Preliminary study concerning the Cytoscreen system importance (Liquid Based Cytology) in gynecologic cytology

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Abstract

Our study aims to present the principal and the advantage of using the Liquid Based Cytology method by Cytoscreen system, as an alternative to the conventional Babes-Papanicolaou test, by reducing the false negative results frequency due to the poor quality of the smears and the epithelial cell screening by the blood elements, mucus or inflammatory exudates. A set of 1 054 female patients was available to be investigated during 2002–2004 both in the Clinical Gynaecology of The Clinical Hospital Filantropia and the Clinic of Oncology of the Clinical County Emergency Hospital of Craiova; the patients were from the rural and urban places as well; they underwent cytologic screening by Cytoscreen method. We simultaneously performed the cytologic exam by using both the Cytoscreen and the conventional Papanicolaou methods in 220 patients; the rest of them, namely 834 patients, were examined just by Cytoscreen method. The samples were processed in the Laboratory of Pathology and Cytology of the Clinical County Emergency Hospital of Craiova. The smears were fixed in the absolute ethanol for minimum five minutes than was performed the Papanicolaou stain. The diagnosis was according to Bethesda System 2001. Most of the patients (85.87%) were 21–50 aged. For the group of those tested by Cytoscrren, the rate of the "satisfactory smears" was significant increase (82.27% compared to 65.45% of the patients examined by using only the conventional method). The positive results were 5.44% compared to 2.27%. More accurate diagnosis of high degrees squamous intraepithelial lesions (1.36% compared to one case – 0.45%), of low degree lesions (4 cases – 1.81% compared to 2 cases – 0.91%) and the atypical squamous cells with undetermined significance (1.36% Cytoscreen tested compared with 0,91%). Cytologic diagnosis was enforced by biopsy with histopathologic exam for 4 of 10 cases; the rest of the patients did not present for biopsy to be performed. In one case, HSIL diagnosis was false negative as the biopsy result was well-differentiated invasive squamous carcinoma. Both the diagnosis sensitivity and the smears fezability were significantly improved by using Cytoscreen method.

Keywords: Cytoscreen, Liquid Based Cytology, screening.

Introduction

Preneoplastic lesions that can develop during many years almost always precede uterine cervix cancer. Therefore, only 10% of them develop invasive cancers and 40–60% are spontaneously remitted [1]. It was noted the uterine cervix cancer frequency decreasement lately, due to the broad using of the conventional Babeş-Papanicolaou as screening method. It is especially performed to an earlier discovering a preneoplastic lesions (also named cervical intraepithelial neoplasia) and it represents the most remarkable which women could do to forewarn cervical cancer.

Nowadays, according to EU statistics, Papanicolaou test (Pap test) succeeded in reducing the uterine cervix death rate with 70–80%. Despite of all these, 25 000 new cases and 12 000 demises of this kind of cancer are annually reported.

The relative large number of demise cases due to the high false negative frequency results, ranging from 2.4% to 26% (mean 10–15%), as well. These are produced by errors in prelevating, processing, reading and interpreting the biopsies.

The technical inherent limits of the conventional cervico-vaginal cytology are responsible for most of the false-negative diagnosis (30–70%).

However, the *prelevation errors* appear under the following circumstances:

– the lesion was not touched or the removed material comes just from one area and it is therefore unrepresentative.

– the prelevated product is very abundant thus causing some thickened displayed smears, with cell overlaps making the morphologic details impossible to be decelated.

– cell transfer from the removing device to the glass slide is incomplete.

Processing errors are due to a defective fixation of the smears, overstaining and lack of removed material standardization.

Reading and *interpreting errors* as a consequence of the defective interpretation of the abnormal cells because of the:

– morphological changes.

– defective processing.

– the reduced number of abnormal cells on the smears (less than 50–100).

– presence of the small atypical "interpolate" cells between the overlapped layers of normal cells.

Another factors, such as the presence of blood or polymorphonuclears covering cells and the weak

staining quality might also compromise the screening and the interpretation of the slides. Therefore, due to the aspects represented by the inadequate removing of the pieces and the suboptimal achievement of the conventional Pap smears, three liquid based cytology methods have appeared (comparable but distinct) to get smears in monostratum: Cytoscreen, Cytyc Thin Prep 2000 and Autocyte Prep (Tripath). They appeared initially to facilitate the automatization of the cytological screening but we noted that comparing to the conventional manual screening, the former could offer many advantages.

