**Editorial Articles**

# Cytodiagnosis in cervical neoplasia: from the Babes/Papanicolaou smear to the actual Bethesda System

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**Summary**

The detection of cell abnormalities by studying cervico-vaginal smears dates from 1927. Papanicolaou and Traut are still considered the fathers of this method even if two Romanian scientists, A. Babes and C. Daniel first published the technique. Cervical cytology since then has become a useful and worldwide used screening test for cervical cancer. It cannot be used as a means of ultimate diagnosis as it has to be confirmed by a tissue diagnosis. The method has an acceptable sensitivity and specificity. In 1988 a new system of cytologic analysis was introduced: the Bethesda System. It provides a uniform format and offers a standardized lexicon for cervical cytopathology reports. The system was revised in 1991 and again in 2001. Recent discoveries about the precursors of cervical cancer and the availability of new cytologic testing methods now make it possible to incorporate new approaches into managing women with cytologic abnormalities.

*Key words:* Screening of cervical cancer; Cervical cytology; The Bethesda System.

The idea of early detection of cervical cell abnormalities by studying cervico-vaginal smears appeared 70 years ago. Few specialists know the truth about the paternity of the method and few recognize the Romanian priority. In 1927, two Romanian scientists, Aurel Babes and Constantin Daniel communicated at the Gynecologic Society in Bucharest, the results of their research: "The possibility of diagnosing cervical cancer by smears". The main idea was that exfoliative cytology could be a method of early detection of cervical cancer. A few months later in March 1927 their presentation was published in the *Journal of Gynecology*, Bucharest. Babes developed the research further and the results were published in *Press Medicale*, April 11, 1928: "Diagnostic du cancer du col uterin par frottis" ("Diagnosis of cervical cancer by smears"). In this article the author presents the coloring technique (Giemsa method) and the cytological criteria for malignancy: *atypia, heterotypia, the disposition of epithelial elements, the nuclear and nucleolar aspects.* Babes was also one of the pathologists who first promoted the idea of intraepithelial cancer insisting on the possibility of diagnosing it by exfoliative cytology [1-3].

On January 4th, 1928 at a conference in Battle Creek, Michigan, USA, Papanicolaou presented the paper "New cancer diagnosis". The communication was published in 1929 in the journal *Growth* with no reference made to Babes' article from *Press Medicale* which was a worldwide known journal at that time.

In 1941 and 1943 Papanicolaou and Traut developed and modified the fixation and coloring technique and published it under the title: "Diagnosis of the uterine cancer by the vaginal smear". There is no doubt about the great merits of Papanicolaou and his co-worker Traut in perfecting the method, however Babes was the first to introduce it as a diagnostic technique. The original Papanicolaou classification was a grading system with classes I-V:

*Class I - normal cells*
*Class II - slightly abnormal, usually indicating inflammatory change*
*Class III - a more serious degree of cellular abnormality*
*Class IV - distinctly abnormal cells, possibly malignant and definitely requiring biopsy*
*Class V - malignant cells.*

Cervical cytology since then become a useful and worldwide used screening test for cervical cancer because it can identify asymptomatic patients at high risk at a point in the disease course when intervention can alter the outcome. It cannot be used as a means of ultimate diagnosis as it has to be confirmed by a tissue diagnosis. Cytology can detect the malignant nature of cells, but it cannot identify where those cells came from with any specificity. The Papanicolaou smear is a broad sampling of cells from the surface of the cervix, not an exploration into the submucosal soft tissues or endocervical glands. Although cytologists may be able to recognize a number of features that are consistent with invasive disease, it is almost impossible to determine whether a given malignant cell is from a mucosal surface or from a focus of connective tissue invaded by cancer. The evaluation of cells is a specialized taks. The procedure is substantially different from making a histological diagnosis and requires special training.

At the same time the method has an acceptable sensitivity and specificity. The true sensitivity and specificity of the method is impossible to evaluate because the standard against which it must be compared is histologic analysis of the entire cervical transformation zone. A cone biopsy on cytologically normal women cannot be made solely to determine these rates, so the objective cannot be attained.

