EVALUATION OF PAP-TEST PERFORMANCE BY A
CYTO-HISTOPATHOLOGICAL AND IMMUNOCYTOCHEMISTRY
STUDY WITH THERAPEUTIC IMPLICATIONS

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Summary
In our study we evaluated on a group of 321 patients, the cytological results obtained
by the conventional Papanicolaou method, and compared them with the histological results
from the colposcopic guided biopsy, along with the immunocytochemical detection of HPV
L1 capsid protein, as a prognostic marker. In order to determine the correlation of different
immuno-cyto-histological categories and the performance evaluation of Pap-test in
identifying squamous intraepithelial lesions, we considered biopsy as the "gold standard" and
used the following screening parameters: sensitivity (Se), specificity (Sp), positive predictive
value (PPV), accuracy (Acc) cytological diagnosis. Our study found 10.26% false-negative
results. The rate of dysplasia has high values in histological HSIL detection. Between the
sensitivity and specificity of cervical cytology there is a high negative correlation. The
positive predictive value increases with the cytological interpretation threshold. The accuracy
of Pap-smear test records moderate values. All cytological categories provided L1+ immuno-
colorings, but with histological CIN1 and CIN2 strict type correspondent. The CIN3
histological category was provided by immunocytochemical colorings L1-. Considering the
detection of the viral capsid as prognostic marker, it was subsequently used for the arbitration
of therapeutic attitude.

Keywords: Pap test, cyto-histological correlations, immunocytochemistry.

Introduction
The standard Papanicolaou test (conventional method) is most commonly
used test in cytopathology laboratories for
detection of cervical cancer and its
precursors. One of the frequent
mislconceptions of the public, but also of
many physicians, is that the Papanicolaou
test is an accurate diagnosis because the
examination of cytological smears is always
associated with a more or less risk of error.
The comparison of cytological results with
the histopathologic ones is essential in
cytopathology laboratories and is one of the
recommendations of the European
guidelines for their quality assurance, for
the development of laboratory performance
and, in particular, to reduce false-negative
results (Wiener et al., 2007).

Done alone, the Pap test showed
moderate accuracy, which is why it does
not have a high sensitivity and specificity
(Nanda et al., 2000). Cytological methods
in cervical screening have a sensitivity
ranging from 11% to 99% with an average
of 58% and specificity between 14% and
97% with an average of 69%, often a high
specificity being associated with a low
sensitivity (Fahey et al., 1995).

Understanding the molecular biology
of human Papillomavirus infection and the
mechanism involved in the apparition of
cervical cancer, contributed to the
development of molecular tests, which, combined with the microscopic
examination of Pap-test, ensures an increase in sensitivity of cervical screening tests (Doorbar and Cubie, 2005). Prognostic biomarkers are useful in differentiating patients with aggressive management recommendation or not, thus ensuring their quality of life and preserving the health system resources (Hilfrich and Hariri, 2008).

We plan to evaluate, on a group of 321 patients, the cytological results obtained by conventional Papanicolaou smear, that we will compare with the histopathological of colposcopic guided biopsies, along with immunocytochemical detection of HPV L1 capsid protein as prognostic marker. For the evaluation of Pap-test performance we will consider biopsy as the "gold standard" and the detection of viral capsid will influence therapeutic management.

Material and Methods
Our study was conducted in a private cytopathology laboratory in Slatina, in 2007-2010, on a subset of 321 patients, with the average age of 34.5 years, from who were collected cervical cytology smears before colposcopy procedures. For each patient, an additional smear was collected, for the immunocytochemistry study with Viroactiv HPV-HR Kit. Subsequently, in patients with clinically suspicious lesions were harvested by biopsy or diathermalexcision tissue fragments, colposcopically directed. In cases where colposcopy was unsatisfactory, endocervical curettage was performed. Histopathological diagnosis was making in the Pathology Service of the County Emergency Hospital of Slatina.

The collected slides for cytological examination were rapidly included in fixing ethanol solution from 15 minutes to 24 hours. They were stained by the Papanicolaou method with the Merck kit KGaA 64271), Darmstadt, Germany.