Material and methods

Our study based on a set of 1 054 female patients, 834 of which underwent cytological screening by Cytoscreen method; for a number of 220 patients cytological exam was performed both by Cytoscreen and the conventional Papanicolaou methods.

Cytoscreen tests were achieved by removing pieces with a special device called Cytoprep (exo-endocervical brush); we used an Ayre spatula to remove pieces for the conventional examination: one from the exocervix and the other from the endocervix levels. We mention that, in the patients tested by the two methods, the liquid environment removing followed the conventional one.

All the tests were accompanied by labels containing: the patient's name and the Christian name; the date of the last menstra or the day of the cycle; pathologic personal antecedents in gynecology or other data which could influence the cytological assessment: pregnancy, nursing/suckling, intrauterine device, radiotherapy antecedents, electro-cauterization, hormonal treatment or abnormal smears. After removing was done and the endhead of the brush was sunk in a preservation liquid phial, the liquid cytology pieces were processed in the Laboratory of Pathologic Anatomy and Cytology within the Clinical County Emergency Hospital of Craiova, by using Cytoscreen method.

The following stages can be mentioned:

– the phial with the removed device (Figure 1a) was placed in a shaker (Figure 1b) to take the cells out off of the removing device and get an homogenous cell suspension.

– automate reading of the cell sample density by using a nephelometer (Figure 2).

– cells deposition in monostratum after centrifugation (Figure 3), resulting a round shaped smear with a diameter of 17 mm.

Also, the conventional smears from the 220 patients undergoing screening by both the methods were fixed in absolute ethanol for minimum 5 minutes, and then they were stained by Papanicolaou method. For each case, we assessed the smears fezability according to Bethesda System from 1999 (with its changes since 2001) for both the conventional and the Cytoscreen methods (Figure 4).

Therefore, the smears were grouped in four groups such as: *satisfactory*, *satisfactory but limited* by the absence of an endocervical component or by the presence of the factors covering the cells and/or prevent the interpretation (haematiae, polymorphonuclears, defective fixation, artifacts) (just for the conventional smears) and *non-satisfactory*.

More than that, to assess how adequate the smears by Cytoscreen were, we considered a macroscopic criterion represented by the cell density automatically assessed by using a nephelometer; at least class A2 is necessary for a smear to be adequate (Table 1).

Table 1 – *Cell classes in Cytoscreen system*

Class	Cell density	Sample quantity
A	Very small cellularity	5 ml
A2	Small cellularity	2.5 ml
B+	Intermediate cellularity	1.2 ml
B	Large cellularity	700 µl
B2	Dense cellularity	350 µl
C	Very large cellularity	200 µl
C2	Extremely large cellularity	100 µl

We achieved the cytological diagnosis groups describing by using the Bethesda System; for the cell anomalies we used the terms: atypical squamous cells of undetermined significance (ASCUS, with the two subgroups: ASC-US and ASC-H), low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL).

Results and discussions

Patients available for our study were aged between 17 and 74 years, an average of 36.85 years (Table 2), most of them are coming from the urban places (924 cases) and the rest of them from the rural side (130 cases). In all the cases, we carefully pursued to assess the cell concentration, the cell presence at the level of transformation zone, the presence of infection with different microorganisms (*Candida*, *Trichomonas*, *Gardnerella*, *Leptotrix*), the presence of reactive benign cellular changes and the identification of squamous and glandular cell anomalies, eventually with histopathologic confirmation.

In 220 cases where the cytologic screening was performed by using both methods, we comparatively assessed the following parameters: smear adequability, the sensibility in discovering the precancerous lesions and the specificity of the method.

Table 2 – *Distribution on groups of age*

Age group	No. of cases	%
10–20 years	35	3.32
21–30 years	253	24.02
31–40 years	463	43.92
41–50 years	189	17.93
51–60 years	78	7.40
61–70 years	21	1.99
over 71 years	15	1.42

As to assess the Cytoscreen smears fezability, we used two criteria: macroscopic one, represented by the cell density, standardized by means of nephelometer, and a microscopic one, represented by the presence on the smear the cell from the squamo-cylindric junction level. Evaluating the cell density using the Cytoscreen method, we established that the sampling was correctly performed (adequate smears to be interpreted), as in 932 cases (88.42%) the samples had mean and high concentrations (B and B2 classes).