The accuracy of cytology depends on the prevalence of cervical intraepithelial neoplasia (CIN) in the population being screened. Populations with a high proportion of high risk women merit more intensive screening. The incidence of CIN is far higher than that of invasive cancer of the cervix. This is probably a result of both the natural history of CIN and the identification and eradication of CIN in screened populations before invasion develops.

The false-negative rate appears to decrease significantly when serial smears are taken. The American Cancer Society recommends that cervical cancer screening begins with cytologic evaluation at the onset of sexual activity, or age 18, continues annually until three consecutive normal smears and then the screening can be decreased to 3-years intervals. Populations with a high risk should be screened annually.

In time, cervical cytology proved its superiority to other techniques (inspection, palpation, iodine staining, blind biopsy) and has remained the standard tool for cervical cancer screening. Several studies showed significant declines in the incidence of cervical cancer when this method was used in widespread programs. Unfortunately many patients fail to comply with screening recommendations and many women with cervical cancer admit to inadequate screening. Compliance has been shown to improve with education.

Inadequate cervical sampling can be a reason why malignant or premalignant lesions may progress untreated.

Papanicolaou and Traut described the vaginal pool aspirate as source of cytologic material. Modern understanding of the location of CIN has led to new techniques for sampling the cervical transformation zone. The sampling must be done from both the ectocervix and the endocervix. For this reason there are a variety of instruments to facilitate the sampling but none is ideal and few studies in the literature have tried to determine their comparative efficacy. Another reason for missed lesions is improper handling. Rapid fixation is essential to proper reading. Air drying introduces artifacts that alter the diagnosis. The results of the cytologic analysis can also be affected by the quality of the cytotechnicians. The miscommunication between physician and cytopathologist is another source of mistakes in diagnosis [4, 5].

In order to standardize the nomenclature and to minimize misunderstandigs in December 1988, at a workshop in Bethesda, a new system of cytologic analysis for cervical screening was introduced: the Bethesda System (TBS). TBS provides a uniform format and offers a standardized lexicon for cervical cytopathology reports. It has received general support from professional societies and has gained widespread acceptance in laboratory practice.

## THE BETHESDA SYSTEM:

### ADEQUACY OF THE SPECIMEN

**Satisfactory for evaluation**

Satisfactory for evaluation but limited by ... (specify reason)

Unsatisfactory for evaluation ... (specify reason)

GENERAL CATEGORIZATION (optional)

Within normal limits

Benign cellular changes. See descriptive diagnosis
Epithelial cell abnormality. See descriptive diagnosis
DESCRIPTIVE DIAGNOSIS
**BENIGN CELLULAR CHANGES**
**INFECTION**
**Trichomonas vaginalis**
**Fungal organisms morphologically consistent with Candida spp**
**Predominance of coccobacili consistent with shift in vaginal flora**
**Bacteria morphologically consistent with Actinomyces spp**
**Cellular changes associated with herpes simplex virus**
**Other**
**REACTIVE CHANGES**
**Atrophic with inflammation ("atrophic vaginitis")**
**Reactive cellular changes associated with:**
**Inflammation (includes typical repair)**
**Atrophy with inflammation ("atrophic vaginitis")**
**Radiation**
**Intrauterine contraceptive device (IUD)**
**Other**
**EPITHELIAL CELL ABNORMALITIES:**
**SQUAMOUS CELL**
**Atypical squamous cells of undetermined significance (with qualification)**
**Squamous intraepithelial lesion (SIL)**
**Low-grade SIL (LSIL) encompassing: HPV; mild dysplasia; CIN I**
**High-grade SIL (HSIL) encompassing: moderate dysplasia/CIN II; severe dysplasia/CIN III**
**Squamous cell carcinoma**
**GLANDULAR CELL**
**Endometrial cells, cytological benign, in a postmenopausal woman**
**Atypical glandular cells of undetermined significance: qualify**
**Adenocarcinoma**
**Endocervical**
**Endometrial**
**Extrauterine**
**No obvious site**
**OTHER EPITHELIAL MALIGNANT NEOPLASM: specify**
**HORMONAL EVALUATION (applies to vaginal smears only)**
**Hormonal pattern compatible with age and history** [6]

