

## The importance of monitoring protocols in cervical carcinoma screening

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### Abstract

Romania has supremacy in terms of European statistical indicators of cervical cancer, a fact attested by the studies made by international organizations. The present study is based on cytological evaluation of a group of 9269 cervico-vaginal smears, segregated in various groups that were monitored by standard diagnostic and therapeutic protocols, attitude based on an excellent collaboration with the gynecologist. This cooperation led to the elaboration of a set of protocols for follow-up of patients assessed by Babes–Papanicolaou test, in order to assure an adequate management for all cervical lesions. An important feature of this study is that histopathological examination of cases cytologically designated as HSIL showed, along with changes of HSIL–CIN2 and HSIL–CIN3, also carcinoma *in situ* and invasive squamous cell carcinoma lesions, emphasizing the importance of the pathologic diagnosis of certainty. This idea is also supported in cases of glandular cell atypia, whose microscopic evaluation identified premalignant and malignant lesions, both in endometrial and endocervical site. A particular aspect of the analyzed batch consists in the description of a subgroup of false-negative cytodiagnostic results associated with cervical carcinoma, highlighting the causes and the possibilities to avoid further errors. Overall analysis of results reveals major involvement of the pathologist in providing the sequence from cytology to histopathological diagnosis and to establish diagnostic continuity.

**Keywords:** squamous intraepithelial lesion, atypical squamous/glandular cells of undetermined significance, cervical carcinoma, oncogenic HPV.

### Introduction

Romania has supremacy in terms of European statistical indicators of cervical cancer, a fact attested by the studies made by international organizations. According to WHO database, since July 2008, Romania also ranked first in Europe both in the incidence of cervical cancer, with 29.9 in 100 000 women and mortality caused by this type of cancer, with 12.06 in 100 000 women [1]. This is due to increased incidence of risk factors and to the use with decreased frequency of both screening tests (Babeș–Papanicolaou smear examination) and additional methods (detection of oncogenic HPV types). Cervical carcinoma often occurs during the period of professional and familial evolution of the woman (over 50% of cases occur between 35–55 years) and treatments for advanced forms associate potential complications, including infertility and deteriorating quality of life [2]. These considerations plead for the need of early detection of this disease with major impact on physical, mental and social levels.

Factors favoring the development of cervical cancer are various, related to individual behavior (sexual habits, use of oral contraceptives, multiparity, smoking) and, better certified, to sexually transmitted HPV (*Human Papilloma Virus*) infection [3]. This is the only factor considered etiologic agent in the pathogenesis of cervical cancer, an epitheliotropic virus that mainly

affects the skin and mucous membranes and causes epithelial proliferation associated with characteristic changes at the site of infection [4]. The association between HPV infection and cervical carcinoma has been demonstrated by numerous epidemiological, clinico-pathological and molecular studies [5]. Persistent HPV infection may induce in cervical epithelium a range of premalignant lesions, which varies depending on the type of virus:

- infection with low or high oncogenic risk HPV causes squamous intraepithelial lesion of low grade malignancy (LSIL–CIN 1, plane condyloma);
- infection with high oncogenic risk HPV causes squamous intraepithelial lesion of high-grade malignancy (HSIL–CIN 2 and 3) and cervical intraepithelial glandular neoplasia (CIGN) (HPV type 18) [6].

Cytopathology represents the best method for gynecological neoplasia detection, the Babeș–Papanicolaou test being recognized worldwide for its reliability and, especially, for its capacity to offer a rapid diagnosis, in terms of large savings in time and materials used in comparison with histological techniques. *Bethesda System 2001* represents a standardized system for reporting cytological results, including many advantages: facilitates pathologist–gynecologist communication; sets cyto-histological correlations; provides information for research in cervical pathology; generates data for statistics [7, 8].

## Materials and Methods

The present study is based on cytological evaluation of a group of 9269 patients, performed in a laboratory for cytodiagnosis and histopathology of Constanța, between January 1<sup>st</sup>, 2009 and December 31<sup>st</sup>, 2009. The features of analyzed series were as follows:

- wide age range: 16–79 years (Figure 1);
- diverse origin: gynecological practices, medical analysis laboratories, planning practices, hospital clinics, family medicine practices, oncology practices;
- wide range of reasons for referral to the gynecologist: routine control, pregnancy monitoring, leucorrhea, meno-/metrorrhagia, pelvic pain.

