Performance of Conventional Cytology and Colposcopy for the Diagnosis of Cervical Squamous and Glandular Neoplasias

Desempenho da citologia convencional e da colposcopia para o diagnóstico de neoplasias cervicais escamosas e glandulares

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Abstract

Objective To estimate the cytological and colposcopic performances for the diagnosis of cervical neoplasias.

Methods Cross-sectional retrospective study with data from patients’ charts. The participants underwent colposcopy, guided biopsies, and excision when needed. The cytological and colposcopic categorization followed the Bethesda System and the international colposcopic terminologies. The cytology and colposcopy performances were evaluated by sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) analyses with 95% confidence interval (95% CI).

Results From 1,571 participants, a total of 1,154 (73.4%) were diagnosed with cervical squamous intraepithelial neoplasia grade 2 or worse (CIN 2+), 114 (7.2%) with adenocarcinoma in situ or worse (AIS+), 615 (39.2%) presented atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion or worse (ASC-H+) cytology, and 934 (59.4%) presented major or suspicious for invasion colposcopic abnormalities. The SE, SP, PPV, and NPV of ASC-H+ for diagnoses of CIN 2+ and AIS+ were, respectively: 44% (95% CI: 41–47) and 72% (95% CI: 67–76), 79% (95% CI: 77–81) and 79% (95% CI: 75–83), 88% (95% CI: 87–90) and 55% (95% CI: 50–60), and 28% (95% CI: 26–31) and 88% (95% CI: 85–91). The SE, SP, PPV, and NPV of major or suspicious for invasion colposcopic abnormalities for diagnoses of CIN 2+ and AIS+ were, respectively: 62% (95% CI: 60–65) and 86% (95% CI: 83–89), 59% (95% CI: 57–62) and 59% (95% CI: 55–64), 85% (95% CI: 83–87) and 44% (95% CI: 40–49), and 29% (95% CI: 27–32) and 92% (95% CI: 89–94).

Conclusion The SE analyses results of ASC-H+ and major or suspicious for invasion colposcopic abnormalities were higher for diagnoses of glandular neoplasias. These results confirm the role of cytology in identifying women at risk who will have their final diagnoses settled by colposcopy and histology.
Introduction

Well organized invasive cervical cancer (ICC) screening programs based on cytology, colposcopically-guided biopsies, and treatment of precursor neoplasias have led to an important decrease in the ICC incidence and mortality. However, none of these programs could eradicate cervical cancer in any part of the globe.\(^1\) In fact, evidences have shown that in the last decades, the incidence of cervical adenocarcinoma (AC) has risen, especially in younger women, denoting the lack of impact of these programs for this particular histological type.\(^2\)

Although the triage of patients at risk for ICC precursor lesions is based on cytological abnormalities, these do not have adequate specificity to indicate treatment to all women with such findings.\(^3\) Therefore, colposcopically-guided biopsy was added to the system aiming to select which women with abnormal cytology would actually need treatment.\(^4\)

Recently, as expected, a decrease in the prevalence of cervical intraepithelial neoplasias has been observed in developed countries due to high human papillomavirus (HPV) vaccination coverage.\(^5\) It is possible that in vaccinated populations, the neoplasias that will still be found will show more subtle appearance and smaller sizes.\(^6\) At the same time, a better diagnostic performance has been reached with the introduction of new screening programs with high sensitivity, which added DNA detection methods to cytology, resulting in a decrease in false-negatives,\(^6\) and an improvement in cytological detection rates, even for more discrete neoplasias. Nonetheless, knowledge about colposcopy performance is still needed, especially regarding the recognition of subtle neoplasias,\(^6\) which could be missed since colposcopic criterion has not been updated to the new scenario.