In April 1991, the National Cancer Institute sponsored a second workshop to assess the utilization of TBS in actual practice and to consider areas for possible improvement. A brief communication published in 1992 presented an abbreviated summary of the changes resulting from this workshop. The main specifications were:

– Four elements constitute the adequacy of the specimen for detection of abnormalities of the uterine cervix: 1. patient and specimen identification; 2. pertinent clinical information; 3. technical interpretability, and 4. cellular composition and sampling of the transformation zone.

– Cross-sectional studies have repeatedly demonstrated that smears with endocervical cells have a significantly higher frequency and higher grade of squamous epithelial abnormalities detected than do smears lacking such cells.

– The clinician ultimately determines what is adequate sampling for an individual patient based on integrating information from the clinical history, visual inspection of the cervix and the cytopathology report.

– The reactive changes encompass benign cellular changes that are reactive in response to such factors as inflammation, radiation or an intrauterine device; reactive cellular changes associated with atrophy and inflammation, or atrophic vaginitis has been added.

– Hyperkeratosis, parakeratosis and dyskeratosis are not included in the TBS terminology.

– Low and high grade SIL encompass the spectrum of precursors of squamous cell carcinoma of the cervix.

– Terms sucha as *koilocytosis, koilocitotic atypia* and *condylomatous atypia* are not included in the TBS lexicon.

– The diagnosis should, if possible, indicate whether the cells are favored to be of endocervical or endometrial origin. If the origin of the cells cannot be determined, the diagnosis "atypical glandular cells of undetermined significance" (AGUS) is used.

– The diagnosis of adenocarcinoma indicates a probably invasive tumor. The origin of the tumor – endocervical, endometrial or extrauterine – should be specified if possible.

– TBS does not include guidelines for patient management based on TBS diagnoses. The guidelines are focused on areas for additional research and clinical trial to resolve certain unanswered questions regarding the management of atypical squamous cells of undetermined significance [7-12].

In 2001, the American Society for Colposcopy and Cervical Pathology made a revision of the Bethesda System and published a consensus statement, "the Bethesda 2001 System" that would help clinicians better care for women with cervical cytological abnormalities. It was developed with a broad participation and reflects important advances in the biological understanding of cervical neoplasia and cervical screening technology:

– According to the 2001 consensus statement, specimens are deemed either *satisfactory* or *unsatisfactory* for evaluation. The potentially confusing category *satisfactory but limited by ...* was excluded.

– For the first time, the Bethesda System takes into consideration the technology of liquid-based cervical cytology. An adequate specimen now comprises at least 8,000 to 12,000 well-visualized squamous cells for conventional smears and 5,000 for liquid-based preparations.

– The general categories were simplified: *within normal limits* and *benign cellular changes* have been combined into a single designation: *negative for intraepithelial lesion or malignancy.*

– The term *diagnosis* is no longer part of the Bethesda System. The heading *descriptive diagnosis* has been renamed *interpretation results.*

– General interpretation results now fall into two main categories: *negative for intraepithelial lesions or malignancy and epithelial cell abnormalities.* The epithelial cell abnormalities involve *squamous cells* or *glandular cells.*

– The following key terms for squamous cell abnormalities have remained unchanged:

• LSIL; HPV/mild dysplasia/cervical intraepithelial neoplasia (CIN I); and

• HSIL; moderate and severe dysplasia, carcinoma in situ (CIN 2 and CIN 3)

– For findings of *atypical glandular cells* (AGC), the old qualifier AGUS (atypical glandular cells of undetermined significance) has been eliminated. The term *favor reactive* has also been eliminated. Categories AGC include endocervical, endometrial and not otherwise specified (AGCNOS); favor neoplasia and endocervical adenocarcinoma in situ [13-16].

