Conventional cytology versus liquid based cytology in cervical pathology: correspondences and inconsistencies in diagnosis, advantages and limits

NICOLETA SIMION, IRINA-DRAGA CĂRUNTU, ELENA-ROXANA AVADÂNEI, RALUCA BALAN, CORNELIA AMĂLINEI

Department of Morphofunctional Sciences – Histology, "Grigore T. Popa" University of Medicine and Pharmacy, Iassy, Romania

Abstract

Liquid Based Cytology (LBC) has replaced Papanicolaou standard cytology due to its practical advantages. Our study aimed to analyze the diagnosis correlations and differences between the conventional and liquid based cytology. The study group has been composed by 104 patients, diagnosed in the Laboratory of Cytology, Galati County Hospital, Romania by using both methods on the same patients group. Our study revealed a good correlation between the results. Thus, in 78 from the total of 104 (75%) analyzed cases, the diagnoses established by conventional smears have been identical to that obtained in LBC. The diagnoses have been different in 26 (25%) cases, the inherent errors of conventional Pap diagnosis being corrected by LBC. LBC resulted in the diagnosis of some entities missed in conventional cytology, namely: ASC-US (atypical squamous cells of undetermined significance) (one case), ASC-US associated to AGC-NOS (atypical glandular cells not otherwise specified) (one case), and HSIL (high-grade squamous intraepithelial lesion) associated to AIS (adenocarcinoma in situ) (one case). LBC provided the identification of a higher number of cases of associated lesions, as LSIL (low-grade squamous intraepithelial lesion) and AGC-NOS (five versus two cases), and HSIL and AGC-NOS (seven versus five cases). The national experience is mainly based on conventional cytology usage, after Papanicolaou staining method. Extremely rare centers have benefited by the necessary infrastructure for LBC, thus there are no constant reports in the mainstream. The main impediments in large-scale application of this method, in all national screening centers are connected to costs for capital investments and by conditions of exploitation. Our experience and results support the simultaneous use of the two methods.

Keywords: conventional cytology, liquid based cytology, squamous lesions, glandular lesions.

Introduction

Cervical carcinoma is the second cancer as incidence in female gender worldwide, being exceeded only by breast cancer. Lately, because of cytology screening, the incidence and mortality correlated to this cancer type have considerably decreased in all western countries. Liquid Based Cytology (LBC) has replaced Papanicolaou standard cytology due to its practical advantages, with variable circumstances. In relation to this issue, the cell transfer to the slide is standardized, the cells are homogenously distributed on the entire surface, blood, mucus, and inflammatory cells are eliminated by the preparation, and the fixation is efficient and uniform. These characteristics have demonstrated, in numerous studies, a high detection of intraepithelial cervical neoplasia, the decrease in atypical squamous cells of undetermined significance and/or a reduced rate of uninterpretable features [1–13] and an improvement in preinvasive and invasive glandular lesions detection [14, 15]. Supplementary, the residual biologic material may be investigated for biomarkers as human papillomavirus (HPV) and p16, or for miscellaneous infectious agents. These characteristics resulted in immediate and large-scale application, in United States of America and its utilization as a standard screening method in Great Britain and Canada.

LBC contesters are mainly considering the studies design that affirmed its superiority [16, 17]. A recent systematic review [18] demonstrates, in correlation to the criteria established by the authors that, as the study is better, the differences between the results obtained by LBC and Pap smear is decreasing. Considering these aspects, although some countries already use LBC for cervical cancer screening, valuable studies regarding its adequacy are limited and scarce. The majority of published researches are based on the comparative studies of non-randomized populations or on a double testing of the same patients. The design based on double testing may lose the cells used in conventional procedure, which is firstly performed, resulting in a LBC subevaluation [19]. Moreover, only few published researches have been concentrated on confirmed cervical intraepithelial neoplasia and, in a relatively reduced number of cases, the diagnoses have been verified by colpo-histology.

Indubitably, cytological interpretation is highly subjective, the examiner’s experience having an essential role. The accumulate national experience is mainly based on conventional cytology usage, after Papanicolaou staining method. Extremely rare centers have benefited by the necessary infrastructure for liquid based cytology, thus there is a reduced experience in this method, and no constant reports in national specialty literature. We consider that the main impediments in large-scale application of this method, in all national screening centers are connected to costs for capital investments and by conditions of exploitation.
Within this context, our study aimed to analyze the diagnosis correlations and differences between the two cytological approaches, by methods application on the same patients group and comparison of the results.

Materials and Methods

The study group has been composed by 104 patients, diagnosed in the Laboratory of Cytology, Galați County Hospital, Romania.

