(12)</sup>. The arguments that support this statement are the fact that patients with a low socio-economic status associate a low level of health education and do not present for screening investigations, and reinforced by reports that place Romania in first place in the number of underage mothers in Europe, we conclude the early age of starting sexual life and therefore a greater exposure to HPV infection<sup>(16)</sup>.

Likewise, a meta-analysis published in 2010, which included over a million women with normal cytology, showed a higher incidence of HPV infection in Eastern Europe compared to other European regions, and this fact associated with socio-economic status lower<sup>(17)</sup>. In the present study, we note a high percentage of HR strains. Of course, this percentage is a relative one, not including cases with HPV present (either HR or LR) and with a normal cytology. We note the fact that in 30% of the cases of HPV HR infection, biopsy was not necessary as a result of dysplasia remission

following the medical treatment, in this case the lesions were of ASCUS type (83.3%) and LSIL (16.7%). The remission of dysplasia does not necessarily imply the disappearance of the HPV infection, as there is a risk of persistence of the infection and the reappearance of dysplasia that requires a subsequent biopsy. The essential contribution of HPV infection in the development of high-grade dysplastic lesions is manifest when we analyze the abnormal cytological results that reported ASCH. Of the 15 cases encountered, 40% associated HPV HR infection, and 60% presented HPV LR or the absence of infection.

Biopsy of these lesions revealed high-grade HSIL cervical dysplasia in 83.33% of cases with HPV HR infection and only in 22.22% of the other cases. This data validates the important role of HPV infection in the occurrence of high-grade dysplasia and invasive neoplastic lesions. Regarding the strain of HPV present, the data highlights the presence of a high percentage of infection with subtype 16 and a relatively low frequency of subtype 18. The most common type in terms of occurrence is strain 31, also classified as high-risk. A study published in 2020 by Gultekin et al., including more than 4 million women, identified HPV 31 infection as the third most common after subtype 16 and 51<sup>(18)</sup>. The HPV 16 infection rate overlaps results in the literature where it is reported as being among the most common subtypes<sup>(18, 19)</sup>. The introduction of the Bethesda classification for the stratification of cervical cytology results allowed a better understanding of the process of appearance and development of neoplasias. At the same time, this stratification allows more specific sorting of cases that require a simple local anti-inflammatory drug treatment and follow-up through cervical cytology, and cases that require additional investigations such as colposcopy and biopsy. The advantages of this more complex triage are, from a medical point of view, the reduction of costs through unnecessary investigations, and from the patient's point of view, the limitation of the psychological impact that an abnormally benign result can have.

The limitations of this study are represented by the small sample of subjects included in the study. Also, molecular examinations are more specific and can bring additional information in this regard. However, appreciating the failure rate of the implementation of screening methods in developing countries, reflected in a high diagnosis rate of cervical cancer in an advanced stage, health education and

primary screening methods represent the first step in prophylaxis and cervical cancer prevention.

## Conclusions

Cervical cytology remains the main screening method for detecting preneoplastic and neoplastic lesions of the cervix. Regarding the sequence of neoplasia development, an important role is attributed to HPV infection. Despite the existence of these screening methods, cervical cancer is responsible for significant morbidity and mortality worldwide. The diagnostic and treatment protocols in this sense, take into account the presence of HPV infection, without currently existing a specific treatment method for active HPV infection.

In developing countries, access to screening methods and health education limits the early diagnosis of cervical dysplasia and especially the presence of HPV HR infection, which is why the establishment of programs at national and international level is essential in trying to reduce mortality and morbidity.

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*Funding:*

*This work was supported by the grant Partnership for the transfer of knowledge in biogenomics applications in oncology and related fields – BIOGENONCO, Project co-financed by FEDR through Competitiveness Operational Programme 2014 – 2020, contract no. 10/01.09.2016, Code: ID P\_40\_318, MySMIS 105774.*

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