No matter how important these statements about cervical technology are, cytological screening cannot replace histological diagnosis. Recent discoveries about the precursors of cervical cancer and the availability of new cytological testing methods – such as liquid cytology and HPV DNA testing – now make it possible to incorporate new approaches into managing women with cytological abnormalities. The thin layer Pap test may improve the sensitivity in detection of cervical dysplasia and significantly reduce the number of inadequate tests. Screening can be improved upon when the practitioner also understands the principles of diagnostic cytology known to optimize the collection and interpretation of cytological smears and has good communication with the cytopathologist.

## References

[1] Babes A.: "Diagnostic du cancer du col uterin par les frottis". *La Press Med.,* 1928, *36*, 451.

[2] Daniel C.: "Diagnostique par frottis du cancer du col uterin". *Gynecol. Prat.*, 1951, *2*, 153.

[3] Daniel C.: "Istoricul unei metode romanesti pe nedrept considerate de origine straina". *Obstet. Si. Gynecol. (Buc.)*, 1964, *12*, 555.

[4] Massad L. S., Cejtin H. E.: "Cervical Intraepithelial Neoplasia in Gynecology and Obstetrics". J. J. Sciarra (ed.), 1998, Lippincott Williams & Wilkins.

[5] Hatch K. D., Hacker N. F.: "Intraepithelial disease of the cervix, vagina and vulva". In: Novak's Gynecology. J. S. Berek, E. Y. Adashi, P. A. Hillard (eds.), Baltimore, Williams & Wilkins, 1996.
[6] The Bethesda System for reporting cervical vaginal cytologic diagnoses. *Acta Cytologica,* 1993, *37* (2).
[7] National Cancer Institute Workshop: "The 1988 Bethesda System for reporting cervical/vaginal cytologic diagnoses". *JAMA*, 1989, *262*, 931.
[8] "The 1991 Bethesda System for reporting cervical/vaginal cytologic diagnoses: report of the 1991 Bethesda Workshop". *JAMA*, 1992, *267*, 1892.
[9] Jones B. A., Novis D.: "Cervical biopsy-cytology correlation: a College of American Pathologists Q-Probes study of 22,439 correlations in 348 laboratories". *Arch. Pathol. Lab. Med.*, 1996, *120*, 523.
[10] Lee K. R., Manna E. A., Jones M. A.: "Comparative cytologic features of adenocarcinoma in situ of the uterine cervix". *Acta Cytol.*, 1991, *35*, 117.
[11] Biscotti C. V., Gero M. A., Toddy S. M., Fischler D. F., Easley K. A., Ng A. B. P. *et al.*: "Significance of endometrial cells in the detection of endometrial carcinoma and its precursors". *Acta Cytol.*, 1974, *18*, 356.
[12] Jones B. A., Novis D. A.: "Follow-up of abnormal gynecologic cytology: a College of American Pathologists Q-Probes study of 16,132 cases from 306 laboratories". *Arch. Pathol. Lab. Med.*, 2000, *124*, 665.
[13] Smith-McCune K., Mancuso V., Constant T., Jackson R.: "Management of women with atypical Papanicolaou tests of undetermined significance by board-certified gynecologists: discrepancies with published guidelines". *Am. J. Obstet. Gynecol.*, 2001, *185*, 551.
[14] Wright T. C. Jr., Cox J. T., Massad L. S., Twiggs L. B., Wilkinson E. J., for the 2001 ASCCP-sponsored Consensus Conference. "2001 consensus guidelines for the management of women with cervical cytological abnormalities". *JAMA*, 2002, *287*, 2120.
[15] Duggan M. A.: "Cytologic and histologic diagnosis and significance of controversial squamous lesions of the uterine cervix". *Mod. Pathol.*, 2000, *13* (3), 252.
[16] Smith-McCune K.: "Report from the Biennial Meeting of the American Society of Colposcopy and Cervical Pathology, 2002". *Medscape OB/GYN Women's Health,* 2002, *7* (1).

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