The conventional Pap smears have been performed with a wooden (Ayre) spatula from the fornix and portio and with Cytobrush from the endocervix. LBC has been similarly performed with a plastic spatula and a Cytobrush. Consequently, material from fornix, portio, and endocervix has been prelevated from all the investigated patients. Liquid-based monolayer specimens have been immersed in PreserveCyt solution and have been processed with ThinPrep 2000 processor (Cytyc Corporation, Boxborough, Massachusetts, USA). All the slides for conventional and LBC have been stained with Pap staining.

The stained slides have been examined using the microscope (×10 objective), for a general view, by evaluating the staining quality, the cellularity, the acellular material, the bacterial flora, and then with ×40 objective for morphological details identification, avoiding the cellular overlaps and crowding that may result in errors of interpretation of chromatin features, of nuclear-cytoplasmic rate and of cytoplasmic basophilia. The microscopic examination aimed the pathological alterations that appear in smear cellular features, guiding the cytopathologist in the diagnosis of non-neoplastic, inflammatory, preneoplastic, preinvasive and invasive tumors. In conventional smears, the evaluation of the degree of specimen significance and of the presence of endocervical/metallic cells used the Bethesda 2001 criteria. LBC has been considered as significant if the slide contained more than 5000 epithelial cells. Endocervical cells have been considered as present if two or more glandular/metallic cell groups, each of at least five cells or if the slide contained at least 10 glandular/metalastic cells.

Results

Our study revealed a good correlation between the results obtained by the application of both methods, in the same patients (Table 1). Thus, in 78 from the total of 104 (75%) analyzed cases, the diagnoses established by conventional Pap diagnosis have been identical to that obtained in LBC (Table 1). The diagnoses have been different in 26 (25%) cases, the inherent errors of conventional Pap diagnosis being corrected by LBC (Table 2).

Table 1 – Synopsis of concordant diagnoses

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Concordant diagnosis CC-LBC</th>
<th>Diagnosis exclusively obtained in CC</th>
<th>Complete diagnosis obtained in CC</th>
<th>Diagnosis exclusively obtained in LBC</th>
<th>Complete diagnosis obtained in LBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NILM</td>
<td>36 (34.61%)</td>
<td>5 (6.73%)</td>
<td>41 (39.42%)</td>
<td>7 (6.73%)</td>
<td>43 (41.34%)</td>
</tr>
<tr>
<td>ASC-US</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (0.96%)</td>
<td>1 (0.96%)</td>
</tr>
<tr>
<td>ASC-US, AGC-NOS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (0.96%)</td>
<td>1 (0.96%)</td>
</tr>
<tr>
<td>ASC-H</td>
<td>2 (1.92%)</td>
<td>1 (0.96%)</td>
<td>3 (2.88%)</td>
<td>0</td>
<td>2 (1.92%)</td>
</tr>
<tr>
<td>ASC-H, AGC-NOS</td>
<td>1 (0.96%)</td>
<td>1 (0.96%)</td>
<td>2 (1.92%)</td>
<td>0</td>
<td>1 (0.96%)</td>
</tr>
<tr>
<td>LSIL</td>
<td>0</td>
<td>3 (2.88%)</td>
<td>3 (2.88%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LSIL, AGC-NOS</td>
<td>2 (1.92%)</td>
<td>0</td>
<td>2 (1.92%)</td>
<td>3 (2.88%)</td>
<td>5 (4.80%)</td>
</tr>
<tr>
<td>HSIL</td>
<td>11 (10.57%)</td>
<td>3 (2.88%)</td>
<td>14 (13.46%)</td>
<td>1 (0.96%)</td>
<td>12 (11.53%)</td>
</tr>
<tr>
<td>HSIL, AGC-NOS</td>
<td>2 (1.92%)</td>
<td>3 (2.88%)</td>
<td>5 (4.80%)</td>
<td>5 (4.80%)</td>
<td>7 (6.73%)</td>
</tr>
<tr>
<td>HSIL, AIS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (0.96%)</td>
<td>1 (0.96%)</td>
</tr>
<tr>
<td>AGC-NOS</td>
<td>23 (22.11%)</td>
<td>9 (8.65%)</td>
<td>32 (30.76%)</td>
<td>6 (5.76%)</td>
<td>29 (27.88%)</td>
</tr>
<tr>
<td>AGC-FN</td>
<td>1 (0.96%)</td>
<td>1 (0.96%)</td>
<td>2 (1.92%)</td>
<td>1 (0.96%)</td>
<td>2 (1.92%)</td>
</tr>
<tr>
<td>Total of cases</td>
<td>78 (75%)</td>
<td>26 (25%)</td>
<td>104 (100%)</td>
<td>26 (25%)</td>
<td>104 (100%)</td>
</tr>
</tbody>
</table>