On the slides fixed in alcohol, sampled for immunocoloring, for the detection of the major L1 capsid protein, we used materials supplied in the kit, following the working protocol described by the manufacturer Viroactiv & Virofem, Diagnostics GmbH, Wiesbaden, Germany).

The collected tissue fragments were fixed in formaldehyde 10%, processed by classical histological methods and stained with hematoxylin-eosin.

The interpretation of cytological results was descriptive done in the Bethesda System terminology 2001 (Solomon and Nayar, 2004), which includes the following entities: NILM - negative for intraepithelial lesion or malignancy, ASC-US - atypical squamous cells of undetermined significance, ASC-H - atypical squamous cells that cannot exclude high-grade lesion, LSIL - low grade intraepithelial lesion, LSIL-H - low grade intraepithelial lesion that cannot exclude high-grade lesion, HSIL – high grade cervical squamous intraepithelial lesion.

The immunocolored slides with at least a red nucleus indicate a positive immunochemical reaction of the sample. The immunocoloring results were reported as positive or negative and were correlated with positive-control slides and the cytohistological data.

Histological sections were gradually evaluated, depending on the pattern of cervical squamous lesions severity, combining the Bethesda nomenclature with Richart nomenclature: LSIL CIN1), HSIL CIN2, CIN3).

The Pap-test performance in detecting squamous intraepithelial lesions by cytohistological correlation analysis was evaluated using the following definitions, used as cytological screening parameters: true positive cytological results Ap) - positive cytological result corresponding to the positive biopsy diagnosis, cytological negative true result An) - negative cytological result, according to the biopsy negative result, false positive cytological result Fp) - positive cytological diagnosis with a negative biopsy diagnosis; false negative cytology result Fn) - negative
cytological diagnosis with a positive biopsy diagnosis.

For the evaluation of Papanicolaou test performance in detecting CIN2 + lesions on the 321 cytomorphological combinations, we considered diagnostic discordance between cytology and biopsy, when one of the two tests provided CIN2 + lesion and the other test a lesion less severe, and we used the screening parameters mentioned above, at different thresholds of cytological interpretation.

Sensitivity Se) requires the cervical cytology ability to correctly identify the presence of cervical lesions or malignancy in the biopsy; we calculated sensitivity by dividing the true positive results to the sum of the true positive and false negative results \( \left( \frac{Ap}{Ap + Fn} \times 100 \right) \).

Specificity Sp) – the ability of cervical cytology to correctly identify the absence of lesions or cancer on biopsy; we calculated the specificity by dividing the true negative results by the sum of true negative and the false positive results \( \left( \frac{An}{An + Fp} \times 100 \right) \).

Positive predictive value PPV) - the ability of the cervical cytology to predict the lesions in the cervical biopsy; we calculated the PPV by dividing the true positive results by the sum of true positive and false positive results \( \left( \frac{Ap}{Ap + Fp} \times 100 \right) \).

Diagnostic accuracy Acc) - is the proportion of all cytology results, both positive and negative, that are correct, representing the ratio of the sum of true positive, true negative, false positive and false negative results \( \left( \frac{Ap + An}{Ap + An + Fp + Fn} \times 100 \right) \).

Results

The cytological examination performed in the 321 studied patients provided the following results: 39 NILM cases, 154 ASC-US results, 25 ASC-H cases, 76 LSIL smears, 8 LSIL-H cases and 19 HSIL smears. The histological results for these categories are described in Table 1.

Table 1. Histological results for cytological categories studied

<table>
<thead>
<tr>
<th>Cytological category</th>
<th>№</th>
<th>Histologically negative</th>
<th>LSIL/CIN1</th>
<th>HSIL/CIN2</th>
<th>HSIL/CIN3</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLIM</td>
<td>39</td>
<td>35</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>ASC-US</td>
<td>154</td>
<td>94</td>
<td>36</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>ASC-H</td>
<td>25</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>LSIL</td>
<td>76</td>
<td>9</td>
<td>46</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>LSIL-H</td>
<td>8</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>HSIL</td>
<td>19</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>total</td>
<td>321</td>
<td>144</td>
<td>97</td>
<td>56</td>
<td>24</td>
</tr>
</tbody>
</table>

In the studied group, the NILM interpreted cytology provided two CIN1 lesions 2/39, 5.13%) and two CIN2 lesions 2/39, 5.13%), the rest were histologically negative 35/39, 89, 74%) .10.26% of NILM smears were false negative for CIN interpretation threshold. In patients with ASC-US cytology the histological examination revealed: 94/154 61.04%) cases with reactive benign morphology, associated with chronic cervicitis or endocervical polyp, 36/154 23.38%) CIN1 cases, 20/154 12.99%) CIN2 cases, respectively 4/154 2.59%) CIN3 cases. Dysplasia rate was 38.96% for CIN CIN1-23, 38%, CIN2-12, 99%, CIN3-2, 59%.