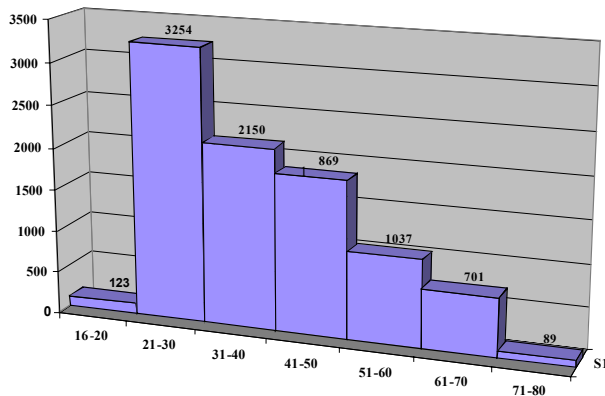


Figure 1 – Age-related distribution of cases.

Information accompanying cervico-vaginal smears included the date of the last menstrual period and summary information on each patient's history (use of oral contraceptives or IUD, history of abnormal cervical screening, cauterization of a previous cervical lesion, resection of an endocervical polyp), in order to achieve a correct interpretation of the clinico-pathological context.

For staining of conventional cervico-vaginal smears, Papanicolaou kit manufactured by Merck was used.

The adequacy of smears was “satisfactory for evaluation” in 9223 cases; the remaining 46 smears were not taken into consideration because of pauci-cellularity, thick layer of cervico-vaginal secretions, predominance of inflammatory elements or red blood cells.

The cases were segregated in various groups that were monitored by standard diagnostic and therapeutic protocols, attitude based on an excellent collaboration with the gynecologist. This cooperation led to the elaboration of a set of protocols for follow-up of patients assessed by Babeş–Papanicolaou test, in order to assure an adequate management for all cervical lesions. The main principles that guided us in drawing up these screening tables were based on the criteria issued by worldwide-recognized societies for cervical pathology, but adapted to the peculiar features of Romanian female population and modified by our experience [9–11].

## Results

The lesional configurations of the examined batch and attitude of pathologist in each case are described below.

8523 patients were “negative for intraepithelial and malignant lesions” (NIEML), of which 8402 had infections with various microorganisms and reactive cellular changes (RCC) associated with particular situations (inflammation, intrauterine devices, the presence of typical glandular cells, post-subtotal hysterectomy, hyperkeratosis, parakeratosis, atrophy, cytolysis). The pathologist's recommendations consisted in application of specific antimicrobial therapy (indicated by the gynecologist), followed by control, which showed, in most cases, normalization of vaginal flora or, rarely, changes in the type of the microorganism involved. Long-term management is applied to this category of patients in routine annual inspection and for women over 65 years, after three consecutive annual NIEML results, the indication was for discontinuation of monitoring (Table 1).

Table 1 – Protocol for monitoring NIEML patients

“Negative for intraepithelial and malignant lesions”	
Patients without history of cervical screening	Routine recall after one year
Patients with history of negative cervical screening	Routine recall after one year
Patients over 65-year-old without cervical screening history	Control for three consecutive years ↓ negative results ↓ discontinuing screening
Patients with abnormal cervical screening history	Follow-up according to the protocol for LSIL
Patients previously treated for CIN	Follow-up according to the protocol
Patients with untreated CIN I history	Control at 6 months interval ↓ negative results ↓ routine recall
Patients with vaginal infections	Management of vaginal infection Control after treatment

Two hundred and sixty patients had “atypical squamous cells of undetermined significance” (ASCUS) and they were monitored under the following protocol. The associated vaginal infections (112 cases) required appropriate therapy, followed by cytological examination after therapy, when it was found that the changes initially interpreted as ASCUS, after treatment of infection, were falling into the category of reactive cellular changes – RCC (squamous metaplasia, regeneration, parakeratosis). In postmenopausal patients (68 cases), local estrogen treatment was recommended for one week, followed by control smear, which showed improvement of cellular changes due to cervico-vaginal epithelium atrophy. Fifty-five patients with ASCUS had cellular changes associated with HPV infection and, even though they did not meet all the criteria for belonging to the LSIL category, they were directed to a laboratory to investigate the HPV genotype. In 25 cases, the review carried out after six months showed clear lesions of HSIL, which required the application of standard procedures sequence: gynecological examination, colposcopy, cervical biopsy and/or curettage, HPV genotyping. Histopathological examination of specimens taken from the cervix in the 25 cases showed

squamous intraepithelial lesions type HSIL–CIN2 (18 cases) and type HSIL–CIN3 (seven cases), which required cone biopsy procedure (Table 2).