CC: Conventional cytology; LBC: Liquid Based Cytology; NILM: Negative for intraepithelial lesion or malignancy; ASC-US: Atypical squamous cells of undetermined significance; AGC-NOS: Atypical glandular cells not otherwise specified; ASC-H: Atypical squamous cells – cannot exclude high-grade squamous intraepithelial lesion; LSIL: Low-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial lesion; AIS: Adenocarcinoma in situ; AGC-FN: Atypical glandular cells, favor neoplasia.

Table 2 – Synopsis of discordant diagnoses

<table>
<thead>
<tr>
<th>Bethesda diagnosis in CC</th>
<th>Bethesda diagnosis in LBC</th>
<th>Cases (#)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upgrade diagnostic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NILM</td>
<td>ASC-US, AGC-NOS</td>
<td>1</td>
<td>Atypia identification without categorization in a type of squamous lesion; identification of non-specific glandular lesion.</td>
</tr>
<tr>
<td>NILM</td>
<td>LSIL, AGC-NOS</td>
<td>2</td>
<td>Low-grade squamous lesion identification; identification of non-specific glandular lesion.</td>
</tr>
<tr>
<td>NILM</td>
<td>HSIL</td>
<td>1</td>
<td>High-grade squamous lesion identification.</td>
</tr>
<tr>
<td>NILM</td>
<td>AGC-NOS</td>
<td>1</td>
<td>Glandular non-specific lesion identification.</td>
</tr>
<tr>
<td>ASC-H</td>
<td>AGC-NOS</td>
<td>1</td>
<td>Refinement of cellular details providing a different categorization of the lesion (glandular versus squamous).</td>
</tr>
<tr>
<td>LSIL</td>
<td>LSIL, AGC-NOS</td>
<td>1</td>
<td>Identification of associated non-specific glandular lesion.</td>
</tr>
<tr>
<td>HSIL</td>
<td>HSIL, AIS</td>
<td>1</td>
<td>Identification of associated high-grade glandular lesion.</td>
</tr>
<tr>
<td>AGC-NOS</td>
<td>AGC-FN</td>
<td>1</td>
<td>Refinement of cellular details providing a glandular lesion upgrade.</td>
</tr>
</tbody>
</table>
Bethesda diagnosis in CC | Bethesda diagnosis in LBC | Cases (#) | Remarks
---|---|---|---
AGC-NOS | HSIL, AGC-NOS | 4 | High-grade squamous lesion identification associated to glandular lesion.
AGC-FN | HSIL, AGC-NOS | 1 | High-grade squamous lesion identification associated to non-specific glandular lesion.

**False positive results removal**

<table>
<thead>
<tr>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exclusion of squamous and glandular lesion.</td>
</tr>
<tr>
<td>Squamous lesion categorization in non-specific type.</td>
</tr>
<tr>
<td>Squamous lesion exclusion.</td>
</tr>
<tr>
<td>High-grade squamous lesion exclusion; identification of a non-specific glandular lesion.</td>
</tr>
<tr>
<td>Squamous and glandular lesions exclusion.</td>
</tr>
<tr>
<td>High-grade squamous lesion exclusion.</td>
</tr>
<tr>
<td>Non-specific glandular lesion exclusion.</td>
</tr>
</tbody>
</table>

CC: Conventional cytology; LBC: Liquid Based Cytology; NILM: Negative for intraepithelial lesion or malignancy; ASC-US: Atypical squamous cells of undetermined significance; AGC-NOS: Atypical glandular cells not otherwise specified; LSIL: Low-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial lesion; AIS: Adenocarcinoma in situ; AGC-FN: Atypical glandular cells, favor neoplasia; ASC-H: Atypical squamous cells – cannot exclude high-grade squamous intraepithelial lesion; HPV: Human papillomavirus.

Figures 1–8 illustrate similar lesions, in conventional cytology and LBC, respectively.

The diagnosis has been NILM (negative for intraepithelial lesion or malignancy) associated with reactive cellular changes in 41 (39.42%) cases of conventional cytology, but in five of these cases (12.19% of NILM cases) squamous intraepithelial lesions have been identified (one case of HSIL – high-grade squamous intraepithelial lesion), or glandular lesions (one case of AGC-NOS – atypical glandular cells not otherwise specified), squamous atypia associated to glandular lesions (one case of ASC-US – atypical squamous cells of undetermined significance, associated to AGC-NOS and two cases of LSIL – low-grade squamous intraepithelial lesion associated to AGC-NOS).