In the ASC-H category there were recorded 6/25 24%) negative histological
results, 5/25 20%) CIN1 results, 6/25 24%) CIN2 diagnosed and 8/25 32%) CIN3 cases, that were 56% CIN2 +. The detection rate of CIN in patients with ASC-H cytology is 76% CIN1 - 20%, CIN2 CIN3 - 56%.

Patients with LSIL cytology have provided 9/76 11.84%) negative histology, 46/76 60.52%) CIN1, 20/76 26.31%) CIN2 and 1/76 1.32%) CIN3 with 88.15% dysplasia rate for CIN and of 27.63% for CIN2 +. In patients with LSIL-H cytology, histologically there were found 3/8 37.5%) CIN1 lesions, 4/8 50%) CIN2 and 1/8 12.5%) CIN3, with 100% rate of dysplasia for CIN and 62.5% for CIN2 +. HSIL patients provided 5/19 26.31%) CIN1 lesions, 4/19 21.05%) CIN2 lesions and 10/19 52.63%) CIN3 lesions with 100% dysplasia rate for CIN and 73.68% for CIN2+

In a first analysis, we investigated the ability of any abnormal cytology, ASC-US/ASC-US +, to histologically detect CIN2 +. True positive cases were defined as those with cytological interpretation as ASC-US+ and CIN2 + on biopsy. The true negative ones as cytological NILM and histological less than CIN2 +, false positives as ASC-US+ cytology and less than CIN2 + on biopsy and false negative for NILM cytology and CIN2 + histology. Using these definitions, we found results Ap, An, Fp and Fn at values of 78, 37, 204 and 2, respectively. Calculating Se, Sp, PPV and Acc for the LSIL+ cytology threshold in detecting CIN2 + histology, we found the following values: 67.5%, 69.29%, 42.18%, 68.84%.

In the third analysis, for the HSIL cytological interpretation threshold, we removed combinations of equivocal categories, like ASC-US and ASC-H and LSIL-H smears were included in HSIL. Thus, the results HSIL/CIN2 +, present in both the Pap-test and cervical biopsy, we considered truly positive; the NILM cytological and nonCIN histological results - true negative; in the group of false positive results, we included the HSIL cytology results with histological interpretation less severe than CIN2 +, for example CIN1 or nonCIN ,and false negative results when cytologically expressed NILM or LSIL and histological CIN2 +. We thus calculated, the results Ap, An, Fp and Fn with values of 19, 35, 8 and 23. Calculating Se, Sp, PPV and Acc for the HSIL cytological threshold interpretation in identifying CIN2 + histology, we found the following values: 45.24%, 81.39%, 70.37% and 63.52% Table 2, Figure 1).

<table>
<thead>
<tr>
<th>Definition thresholds of Pap-test</th>
<th>ASC-US</th>
<th>LSIL</th>
<th>HSIL</th>
</tr>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>97.5%</td>
<td>67.5%</td>
<td>45.24%</td>
</tr>
<tr>
<td>Specificity</td>
<td>15.36%</td>
<td>69.29%</td>
<td>81.39%</td>
</tr>
<tr>
<td>PPV</td>
<td>27.66%</td>
<td>42.18%</td>
<td>70.37%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>35.82%</td>
<td>68.84%</td>
<td>63.52%</td>
</tr>
</tbody>
</table>

Table 2. Pap-test performance in detecting CIN2 + with different thresholds of cytological definition
Figure 1. Graphical representation of Se, Sp, PPV at different thresholds of cytological interpretation

Immunocytochemical staining of smears obtained from the 321 patients to identify the prevalence of L1 capsid protein, provided the following results (graphic no.2):