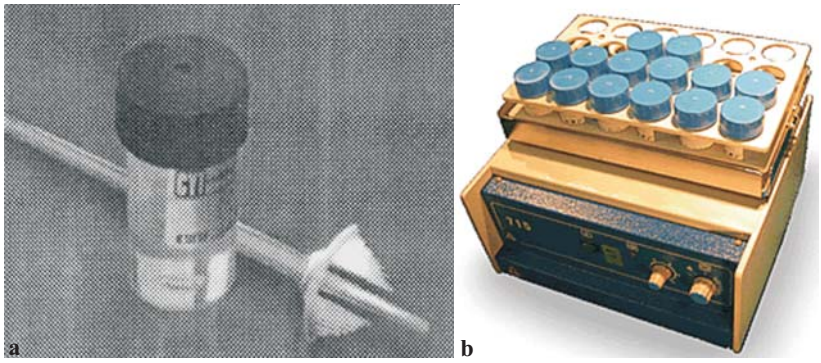

Figure 1 – a) ***Cytoprep brush and vial with conservation liquid*****; b)** ***shaker***

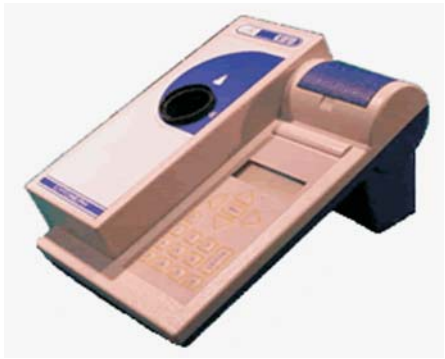

Figure 2 – ***Nephelometer***

Figure 3 – ***Centrifuge***

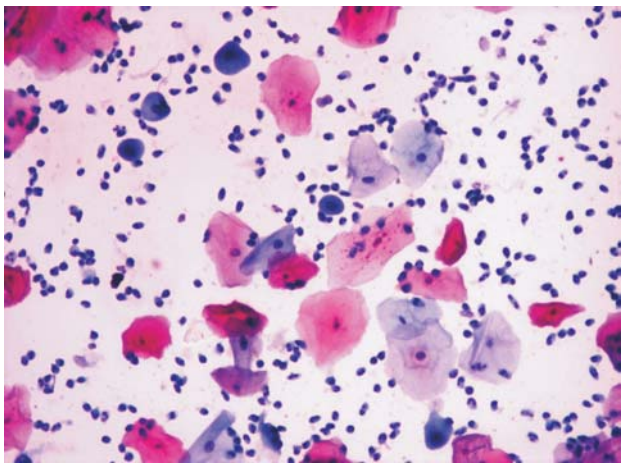

Figure 4 – ***Inflammatory smear, Papanicolaou staining, ×200***

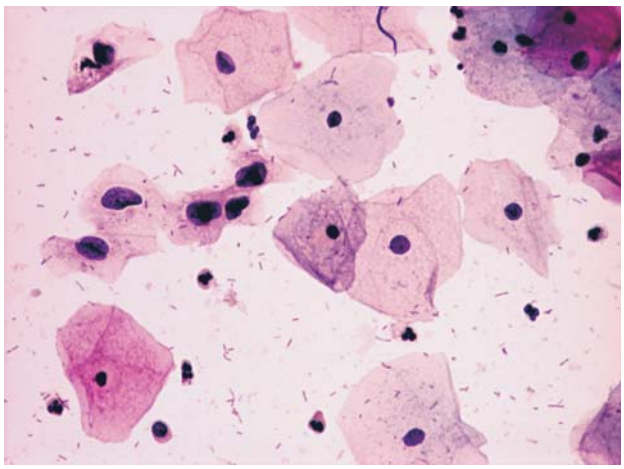

Figure 5 – ***Low-grade squamous intraepithelial lesion (LSIL), Papanicolaou staining, ×200***

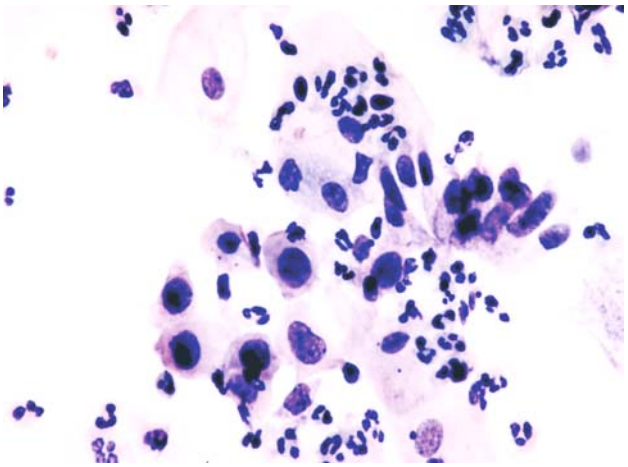

Figure 6 – ***High-grade squamous intraepithelial lesion (HSIL), Papanicolaou staining, ×200***