From the three cases of ASC-H (atypical squamous cells – cannot exclude high-grade squamous intraepithelial lesion) in conventional cytology (2.88%), one case (33.33% of ASC-H cases) has been diagnosed in LBC as a glandular lesion (AGC-NOS).
One case (50%) from the two cases diagnosed with ASC-H associated with AGC-NOS, in conventional cytology (1.92%), has been diagnosed as NILM with reactive cellular changes in LBC.

Between the three (2.88%) cases diagnosed as LSIL in conventional cytology, a glandular non-specific lesion has been associated in LBC in one case (33.33% of total cases of LSIL), the other two (66.67%) cases being re-diagnosed as ASC-US and NILM, respectively. The two (1.92%) cases diagnosed as LSIL associated to AGC-NOS in conventional smear had correspondent diagnosis in LBC.

HSIL has been diagnosed in 14 (13.46%) cases by conventional cytology, 11 of these cases being diagnosed with the same diagnosis in LBC (78.57% of total HSIL cases). In one case (7.14% of total HSIL cases), LBC provided a diagnosis completion by the identification of a high-grade glandular lesion (AIS). The other two cases (14.28% of total HSIL cases) have been re-diagnosed as non-specific glandular lesions AGC-NOS, HSIL result being considered as false positive.

From the total number of 32 (30.76%) cases diagnosed as AGC-NOS in conventional cytology, in 23 (71.87%) cases a diagnostic correspondence has been revealed by monolayer cytology. LBC upgraded the diagnosis to AGC-FN (atypical glandular cells, favor neoplasia) in one case (3.12% of total AGC-NOS cases) and the identification of an associated high-grade squamous lesion – HSIL in four cases (12.5% of total AGC-NOS cases). In four cases (12.5% of total AGC-NOS cases), LBC excluded HSIL lesion, by exclusively revealing reactive cellular changes.

Among the two (1.92%) cases diagnosed as AGC-FN in conventional cytology, one case (50% of total AGC-FN) had been similarly evaluated in LBC but in the other case (50% of total AGC-FN cases), HSIL associated to AGC-NOS has been identified in LBC.

**Discussion**

Papanicolaou smear is the only screening test that resulted in a decrease of incidence and of mortality rate of a type of cancer, namely cervical cancer. The consensus between American Cancer Society, National Cancer Institute, American College of Obstetricians and Gynecologists and American Academy of Family Practice adopted in 1987 recommended smears to any woman over 18 years, which have been/are sexually active [20]. High-risk population (early sexual initiation, multiple
AGC-NOS has been achieved. The possibility of diagnosis completion of LSIL with LBC previously mentioned) with LSIL and ASC-US, respectively.

In the present study, by application of the both cytological methods in the same patients, we have noticed a supplementary detection of endocervical and high-grade squamous lesions in LBC, in 14 cases (13.46% from the total analyzed group of study), the coordinated cytological and histological follow-up being necessary for the confirmation of the results accuracy. In a correlative manner, the elimination of the material that prevented the cytological details, permitted in LBC the false positive diagnoses exclusion in 12 cases (11.54% of the total analyzed cases).

These results worth an evaluation in light of specific situations identified in our study, materialized in a different diagnosis by LBC examination. The diagnosis differences are given either to the identification of new lesions, either by excluding those established in conventional smear.

**Diagnosis differences by identification of new lesions in LBC versus conventional cytology**

It would be worth considering the fact that LBC offered the possibility of per primam identification, in three cases, of HSIL, mainly as one of these cases had been diagnosed as NILM. In the other two cases, HSIL completed the initial diagnosis of glandular lesion. LBC created the possibility of per primam diagnosis of LSIL and the identification of ASC-US, in two cases considered as NILM in conventional cytology.

The monolayer display resulted in cellular or nuclear overlaps removal, providing an exhaustive disclosure of the cytological details of the glandular component. Consequently, LBC identified per primam, in six cases (5.77% of total analyzed group), glandular cells atypia missed in conventional preparations. In three of these six cases, the diagnosis of conventional cytology has been of NILM, with the special mention that the non-specific glandular lesion has been associated in two cases (as previously mentioned) with LSIL and ASC-US, respectively.

The possibility of a clearer cyto-morphological visualization resulted, in a case, to a change of diagnosis categorization, from ASC-H to AGC-NOS. For another case, the possibility of diagnosis completion of LSIL with AGC-NOS has been achieved.