The histopathological diagnosis of specimens obtained using colposcopically-guided biopsy has been traditionally considered the gold standard for cytological and colposcopic analyses. However, this assumption has an intrinsic flaw.\(^4\) Any mistakes made when choosing the site to take the biopsies, due to a misinterpretation of the colposcopic images, would necessarily lead to a bias, compromising the results of these analyses. This study estimated cytological and colposcopic performance to predict the final diagnosis of squamous and glandular neoplasias and determine the performance of these diagnostic tests used in clinicians’ daily practice, considering that the final diagnosis was based on excisional specimens.

Methods

This cross-sectional epidemiological study was based on data collected over a period of 24 years, from April 16, 1991 to November 26, 2015, in a private colposcopy health unit in...
Goiânia, GO, Brazil. The project was approved by the Research Ethics Committee of the Hospital das Clínicas of the Universidade Federal de Goiás (CAAE no. 58228016.1.0000.5078).

A total of 11,999 medical records of patients referred to colposcopy were reviewed. Among them, 1,527 participants were selected for having their final diagnoses settled by histopathological analyses of transformation zone excision (TZE) pieces, 7 by analysis of cold knife conization (CKC) pieces, and 37 with invasive cervical neoplasias diagnosed in the initial biopsy fragment. Therefore, the final sample was composed of 1,571 participants.

All the patients without a histopathological analysis of an excisional specimen were excluded from the study, even if they had cytological abnormalities, and regardless of whether they had normal or abnormal histopathology. Exceptions were made only in the cases with stromal invasion observed in the initial biopsy fragment, because once invasion is found, no worst diagnosis is possible, making a subsequent excisional procedure unnecessary in most cases.

The data obtained from patients’ charts, colposcopic reports, and computer software Diagnose Pro 6, Ginecologia e Obstetrícia, prontuário eletrônico e captura de imagens, (LPT4 sistemas de informação, Curitiba, Paraná, Brazil) and Zscan 7 Gineco, version 7.4 (Zscan Software, 2001-2016, Goiânia, Goiás, Brazil) image files were coded and kept on a 2013 Excel spreadsheet (Microsoft Corp., Redmond, WA, USA). The cytological, colposcopic, and histopathological data included referral cytology, colposcopic findings, visualization of squamocolumnar junction (SCJ), the histopathological diagnosis of an excision piece or a hysterectomy piece, and the histopathological report of biopsy fragments.

The cytological abnormalities were classified as proposed by the Bethesda terminology, updated in 2014:7 atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H), high-grade squamous intraepithelial lesion (HSIL), squamous cell carcinoma (SCC), atypical glandular cells (AGC), adenocarcinoma in situ (AIS), and adenocarcinoma (AC).

The ASC-H group had a cut-off point settled in the cytological results of ASC-H or worse, a threshold in which the patients’ management changes to immediate referral to colposcopy rather than the mere cytological follow-up.8 This group included all patients with cytological abnormalities classified as ASC-H, HSIL, SCC, AIS, and AC.

A single colposcopist performed the exams using at first a 5-fold DFV videocolposcope (D. F. Vasconcellos, Valença, RJ, Brazil) and afterward a Medpej PE 7000 MDL videocolposcope also with five levels of magnification (6x, 10x, 15x, 25x and 40x). Initially 5% and 10% acetic acid solutions were applied followed by the spraying of Schiller’s solution, at this point, the needed biopsies were taken with Gaylor-Medina forceps. Endocervical curettings with a Kevorkian curette were performed whenever necessary.

The colposcopic images were reviewed by the examiner and the 2011 International Federation of Cervical Pathology and Colposcopy (IFCPC)9 terminology was used to group them as follows: normal, minor, major findings, major findings, or suspicious for invasion. Minor findings elementary images included: fine mosaic, fine punctuation, and thin acetowhite epithelium with geographic borders. Major findings included: coarse mosaic, coarse punctuation, and dense acetowhite epithelium with sharp border, with or without ridge and inner border sign, and also cuffed crypt openings. The images considered suspicious for invasion included: atypical or fragile vessels, irregular surface, exophytic lesions, necrosis, necrotic ulceration, and gross neoplasm.9

Moreover, new colposcopic images similar to those described by Wright et al10 were added to the major findings category. It is necessary to emphasize that these images are not accredited by IFCPC terminology.