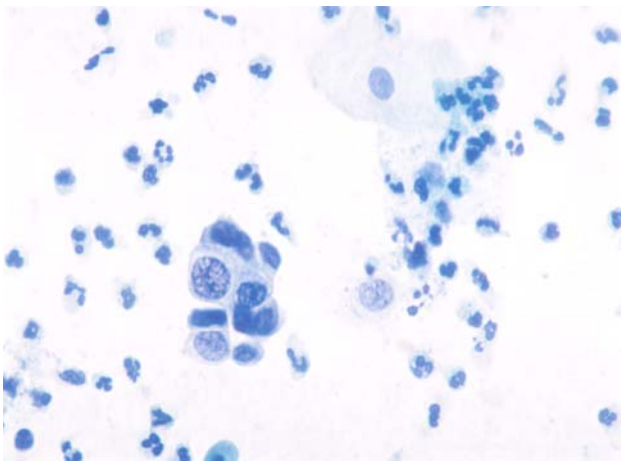

Figure 7 – ***Invasive squamous carcinoma, Papanicolaou staining, ×200***

Endocervical or metaplastic cells were presented on the monostratum smears from the most of the cases (547 cases – 72.22%), therefore the possibility to diagnose the endocortex lesions being much increased.

By examining the removed smears by both the methods, we established that the rate of the cases with satisfactory smears to be cytologically interpreted was significantly increased by the Cytoscreen method (181 cases – 82.27%) compared to the conventional one (144 cases – 65.45%). This difference was due to both the reduction of the number of the smears classified as satisfactory but limited by the absence of an endocervix component and those of the unsatisfactory ones made by the conventional method.

Thus, the endocevix component (endocervical or metaplastic cells) was absent in a very small number of the cases tested by Cytoscreen method (36 patients – 16.36%). Otherwise, a large number of cases conventionally tested were considered as satisfactory but limited by the lack of the endocervix component (128 cases – 58.18%) or unsatisfactory for cytological interpretation due to the some other reasons: reduced cellularity, epithelial cell covering by haematias or polimorphonuclear leukocytes, defective fixation (55 cases – 25%).

As concerning the cytological diagnosis according to Bethesda System, 364 patients (34.35%) had smears within normal limits and in 335 cases (31.78%) the smears were inflammatory not otherwise specified. In 155 patients, the inflammation was due to the presence of various microorganisms: *Trichomonas vaginalis* in 63 cases (5.98%) and 99 cases with mycotic infected inflammation (8.73%). The results were similarly to those conventionally performed smears.

Another assessed parameter was represented by the sensitivity of discovering precancerous lesions comparatively by the two methods (liquid based cytology and conventional ones).

As a consequence we revealed atypical squamous cells with undetermined significance (ASCUS) in three of the patient's smears (2.27%) from the group of 220 cases; in only two cases (0.91%) conventionally tested, another kind of cells were identified and we recorded them in the reactive benign cellular changes group induced by the regeneration and repairing after electrocauterization.

On the smears made by Cytoscreen method we diagnosed 4 cases (1.81%) with low-grade squamous intraepithelial lesion (LSIL), 3 cases (1.36%) of high-grade squamous intraepithelial lesion (HSIL), two of which (0.91%) and 1 case (0.45%) having a counterpart on the conventional smears (Figures 5 and 6).

In only one patient, the cytological diagnosis was invasive squamous carcinoma on both the conventional and Cytoscreen made methods, with subsequent histopathologic confirm (Figure 7).

Among the patients with HSIL, only in two of them the biopsy was performed, the cytological diagnosis being confirmed in a patient with severe dysplasia (CIN 3) and in one case, the cytological diagnosis HSIL was negatively false as the histologic result was invasive squamous carcinoma well differentiated.

Since 1940, when Georgios Papanicolaou described the conventional Pap smear, this was and is still used to trace out preneoplasic lesions of the uterine cervix cancer. Although the conventional method is technically limited and that is why both removing errors and false negative or false positive results may occur. Some studies showed that the removing errors are entirely due to the medical persons performing the prelevation [1].

