Cytological Identification of the (Pre)Cancerous Cervical Lesions within a Clinically Asymptomatic Female Population

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ABSTRACT The study was carried out on 1637 clinically asymptomatic women who voluntarily requested the Babeş - Papanicolaou cytological examination in our laboratory (throughout a two-year period: 2007 - 2008). 1470 smears presented NLIM modifications, 7 presented ASCUS, 3 - ASC-H, 119 - LSIL, 21 - HSIL, 8 SIL borderline, 6 had AGC-NOS and 3 had carcinomatose.

KEY WORDS precancerous lesions, Babeş - Papanicolaou cytological test

Introduction
Throughout the last 20 years numerous scientific evidence has gathered, that modifies the traditional approach of cervical cancer prevention, by means of detecting precancerous lesions induced HPV, in asymptomatic women, in order to stop their evolution into invasive cancer. HPV (human papilloma virus) is the etiologic agent responsible for most cervical cancers and precursory lesions (1, 7, 11, 14).

HPV is a universally spread virus and an easily transmitted one, to such extent that any sexually active woman is subjected to the risk of contacting HPV. The risk to develop HPV infection is initially present on the first sexual contact and demurs throughout the entire sexually active life of the woman.

Despite the facility with which cervical cancer can be determined (clinically, colposcopically and cytologically), cervical cancer is the second most frequent malignant neoplasm found in women all over the world, representing approximately 10% of all cancers (13). In Europe, it is rated as the seventh cause of cancer caused deaths (6).

Within international comparative statistics furnished by WHO, Romania presents the highest mortality rate by cervical cancer death in Europe and, throughout the world, it is situated immediately subsequent to Latin America states. The Babeş - Papanicolaou cytological test constitutes a reliable method, swift and economical of cervical cancer and its precursors’ investigation and detection (7, 9, 16, 17).

The test is composed of sampling a set of cells from the cervix uteri, which will be examined under microscope in order to detect the virtual abnormalities and in order to prevent cancer.

Material and Methods
The study included a number of 1637 clinically asymptomatic patients selected at our laboratory, in a two-year time frame, respectively 2007 – 2008. The patients voluntarily requested the Babeş - Papanicolaou cytological test. We wish to mention that we excluded from our study the patients with clinical complaints (abundant leucorrhea, post-coital bleedings, dyspareunia, pelvic inflammatory disorder, pelvic neuralgia) or, the ones with menstrual bleedings.

We collected the sample cells with the cervix-brush, from the exocervix and the squamous-cylindrical junction and we placed them in as thin and uniform layers as possible on two slides (cleaned and alcohol-degreased) obtaining conventional smears. The slides were quickly introduced in fixative solution (absolute ethylic alcohol) for at least 20 minutes. They were colored in the Papanicolaou method (nuclear color agent: Harris hematoxylin; citoplasmatic color agents: orange G & polychrome mixture EA51). Result interpretation was carried out descriptively in Bethesda 2001 nomenclature system.

Regardless all our diligence, we assume the falsely positive or falsely negative smears, which emerge from the fact that cervical cytology – as any other exploration method – retains inherent limits, discordances, confusions and errors: unsatisfactory samples for evaluation, screening neoplastic cells by means of haematids, mucus...
and fibrin, especially in the conventional methods and, even more importantly, the fact that neoplastic cellular features may sometimes be subtle, rendering their identification difficult.

**Results:**

The study included a number of 1637 clinically asymptomatic patients cytologically tested with the Babeş - Papanicolaou test. Microscopic examination of conventional smears and their interpretation in the Bethesda 2001 algorithm revealed the following lesion categories:

1470 negative smears for an intraepithelial lesion and malignancy (NLIM). We identified normal, inflammatory or inflammatory-reactive smears – detecting the presence of various organisms: Trichomonas vaginalis, candida, gardnerella, cocobacillus and IUD reactive, atrophic ones (fig. 1).

![Fig. 1: NLIM, Ob. x200, Papanicolaou stain](image)

7 smears with atypical squamous cells of undetermined significance (ASCUS) in which abnormal superficial cells are present (S) or intermediate ones (I), but they are not modified enough to pronounce a low grade intraepithelial lesion diagnosis (LSIL); nuclei are two or three times bigger than normal, round-oval with irregularities, hyperchrome. The diagnostic was that of exclusion (outside inflammation and reactive processes).

3 smears with atypical squamous cells which cannot exclude a high-grade intraepithelial lesion (ASC-H): cytological anomalies were revealed, parabasal (PB) and basal anomalies (B), suggesting a high-grade intraepithelial lesion (HSIL), but quantitatively insufficient for a final interpretation. Atypical metaplastic cells or of atypical reshuffling, with hyperchrome, irregular nuclei and non-uniform cromatine.