- in the NILM category out of 39 cases, 4 were L1+, 2 with CIN1 histology, 2 with CIN2 histology;
- for the ASC-US category out of 154 cases, 20 were L1+, 16 with CIN1 biopsy correspondent, 4 cases with CIN2 histology;
- from 25 ASC-H cytology cases only 3 were L1+, 1 case with CIN1 histology, and 2 - with CIN2 histology;
- in the LSIL cytology category out of 76 cases, 23 were L1+, 20 corresponding with CIN1 histology and 3 with CIN2 histology;
- the category LSIL-H out of 8 immunocolored cases, 3 were L1+, all corresponding to CIN1 biopsy;
- out of 19 HSIL cytology cases, 3 were L1+ from CIN1 histological category.

We observed on immunocolored smears, red nuclei, at the squamous intermediate cells Figure 3.1, 3.2) or at metaplasia cellular level, of basaloid or parabasaloid cells Figure 3.3, 3.4).

Figure 2. Graphical representation of the number of smears L1+ / L1-

Figure 3. Smears CIN1 L1+ (1,2); Smears CIN2 L1+ (3,4)
144 histologically negative smears were L1-. Out of the 97 cases histologically confirmed CIN1, 46.39% (45/97) of cytology cases were L1+, 53.61% (52/97) were L1-. 19.64% (11/56) of CIN2 histology cases, were cytologically associated with L1+, the majority, 80.36% (45/56) were cytologically L1-. All cytology categories 24/24, 100%) with CIN3 histology were L1-.

Discussion

The rate of false-negative results is a continuous stress even in the best cytopathology laboratories, the purpose of many quality control actions being the reduction, if possible, of the laboratory error rates (Beeby et al., 1993).

In various international studies, there is a wide variation in false-negative reported results, from 6-55% (Shingleton et al., 1995). In our study, 10.26% of NILM cytology cases were false negative, which puts us in an intermediate position as compared to many other studies (Berkowitz et al., 1979; Alawattegama, 1984; Gay et al., 1985; van der Graaff et al., 1997).

The rate of dysplasia in ASC-US patients is reported in the literature, with large variations from 23 to 51%, or from 13.5% to 61.3% (Grenko et al., 2000). In our patients, the dysplasia rate for the detection of CIN2+, has a higher value as compared to many other studies (Berkowitz et al., 1979; Alawattegama, 1984; Gay et al., 1985; van der Graaff et al., 1997).

In ASC-H patients, some studies report a histologic HSIL detection rate between 25-90% (Barreth et al., 2006) and others between 26-68% the variability explanation being the different thresholds of ASC-H and HSIL cytomorphological interpretation (Rekhi et al., 2010). We observed a detection rate of 56% HSIL, which confirms that ASC-H is associated with a significantly higher risk of HSIL as compared to ASC-US.

The cytological LSIL category provided the highest histological rate of CIN1 of all studied categories, and the association with CIN2 and CIN3 of 27.63% fall in the range 15-30% quoted in the specialty literature (Jones and Novis, 1996; Massad et al., 2001). Our results were slightly elevated as compared to other cytohistologic studies (Shidham et al., 2007; Alsharif et al., 2009; Hong et al., 2010; Ince et al., 2011).

In patients with LSIL-H cytology, we see an intermediate status between LSIL and HSIL, the rate of dysplasia to CIN2 + being of 62.5%, close to that of ASC-H 56%) and intermediate between LSIL 27, 63%) and HSIL 73.68%). Our observations are similar to those drawn from several recent studies that have documented this cytological category (Shidham et al., 2007; Alsharif et al., 2009; Owens et al., 2007).

The rate of dysplasia in our study, for HSIL cytology interpretation is similar to other international studies (Herbert et al., 1995; Jones and Novis, 2000), but higher than other reports (Massad et al., 2001; Sherman et al., 2001; Dunn et al., 2003; Alvarez et al., 2007).

One of the objectives of our study was to evaluate the performance of conventional cytology in detecting CIN2 + histology threshold at different thresholds for cytological interpretation. Our study found high negative correlation between sensitivity and specificity of cervical cytology.

