INTRODUCTION: Romania ranks first in Europe in terms of cervical cancer mortality (10.77%), over 6 times more than the average of European Union countries. Of all cancers in women, occupies 4th place in terms of mortality rate after breast, colorectal and lung cancer. Cervical cancer is caused by persistent and common infections of the female genital tract by human papilloma virus (HPV). Currently there is no treatment for HPV, reducing the incidence of cervical cancer can be achieved through organized screening programs and anti-HPV vaccination of female patients at young age. European Union Council recommends early detection of cervical cancer through programs organized for the population, providing quality services at all levels.

THE AIM of the study was the identification of determinants for squamous intraepithelial lesions and assess the importance of classical cytology in detecting cervical cancer at an early stage.

MATERIAL AND METHODS: We selected a group of 405 patients with positive Babes-Papanicolaou test results from the total number of women tested during 2015 by the screening program organized at the Emergency County Hospital in Tg-Mureș. We performed a retrospective study on the previously mentioned group of women, demographic, obstetric and medical history data were collected from FSI screening forms belonging to the Centre Region Management Technical Assistance Unit of the active screening program for early cervical cancer. The study group included women aged 25-64, asymptomatic and without known disease in the genital area.

RESULTS: The accessibility of women to Babes-Pap testing has become more visible since the implementation of organized screening program. The accessibility of women to Babes-Pap testing has become more visible since the implementation of organized screening program. In the studied group, 52.25% of the women had regular menstrual cycle and 32.5% of them were at menopause. Batch analysis of the studied group of patients showed that in case of 47.75% of the women the presence of lesions at macroscopic examination of the cervix has been identified. In 12% of the patients the presence of leucorrhrea was noted and bleeding of the cervix has been described in 2% of the women. Only 0.75% of the women included in the study benefited from hormonal treatment and a percentage of 1.25% were pregnant at the time of the Babes-Pap test.

Analysis of the group of women from the point of view of microbiological examination showed infection with Gardnerella vaginalis in 44% and Trichomonas co-infection in 42%. Of patients selected by interpreting the results using the Bethesda cytology method, the majority (42.23%) were classified in the cytodiagnostic ASCUS class, and a percentage of 26.75 were included in the ASC-H class. Management of intraepithelial lesions presented in 5 cases showed investigations applying differentiated therapies and the importance of follow-up screening.

CONCLUSIONS:

- Implementing screening of cervical cancer remains a challenge as it involves complex coordination of medical services. Urban women benefit from easier access to specialized medical services, but still a large percentage of women included in the Regional Units' screening underwent Pap test for the first time in life. Our study identified a small percentage of high-grade squamous intraepithelial lesions, but for these women case management is essential for investigation and early treatment to stop the development of dysplasia. The major benefit of screening for cervical cancer is especially identifying these cases of high-grade dysplasia, treatment applied in time decreasing the incidence of cervical cancer.

Keywords: cervix, screening, cytology, dysplasia

CYTOLOGICAL ASPECTS OF CERVICAL CANCER SCREENING

Cosmina Cristina UZUN¹, *Eniko Nemes-NAGY⁵, Al-Aisa Abtessam NARIMAN³, Bogdan Petru UZUN⁴, Daniela Edith CEANĂ¹, Mariana Moldovan–NEGOITĂ⁶, Florin Francisc ROSZNAŸ⁵, Strat ALEXANDRU⁸, Septimiu VOIDÁZAN⁴¹

¹assistant professor, University of Medicine and Pharmacy, Tg-Mureș, Department of Medical Biochemistry
²l Lecturer, University of Medicine and Pharmacy, Tg-Mureș, Department of Medical Biochemistry-coresponding author
³sudent, University of Medicine and Pharmacy, Tg-Mureș
⁴senior urologist,Clinical County Hospital, Tîrgu Mureș, Clinic of Urology,
⁵l, University of Medicine and Pharmacy, Tîrgu Mureș, Department of Public Health,
⁶assistant professor, University of Medicine and Pharmacy, Tg-Mureș, Department of Obstetrics and Gynecology
⁷l, University of Medicine and Pharmacy Tg-Mureș, Department of Surgery,
⁸l tractor, University of Medicine and Pharmacy Tg-Mureș, Department of Epidemiology
partners. Viral types with cutaneous tropism cause localized wart type damage (skin warts) and those presenting tropism for cutaneous epithelium include more than 30 types that infect the lining of the cervix: HPV that cause genital lesions with very low risk of progression to cancer (ex. 6, 11, 40, 42, 43, 44, 70, 71), HPV with moderate and high risk of progression to cancer (16, 18, 31, 33, 45, 51, 56, 82 etc.) and HPV with probably high risk (26, 53, 66) [3].