![Fig. 2: ASCUS, Ob. x200, Papanicolaou stain](image)

119 smears presenting low-grade intraepithelial lesion (LSIL) with HPV – type S and I koilocytes, nuclear atypicalities of simple dysplastic type, nuclei 4 to 6 times bigger, or less than 2 times (when viral HPV cytopathic effects are emphasized), bi-nuclei, hyperchromasia, irregular nuclear envelope, dyskeratosis orangeophilic cells, with or without inflammations.

![Fig. 3: ASC-H, Ob. x100, Papanicolaou stain](image)

3 smears with high-grade undetermined lesions (SIL-borderline), with cytological features running between LSIL and HSIL; I nuclei increased 3 times with the uniform or degenerated cromatine (LSIL); increased nuclei-plasmatic ratio, irregular nuclear envelope, granular cromatine, evident cytoplasm or quantitatively reduced cytoplasm – on PB cells.

![Fig. 4: LSIL, Ob. x200, Papanicolaou stain](image)
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**Fig. 5: SIL - borderline, Ob. x200, Papanicolau stain**

21 high-grade intraepithelial lesion (HSIL) smears with atypical type PB and B cells, isolated, grouped or syncytial cells with or without koliocytotic atypicality, increased nuclei, hyperchromaxis, emphasized increased in nucleus – cytoplasm ration, indistinct or immature cytoplasm, nuclear contour irregularities. Frequent isolated dysplastic cells.

**Fig. 6: HSIL, Ob. x200, Papanicolau stain**

6 smears with atypical glandular cells, not otherwise specified (AGC-NOS), with glandular cells of nuclear supraposition, great anisocaria nuclei, rougher cromatine, unequally distributed, granular or vacuolized cytoplasm.

**Fig. 7: AGC-NOS, Ob. x200, Papanicolau stain**

3 carcinomatose smears, with tumor cells presenting marked pleomorphism, eerie nuclear forms, budded aspect with irregularly engrossed nuclear membrane, marginal cromatine or piled cromatine, atypical mytoses, inflammatory smears with haematids and cytonecrosis detrituses (fig. 8).

**Fig. 8: Carcinomatous smear, Ob. x100, Papanicolau stain**

**Discussion**

The study included a number of 1637 clinically asymptomatic patients who voluntarily requested the Babeş - Papanicolaou cytological test. No clinical complaints were registered (at most, a ‘normal’ secretion), i.e. patients having a firm conviction that they were under no abnormal smear risk. However, smear evaluation within the Bethesda 2001 system identified 167 (approximately 10%) abnormal cytology smears: 7 ASCUS, 3 ASCH, 119 LSIL, 21 HSIL, 8 SIL borderline, 6 AGC-NOS, and 3 carcinomatose smears.

The psychological impact on learning of an unwanted diagnostic was invariably negative (uneasiness, depression, fear, anxiety or shock).

For this reason, encouraging and motivating women to take the Babeş - Papanicolaou cytological test, which is both professional accuracy within method limits and also a risk as regards falsely positive and falsely negative results, is the only procedure by which they may understand what happens when requesting a cytological examination and also what may happen if they choose not to take this test.

Generally, the evolution modality of SIL lesions is difficult to predict, but it is believed that LSILs have a greater tendency to regress, while HSILs persist or progress (10). There are solid molecular and epidemiologic proofs that demonstrate the close relationship between persistent HPV infections with certain types of high-risk HPVs and the development and progression of cervical cancerous disorders (7). HPV 16 and 18 presence increases the relative risk of developing HSIL by approximately 200 times, in a timeframe of merely two years from detection (12). In this context, immunocytochemical identification of HPV HR (high-risk HPV), suggested in a combined screening, seems to be a
promising tool in anticipating SIL behavior associated with HPV, offering the certainty of identifying preinvasive cervical lesions caused by HPV HR infection, thus avoiding aggressive treatment in patients with low-grade lesions (4, 5, 8, 18).

Conclusion:
Few women request the Babeş - Papanicolaou test and too few doctors recommend it to their patients. Gynecologists’ statistics show that every year in Romania 2,500-3,000 new cancer instances appear, in advanced stages, 1,400-1,500 deaths are recorded annually and there are more than 15,000 cervical cancer patients registered and monitored (2,3,13). In these circumstances, the vital message that must reach women is that, although the Babeş - Papanicolaou test is not perfect-proof, voluntary testing remains the best method of preventing cervical cancer, as in this case, prevention ratio is of 80 – 90% (1, 7).

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Abbreviations
NLIM = negative for intraepithelial lesion and malignancy;
ASC-US = atypical squamous cells of undetermined significance;
LSIL = low-grade squamous intraepithelial lesion;
ASC-H = atypical squamous cells, cannot exclude an HSIL;
HSIL = high-grade squamous intraepithelial lesion; AGC-NOS = atypical glandular cells, not otherwise specified.

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