There are studies that at the same threshold of cytological interpretation ASC-US +) show a sensitivity similar to that in our study, but with much greater specificity than the one reported by us (Jones and Novis, 1996; DiBonito et al., 1993; Anschau and Guimarães Gonçalves, 2011). As with other reports, once with increasing cytological threshold, the values become comparable (Abali et al., 2011). There are studies that find, at all thresholds of cytological interpretation, values of sensitivity, specificity, and positive
predictive value, similar to those reported by us (Carns and Fadare, 2008). In agreement with other reports, the positive predictive value increases with increasing cytological threshold interpretation (Abali et al., 2011). As with other studies (Massad et al., 2001; Kim et al., 2003), the accuracy records moderate values, that increase with the dysplasia cytological threshold, but which are lower than values reported by other authors (Saha and Thapa, 2005). In our study, the highest accuracy value we recorded at the cytologic LSIL+ threshold.

It turned on prospective studies that immunodetection of HPV-L1 can be postulated also on borderline cytology, such as ASC or LSIL-H or on CIN2 cytology as part of HSIL, so that mild and moderate L1+ dysplastic lesions reflect a low progression risk, and mild and moderate L1- lesions, as unproductive viral infections or precancerous lesions have a high potential for progression, similar to CIN3CIS (Griesser et al., 2009; Xiao et al., 2010). There are authors who, in a certain percentage of cases of 27.6%, observe lesion progression even in the presence of HPV-L1 (Hilfrich and Hariri, 2008), as there are studies in which a small number of cases, 5.2%, although L1– showed lesion remission (Griesser et al., 2009).

In our study, 144 nonCIN histologically corresponding smears, provided for most cytologic categories were negative immunocolored. It has been shown that even this situation is no evidence that benign lesions are not infected with HPV (Hilfrich and Hariri, 2008). Patients with nonCIN L1- histology without HPV infection), were recommended for routine screening smear test every year). 46, 39% of CIN1 histologically confirmed cases were found to be L1 +, which allowed us to anticipate regression of these lesions. Both in these patients HPV infection with good prognosis) there was recommended annual cytological examination. In CIN1 L1- patients HPV infection with potential for progression) – there was recommended cytological examination at 6 months.

19.64% of CIN2 lesions were cytologically associated with L1 +, thus a relatively small number of cases in this category have potential for remission, while the majority of cases 80.36%) have a high potential for progression. In CIN2 L1+ patients HPV infection with good prognosis) there was recommended the closely follow-up with cytological examination at 3-6 months, possibly colposcopic examination and histological verification. In CIN2 L1- patients HPV infection with potential progression) there was recommended the excisional treatment so as to prevent progression.

In our study, no smear with CIN3 biopsy correspondent was positively immunocolored which indicates a risk of progression of 100% of these lesions. In CIN3 L1- patients, it was recommended excisional or ablative treatment, depending on the colposcopy cartogram.

Conclusions

This study was conducted on a total of 321 patients, tested combined for cytohistopathology and immunocytochemistry, in order to evaluate the performance of Pap-test in capturing CIN and in particular of CIN2 + when the lesion exists and to identify the HPV-L1 viral capsid as a prognostic marker in the evolution of the lesions, with the therapeutic target and subsequent follow-up of the patient. This study, like many others, show that the Pap test is imperfect.

Although both Pap-test and biopsy were collected in the same day by the same colposcopist and evaluated by the same pathologist, the rate of false-negative results was of 10.26%. The dysplasia rate for different thresholds of cytological interpretation had increased values, especially in detecting the histological HSIL. Our study found highly negative correlation between sensitivity and specificity of cervical cytology: sensitivity increases substantially by lowering the
threshold for cytological interpretation, with a significant cost of specificity and vice versa. Positive predictive value increases with increasing the threshold for cytological interpretation.

The Pap-test accuracy in predicting the histological diagnosis records moderate values, the highest value being registered at LSIL + cytology threshold.

All cytological categories provided positive immunocolored, L1 +, but with CIN1 and CIN2 histological strict type correspondent. CIN3 histological category was provided by negative L1-immunocoloring. Considering the detection of viral capsid as prognostic marker, it was used later for the arbitration of therapeutic attitude.

References