Currently there is no treatment for HPV, reducing the incidence of cervical cancer can be achieved through organized screening programs and anti-HPV vaccination of female patients at young age. European Union Council recommends early detection of cervical cancer through programs organized for the population, providing quality services at all levels [5].

The aim of the study was the identification of determinants for squamous intraepithelial lesions and assess the importance of classical cytology in detecting cervical cancer at an early stage.

Material and Methods: We selected a group of 405 patients with positive Babes-Papanicolaou test results from the total number of women tested during 2015 by the screening program organized at the Emergency County Hospital in Tg. Mures.

We performed a retrospective study on the previously mentioned group of women, demographic, obstetric and medical history data were collected from FS1 screening forms belonging to the Centre Region Management Technical Assistance Unit of the active screening program for early cervical cancer. The study group included women aged 25-64, asymptomatic and without known disease in the genital area.

Clinical data were interpreted corroborated with clinical materials analyzed, we selected cases by age, origin, hormonal status (hormone therapy, contraception, pregnancy, menopause, postpartum and breastfeeding), personal history, quality of smear, description of the smear and infections, changes in non-neoplastic cells, squamous epithelial cell abnormalities and abnormalities of glandular epithelial cells.

Processing of the pathological products was made by staining technique Pap (Babes-Papanicolaou) and interpretation of cervical-vaginal smears was made according to the American Bethesda System 2001.

Microbiological examination of vaginal secretion was achieved by seeding the culture media. We evaluated also the possible presence of infection with Trichomonas, Candida, Gardnerella vaginalis, Actinomyces, herpes simplex virus and other infections.

Statistical analysis was performed with GraphPad InStat3 and the processed data have been regarded as nominal or quantitative variables. Nominal variables were characterized using frequencies. Quantitative variables were tested for normal distribution using the Kolmogorov-Smirnov test and were characterized by median and percent-ages (25-75%) or mean and standard deviation (SD), as appropriate. Quantitative variables were compared using the t-test, ANOVA or Kruskal-Wallis test.

Results:
The average age of the study group was 44.54 ± 10.83 years (SD). Demographical data showed that 54% of the tested women were from urban and 46% from rural environment. The accessibility of women to Babes-Pap testing has become more visible since the implementation of organized screening program.

In the studied group, 52.25% of the women had regular menstrual cycle and 32.5% of them were at menopause. Batch analysis of the studied group of patients showed that in case of 47.75% of the women the presence of lesions at macroscopic examination of the cervix has been identified.

In 12% of the patients the presence of leucorrhoea was noted and bleeding of the cervix has been described in 2% of the women. Only 0.75% of the women included in the study benefited from hormonal treatment and a percentage of 1.25% were pregnant at the time of the Babes-Pap test.

Analysis of the group of women from the point of view of microbiological examination showed infection with Gardnerella vaginalis in 44% and Trichomonas co-infection in 42%. Histopathological analysis of the cervical secretion showed the highest rate of positive results in women aged 36-45, in the percentage of 34.25.
We observed in this group of patients 42.02 percent of women who did not have mucosal damage of the cervix revealed by macroscopical examination or at the cytological secretion sampling time, respectively. Moreover, these macroscopic damages were identified only in a percentage of 28.26%.

Women in the age group of 56-65 years showed no macroscopic changes of the uterine lining and just in 1 case Trichomonas infection was described.

Of patients selected by interpreting the results using the Bethesda cytology method, the majority (42.25%) were classified in the cytodiagnostic ASCUS class, and a percentage of 26.75 were included in the ASC-H class.

**Pregnant women** tested were aged between 29-49 years, 3 had intraepithelial lesions of the cervix (ASCUS, ASCH) and Gardnerella vaginalis infection was identified in one single pregnant.

*Figure 3 - Patients’ distribution related to Bethesda cytodiagnostic classification*

In the study group we selected a number of 5 completely asymptomatic women in terms of gynecological diseases, therefore healthy women, which had at histopathological examination intraepithelial lesions of the uterine lining.

*Figure 4 - Patient aged 62, ASC-US, menopause, Pap, HE, 20x*

**Figures 5, 6 - Patient aged 49 years, pregnant, ASC-US, LSIL, Pap, HE, 20x**

**Figures 7, 8 - Patient aged 35 years, LSIL with HPV atypia, Pap HE, 20x and koilocyte, Pap, HE, 40x**

HPV is the most important aspect of the case management scheme, then it is the indication to repeat the cytology Pap test, followed by colposcopy, if the HPV test is positive. Positive HPV test should be repeated after one year if the biopsy and colposcopy are negative. In cases of negative HPV is recommended to do a cytological reassessment after 1 year.