In this context, we may highlight the real advantages of LBC use in differentiation of a SIL, mainly of HSIL involving the subjacent endocervical glands, from AIS. Thus, LBC reveals the lack of central cell polarization in HSIL extended to endocervical cells, different from AIS. Moreover, in contrast with conventional smears, nucleoli may be visualized in HSIL, inside the glandular cells, in LBC, but not as prominent as in AIS. These situations require an increased attention, considering the fact of possible association between HSIL and AIS, as in our case. Not the last, we may mention the LBC potential in diagnosis refinement, by superior disclosure of cytological details, thus we could improve the diagnosis categorization of the glandular lesion, as AGC-FN instead AGC-NOS.

**Diagnosis differences by exclusion of some lesions in LBC versus conventional cytology**

The liquid-based preparation examination resulted in a complete exclusion of some diagnoses, which indicated either squamous lesions (high-grade or low-grade), either non-specific glandular lesions, either squamous (high-grade) lesions associated to non-specific glandular lesions. The removal of inflammatory and bloody background, associated to fixation artifacts decrease explain the change of NILM diagnosis in seven cases of conventional cytology to ASC-H, AGC-NOS (one case), LSIL (one case), HSIL, AGC-NOS (one case), and AGC-NOS (four cases). Other three cases benefited from a superior diagnostic categorization in a less severe lesion ASC-US instead LSIL (one case) and AGC-NOS instead HSIL associated to AGC-NOS (two cases). In two cases, AGC-NOS identification could replace HSIL previous diagnosis. A special mention worth highlight, as HSIL diagnosis has been removed in five cases and that of LSIL in two cases of the study group.

**Final remarks on the cytological diagnosis – between conventional and liquid based methods**

Researches and meta-analyses focused on the LBC cytology performances register a large variability of the results [8, 17, 21, 23–26]. The most reports in the main publication flow offer solid proofs, which certify the fact that LBC offers a significantly increased detection of high-grade lesions, in comparison to conventional Pap smears [26–34]. Moreover, numerous studies demonstrate that LBC sensibility is higher than conventional cytology for identification of ASC-US [24–26] and LSIL [31]. There are also divergent opinions, such as decreased predictive value of LBC compared to conventional cytology [26] due to a reduced frequency of abnormal features (usually low grade), without high grade CIN increase in histological diagnosis. Concerning the glandular cervical component, literature data support only subtle cytological and architectural differences of glandular neoplasia revealed by LBC in comparison to conventional cytology [29, 35, 36].

However, LBC advantages cannot be contested. Qualitatively superior material may be obtained due to the method of preparation resulting in a high facility of examination and interpretation, increases the specificity and the sensibility, and the laboratory efficiency, eventually [17, 19, 23].

The comparison of our results, obtained by comparative
analysis of the diagnoses given for conventional cytology and LBC revealed a high degree of diagnosis correspondence. In 78 from the total 104 (75%) analyzed cases, the diagnosis obtained by conventional smears examination has been identical with that established in LBC. Though, considering the number of cases corresponding to each diagnosis entity in conventional cytology, compared to LBC (Table 2), we may highlight the following particular features: (i) LBC resulted in the diagnosis of some entities missed in conventional cytology, namely: ASC-US (one case), ASC-US associated to AGC-NOS (one case), and HSIL associated to AIS (one case); (ii) LBC provided the identification of a higher number of cases of associated lesions, as LSIL and AGC-NOS (five cases versus two cases), and HSIL and AGC-NOS (seven cases versus five cases).

In our study, the lack of some remarkable differences between the results obtained by application of the two methods may be explained by the balance resulted from the supplementation of some diagnoses and exclusion of others. Overall, however, the necessity of highlighting once more the LBC utility is particularly revealed by the exclusion of LSIL and HSIL diagnoses.

Conclusions

LBC creates superior means for diagnosis of a greater number of squamous and glandular lesions compared to conventional cytology, decreasing the number of unsatisfactory results. Our experience and results support the simultaneous use of the two methods.

References

Conventional cytology versus liquid based cytology in cervical pathology: correspondences and inconsistencies...


**Corresponding author**

Irina-Draga Căruntu, Professor of Histology, MD, PhD, Department of Morphofunctional Sciences, “Grigore T. Popa” University of Medicine and Pharmacy, 16 University Street, 700115 Iassy, Romania; Phone +40727–003 700, e-mail: irinadragacaruntu@gmail.com

Received: March 28, 2014

Accepted: December 5, 2014