**Table 2 – Protocol for monitoring ASCUS/LSIL patients**

“Cytological atypia” ASCUS/LSIL	
Patients with associated vaginal infections	Management of vaginal infection Control after therapy
Post-menopausal patients	Estrogen therapy Control after 6 months
Patients with cellular changes due to HPV infection (koilocytes)	HPV genotyping: HPV high risk → colposcopy; HPV low risk → cytological control and genotyping after 6 months)
Patients with three consecutive negative results, at 6 months interval	Routine recall after one year
Patients with three consecutive positive results, at 6 months interval	Gynecological examination Colposcopy Endocervical curettage Exocervical biopsy HPV genotyping

Two hundred and one patients belonged to “atypical squamous cells cannot exclude HSIL” (ASC–H) category and were oriented to gynecologist who, after colposcopy, opted for taking exo- and endocervical samples, which were sent for examination to the pathologist. On biopsy specimens HSIL–CIN2 (70 cases), HSIL–CIN3 lesions (81 cases) and atypical immature squamous metaplasia (50 cases) were objectified. Combined interpretation of HSIL lesions with HPV test results led to the recommendation of cone biopsy procedure or hysterectomy (Table 3). Patients treated for HSIL were monitored following specific algorithms (Table 4).

**Table 3 – Protocol for monitoring ASCH/HSIL patients**

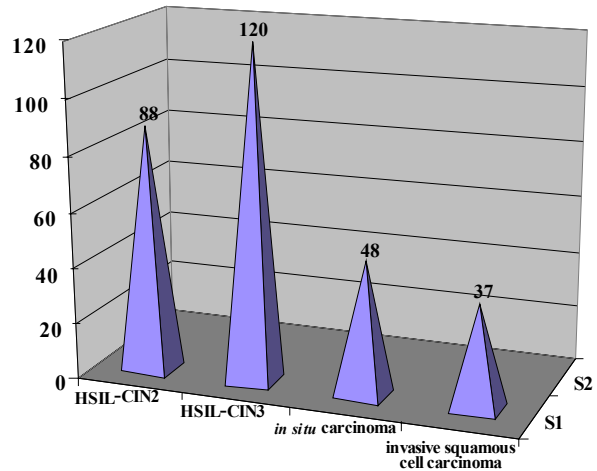
“Cytological atypia” ASCH/HSIL	
Patients with aggravating cellular changes (LSIL→HSIL) or Patients without history of cytological atypia	Gynecological consultation Colposcopy Endocervical curettage Exocervical biopsy HPV genotyping Cone biopsy/Hysterectomy

**Table 4 – Protocol for monitoring patients treated for HSIL**

Monitoring of patients treated for HSIL	
Patients treated by cone biopsy	Cytodiagnostic on the smear taken with endocervical brush (min. 3 tests/5 years) Colposcopy (after 6 months)
Patients treated by hysterectomy	Cytodiagnostic on vaginal smears (at 6–12 months, depending on the gynecologist’s indication)

Two hundred and twenty patients met certain criteria for “squamous intraepithelial lesions” (SIL), of which 103 cases had “low-grade squamous intraepithelial lesion” (LSIL) and 117 cases „high-grade squamous intraepithelial lesion” (HSIL). Patients with LSIL were guided to HPV molecular testing; those detected with high-risk HPV infection were sent to colposcopy and

those with low risk HPV received indications for cyto-diagnosis monitoring every six months. HSIL cases received gynecological examination associated with cervical biopsy, and subsequent histopathological diagnosis showed HSIL–CIN3 lesions (32 cases), carcinoma *in situ* (48 cases), and invasive squamous cell carcinoma (37 cases). Based on these data, the pathologist advised the patient with malignant lesions to follow a complex oncologic therapy, appropriate to each particular situation (Figure 2).



**Figure 2 – Distribution of HSIL cases after histopathological reevaluation.**

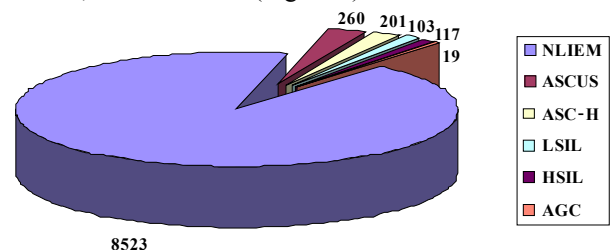
19 patients were placed in “atypical glandular cells” (AGC) category and were oriented to the gynecologist for colposcopy and curettage, which highlighted, in eight cases, lesions of complex endometrial hyperplasia with atypia, and in 11 patients the endocervical changes consisted in adenocarcinoma *in situ* (AIS) (six cases) or invasive adenocarcinoma (five cases). All patients in this category underwent hysterectomy and, in cases of malignancy, radio-chemotherapy schemes (Table 5).