However, another source of error comes from the fact that a significant part of the prelevated material is not placed on the glass slide during the manual preparation of the smear directly, but this is thrown together with the prelevation device [2]. Also, the studies of cytometry flow slowed that, according to the device that was used, up to 90% of the prelevated material is removed away with that instruments [2, 3].

In a study by Hutchinson *et al.* (1994) the quantity of cells transferred by manual extension of smears on the glass slides was shown to vary from 6.5% to 62.5% [4]. That is why such smears can contain an insufficient number of cells necessary for the diagnosis of the uterine cervix lesion.

On the other side, the false negative outcomes still appear either due to the poor quality of the conventional smears (thick layer of smear, cells present on less than 10% of the glass slide surface, cell covering by blood or inflammatory elements, the absence of endocervical component elements) or for the lesion was not reached by using some inadequate removing device (Ayre spatula), and the false positive ones may be due to air drying artifacts of the smears that can cause the cell morphology to be changed [5].

However, to get rid off all those troubles inherent to the conventional Pap method, a new technique has been developed, *Liquid Based Cytology*, base don the cell suspension in a preserving fluid environment and a half-automate method to get the smears.

Nowadays, there are three methods of liquid cytology, but we used the *Cytoscreen method* for our study. As to get smears by this method several well-defined stages should be followed, finally resulting in an uniform distribution of the cells on a surface of 17 mm, without cell overlapping, therefore existing the possibility that polymorphonuclear leucocytes excess should be removed by means of such a solution called *gradient solution*.

Also, due to the composition of the preservation liquid, haematias are lysing, making them unable to mask the squamous or glandular cells. As the removed pieces were not laid down the slides manually to get smears, it made artifacts should be reduced.

The first advantage of Cytoscreen system is represented by the possibility of removing by means of a special device called Cytoprep (exo-endocervical brush). Our patients rapidly accepted this method; it is unpainfull, but sometimes it can cause a light bleeding considering the anatomical features of the patients (atrophic mucosa) or the prelevation hardness.

Because most of the cervical anomalies originated necessary that an optimal cervical smear should contain cells from both the exocervix and the endocervix component levels [6].

In our study, using Cytoprep, all the conditions were carried out, due to its characteristic aspect (Figure 5), the most of the cases (82.27%) had both squamous and endocervical and/or metaplastic cells on the smears, thus corresponding to the data of literature [7].

To improve smears adequability by using the liquid cytology in monostratum method was previously mentioned in other studies, too, where a decreasment of the smears diagnosed as „*satisfactory but limited by the endocervical component absence*" were noted [8, 9].

Also, by using the Cytoscreen method we noted an increasement in the frequency of tracing out the cervical cancer precursor lesions (LSIL and HSIL) compared to the conventional method. That is also due to the significant reduction both in the prelevation errors and those of interpretation ones, as it is shown in other studies as well [10].

In addition, excepting the cases that could not be monitorized, there existed a good concordance between the HSIL cytological diagnosis and the subsequent histologic assessment result. Just in one case with HSIL cytological diagnosis, the result of the biopsy was well-differentiated invasive squamous cell carcinoma. This discordance resulted from that there were very few cells with nuclear anomalies on the smear and the certain diagnosis of malignancy could not be established.

Conclusions

Cytoscreen system is a remarkable alternative of the conventional Papanicolaou test as: it improves the cell removing from the exo- and endocervix level, thus reducing the number of the inadequate smears to be cytologically interpreted; it determines a better preservation and distribution of the cells on smears; the time to examine them is reduced; the mucus and bleeding quantity on smears is reduced. Also, by using this method, the frequency of tracing out the preneoplastic lesions is increased, first of all due to the fact that nothing of the removed material is thrown away, therefore, there is no air drying artifacts which could cause false positive results.

As the removed material is brought into the laboratory namely into the preservation liquid, it can be kept there for a certain period of time. A variable number of smears can be achieved for the same case, without the patient should be called for a new sampling intervention and a lot of tests or additional studies can be achieved – *i.e.*, HPV-DNA testing or DNA probes to trace out *Chlamydia* or *gonococcus*.

Through this new technology implies a more increased price than Babeş-Papanicolaou conventional method, the multiple advantages of the former would impose it in the future as an optimal method to early trace out preneoplastic lesions of the uterine cervix cancer.

The assessment of the Cytoscreen method costs is still in a preliminary stage, but it is supposed that they could be repaid during the time by reducing the screening frequency in the female population with low risk for the uterine cervix cancer.

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