Case management in LSIL (fig.5,6,7,8) consists in repeating smear tests and colposcopic investigation.
Classification of LSIL (low grade squamous intraepithelial lesion), for example, is based on microscopic signs of acute infection with HPV; according to some authors two thirds of cases with LSIL and the majority of cases with HSIL are associated with carcinogenic HPV types [6]. Studies on women who participated in organized screening programs have demonstrated that Pap test prevents cervical cancer. In many countries conventional Papanicolaou test is still the standard for granting the prevention of cervical cancer, perhaps due to the higher cost of cytology in liquid medium [7].

Based on the experience of the Centre Region Unit after four years of implementation of cervical cancer screening, conventional cytology is the only possibility and quality control tests increase their performance.

Co-infections are considered factors favoring the amplification of severity of HPV-initiated changes by inhibiting cellular apoptosis and facilitate the integration of HPV-DNA in the cells [8]. "Clean cervix" at the macroscopic examination does not exclude the presence of microscopic changes, taking into consideration that squamous intraepithelial lesions develop slowly, with progressive evolution.

On the opposite side is the group of women aged 56-65 years who had dysplasia in a percentage of 16.75, which confirms data in the literature on reduced incidence of cervical cancer at older age.

It is known that inflammatory factors are risk factors for preterm birth. The high incidence of Gardnerella vaginalis infection is demonstrated in several studies in the literature, lesions of the cervix having a favorable effect on the progression of the infection caused by this microorganism and in the same time progression favoring vaginal bacteriosis [9].

Predominance of cytdiagnostic LSIL class (84 cases) against HSIL (37 cases) suggest that progression to high-grade squamous intraepithelial lesions is rare. Similar studies indicates that in these cases HPV infection remains transient [8].

Persistent HPV infection causes disruption of cell cycle control by viral oncoproteins and occurrence of intraepithelial neoplastic lesions. It is considered that the peak incidence of HPV infection is at the age of 20 years, the peak CIN3 detection is at 30 years and the incidence of cervical cancer at 40 years [10]. The cases analyzed in our study identify potential moderate/high malignant intraepithelial lesions around the age of 40 years. Randomized trials have shown that cervical cancer screening by HPV DNA test is more sensitive than screening by cytology for detecting CIN3 changes.

Apparenty healthy women who have found through Pap testing that they have intraepithelial lesions, often need medical or psychological counseling for further investigations and possible treatments. Very often a positive result surprises the women, this is true particularly...
in cases with malignant potential; we met women in our practice who initially refused further investigation and treatment after finding out the positive result. For this reason, for assurance of a good quality follow-up of the patients is necessary to provide complex medical structures within the Regional Units, where women receive counseling, investigations and treatments at the level of European standards. Setting up screening centers within the Regional Units which streamlines follow-up of women included in the screening would be beneficial; currently cervical cancer screening is legislated on all components of medical services and women are forced “to walk” between multiple health units (general practitioner-gynecologist-oncologist, etc.) to receive complex treatment. Centralizing all data regarding women included in screening by implementing a regional screening register under electronic form would raise the quality of follow-up and recording efficiency indicators.

An important component of organized screening is implementing HPV testing, as during the last 20 years studies have validated this method as being complementary to Pap testing. Although some studies have shown that the sensitivity of both methods is imperfect, however, the current preferred recommendation remains the method combining cytology with HPV testing [11],[12].

**Conclusions:**
Implementing screening of cervical cancer remains a challenge as it involves complex coordination of medical services. Urban women benefit from easier access to specialized medical services, but still a large percentage of women included in the Regional Units’ screening underwent Pap test for the first time in life.

Based on the data obtained by our study using the Bethesda 2001 cytologic interpretation system, ASC-US class comprised the majority of patients selected (42.85% of cases), followed by ASCH category (26.75%). Regardless of lesion grade, the most affected patients were aged between 36-45 years.

Our study identified a small percentage of high-grade squamous intraepithelial lesions, but for these women case management is essential for investigation and early treatment to stop the development of dysplasia. The major benefit of screening for cervical cancer is especially identifying these cases of high-grade dysplasia, treatment applied in time decreasing the incidence of cervical cancer.

**References**
5. Recomandarea Consiliului Uniunii Europene privind screenigul pentru cancer-2003/878/EC