**Table 5 – Protocol for monitoring AGC/AIS patients**

“Cytological atypia” AGC/AIS	
Patients without squamous cell atypia	Gynecological consultation Colposcopy Fractional curettage
Patients with squamous cell atypia	Gynecological consultation Colposcopy Fractional curettage Exocervical biopsy

**Discussion**

Analysis of the percentage of studied cases showed the following configuration: NIEML – 92.41%, ASCUS – 2.82%, ASC–H – 2.18%, LSIL – 1.11%, HSIL – 1.19%, AGC – 0.20% (Figure 3).



**Figure 3 – Lesional structure of the analyzed group.**

Reassessment of cases of ASCUS and ASC-H changed the distribution of cases as follows: NIEML – 92.41%, LSIL – 1.71%, HSIL – 3.17%, RCC – 1.21%, atrophy – 0.73%, immature squamous metaplasia – 0.54%, AGC – 0.20% (Figures 4 and 5).

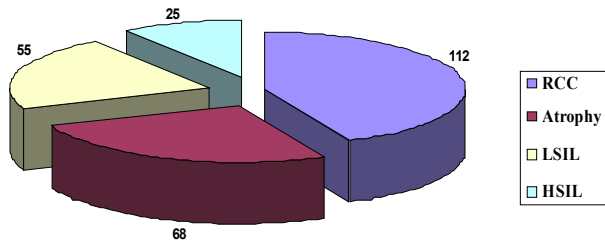


Figure 4 – Structure of ASCUS cases after reevaluation.

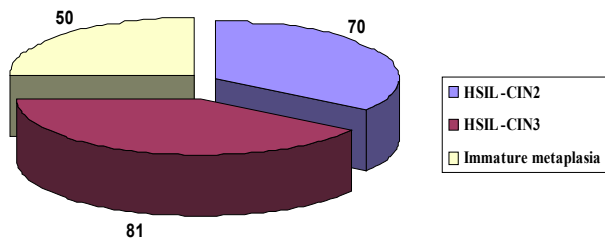


Figure 5 – Structure of ASC-H cases after histopathological reevaluation.

The literature relates an incidence of 2.9% for LSIL and of 0.8% for HSIL, data obtained from a study recently developed in the U.S., in a national program for detection of cervical cancer [11]. The same trial showed that the age range where the rates of LSIL and HSIL reach maximum levels lies between 25 and 29 years.

Results of the present study are not consistent with those reported, HSIL incidence rate being much higher, observation that supports the supremacy of Romania in the incidence of cervical cancer, increased levels of premalignant lesions suggesting high potential for carcinoma development.

Regarding the correlation between HSIL and age of affected women, the examined group revealed two peaks, one in the 21–30 years group and the second, in the 41–50 years group (Figure 6).

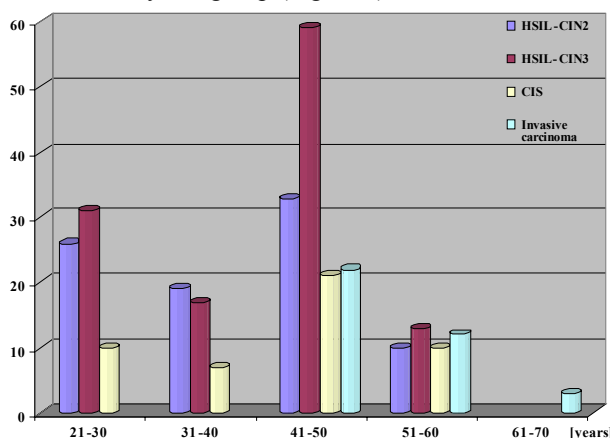


Figure 6 – Age-related distribution of premalignant and malignant lesions.

The first interval is concurrent with the results reported by international trials, but the second age range

suggests several hypotheses related to the patients in this group: tardy infection with high-risk HPV due to change in sexual behavior (increasing number of divorces determines the transition from monogamy to multiple sexual partners), multiparity or development immune depression of various causes, which promotes progression of lesions caused by oncogenic HPV.

Detection by cytodiagnosis of cervical lesions in the stage of HSIL, their histopathological confirmation and therapeutic approach will dramatically reduce the incidence of cervical cancer.

Another feature of this study is that histopathological examination of cases cytologically designated as HSIL revealed, along with changes of HSIL-CIN2 and HSIL-CIN3, also carcinoma *in situ* and invasive squamous cell carcinoma lesions, emphasizing the importance of pathologic diagnosis of certainty. This idea is also supported in cases of glandular cells atypia, where histological evaluation identified premalignant and malignant lesions, both at the endometrial and endocervical site.

Overall analysis of results reveals major involvement of the pathologist in providing the sequence from cytology to histopathological diagnosis and to establish diagnostic continuity.

An important aspect that must be presented consists in the identification of a particular group with false-negative cytodiagnosis associated with cervical cancer. Of all investigated cervico-vaginal smears, eight cases had the result of “negative for intraepithelial and malignant lesions”, but clinical symptoms (leucorrhea, abnormal vaginal bleeding, irradiated pain to the sacral area) has led patients to refer to the gynecologist, who, following clinical examination, decided to carry out, where appropriate, a biopsy of the cervix or a fractional curettage. Collected specimens were interpreted by the pathologist, who established a diagnosis of squamous cell carcinoma in five cases and of endocervical adenocarcinoma in three cases. Also, the review of the prior cervico-vaginal smears allowed the identification of causes for the misdiagnosis of malignant lesion: presence on the smear of only a few atypical cells, the absence of endocervical cells, abundant inflammatory context, the predominance of red blood cells that hide atypical cells, postmenopausal atrophy [12].

Analysis of smears with the presence of atypical cells revealed that in five cases, these were of squamous type and, in the remaining three cases, of columnar epithelial type.

Separate assessment of each case revealed the following features:

- two smears contained a very small number of atypical cells; both cases were diagnosed with histopathological lesions of squamous cell carcinoma;
- one smear showed a moderate number of atypical cells masked by an inflammatory background and by significant hemorrhage; the patient had lesions of squamous cell carcinoma;
- one smear was characterized by fragments of desquamated atypical epithelium, interpreted as having

squamous metaplasia; pathological examination proved that the patient had endocervical adenocarcinoma;

- one smear contained rare atypical cells, in a context of significant cytological changes associated with menopause; histopathological diagnosis was endocervical adenocarcinoma;

- two smears had, as the only indicator of epithelial anomaly, only fragments of atypical cells, difficult to interpret as being associated with malignancy; in terms of pathology, one case was squamous cell carcinoma, and the other, endocervical adenocarcinoma;

- one smear was characterized by the absence of endocervical cells; histopathological diagnosis was endocervical adenocarcinoma.

The study of the false negative smear pattern and grouping of cases that have led to this result intended to identify the reasons for the absence of any evidence of malignancy and to seek ways to improve the cervical screening.

The ideas arising from this analysis were various. Smears without endocervical component do not provide complete information for diagnosis; the seek for endocervical cells on false-negative smears revealed the absence of normal or metaplastic epithelial cells from the endocervix, additional reason for defective interpretation of cases (being known that squamous metaplasia of the endocervical glandular and surface epithelium may be the origin of squamous cell carcinoma located in endocervix) [13].

The presence on the cervico-vaginal smears of fragments of atypical epithelium that seems to belong, at a superficial examination, to the lesional category of squamous metaplasia of the endocervical epithelium, requires a rigorous analysis, because the association of these groups of desquamated atypical epithelial cells with endocervical adenocarcinoma was reported and, less frequently, with squamous cell carcinoma [14].

Double screening of the smears seems to be a remedy for false-negative results, but this procedure will involve additional costs [15, 16].

Implementation of computerized scanning technique would reduce the frequency of failure in recognizing cellular atypia; we consider that this method will prove effective if it is incorporated into laboratory practice prior to conventional screening, each smear being examined by both techniques; the involvement of the pathologist in the process of diagnosis, by the interpretation of lesions on cytological and histological preparations remains unquestionable [17, 18].

The value of cervico-vaginal screening depends on the quality of smears, which requires the development of a satisfactory standardized sampling of smears [19].

## ☒ Conclusions

Analysis of cervico-vaginal smears and their interpretation according to *Bethesda System* is materialized in cytological diagnosis, which allows the pathologist to recommend, through collaboration with the gynecologist, a certain behavior in the case of each

patient, depending on the peculiarities of each case. This way, some lesional groups are defined, that will undergo standard diagnosis and treatment protocols, which assume the existence of a continuum between the pathologist and the gynecologist, whose relationship must take place under optimum conditions.

The elaboration of monitoring protocols assures an algorithmic approach of each lesional entity, which will benefit from adequate diagnostic procedures and targeted therapy.

The accuracy of diagnosis depends on the quality of cervico-vaginal smears, which should be a constant preoccupation of cytodagnostic laboratories, in order to avoid false-negative results.

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