The cut-off point of colposcopic images was settled at images worse than minor findings, since taking biopsies from this type of findings is considered needless by many colposcopists. Thus, the colposcopic findings were sorted into two groups: 1) normal and minor findings; 2) major findings and suspicious for invasion findings.

A Wavetronic 5000 Digital Hf Surgical Unit (Loktal Medical Electronics Ind. Com. Ltda, São Paulo, SP, Brazil) was employed to perform TZE under colposcopic guidance and local anesthesia, using its handswitch pencil and cord with loop electrodes, at 50% of the coagulation power and the shear power regulated to output 8. A single-fragment resection was performed unless a large transformation zone was present.

A single examiner performed all histopathological analyses and categorized the findings following the World Health Organization International Tumors Classification11 and the Richart Classification for cervical intraepithelial neoplasias.12 The final diagnosis was defined as the most severe histopathological diagnosis among specimens of excision or hysterectomy, except when invasion was already found in fragments of initial biopsies.

The data analysis was performed using the Statistical Package for Social Sciences (SPSS) for Windows version 21.0 (IBM Corp., Armonk, NY, USA). Descriptive analyses of the sociodemographic, behavioral, and clinical features as well as cytological abnormalities, colposcopic findings, and histopathological diagnosis were performed.

Diagnostic performance of cytological abnormalities and colposcopic findings to predict the final diagnosis were evaluated by analysis of sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV), with the respective 95% confidence interval (95% CI). Values of SE, SP, PPV, and NPV between 0.00 and 0.40 were considered as poor, between 0.40 and 0.60 as low, between 0.60 and 0.80 as moderate, and between 0.80 and 1 as high.

Results

The sociodemographic and behavioral characteristics of the participants in this survey are shown in Table 1. The mean age of the participants was 31.7 years and at the first intercourse, it was 18.9 years. A total of 615 (39.6%) patients out of 1,571 were referred to colposcopy due to cytological findings of atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion or worse (ASC-H).
Concerning the prediction of CIN 2\(^+\) and AIS\(^+\), they were moderate, whereas PPV and SP (79%) were high, respectively. Regarding glandular neoplasias, SE (72%) and SP (79%) of ASC-H\(^+\) were moderate, whereas PPV (88%) was high. To the best of our knowledge, to date, no studies have simultaneously measured the performance of cytological and colposcopic diagnoses of squamous and glandular neoplasias.

### Discussion

In the present study, the performances of cytological abnormalities ASC-H\(^+\) and colposcopic major or suspicious for invasion findings were evaluated to separately predict the final diagnoses of CIN 2\(^+\) and AIS\(^+\) in a sample of 1,571 participants. The final diagnosis was defined as the most severe report obtained among all biological specimens of patients who underwent excisional procedure, except when invasion was initially found in biopsy fragments.

The SE of ASC-H\(^+\) (44%) to identify squamous neoplasias was low; in contrast, SP (79%) and PPV (88%) were moderate and high, respectively. Regarding glandular neoplasies, SE (72%) and SP (79%) of ASC-H\(^+\) were moderate, whereas PPV (55%) was low. To the best of our knowledge, to date, no studies have simultaneously measured the performance of cytological and colposcopic diagnoses of squamous and glandular neoplasias.

The sensitivity of a test represents its ability to correctly identify unhealthy individuals, while its specificity shows the ability to identify the healthy ones. Therefore, the low sensitivity found implies that ASC-H\(^+\) may not sort out a reasonable number of patients with CIN 2\(^+\), since the false-negative rate was high (56%). Similarly, the SP of 79% obtained means that absence of ASC-H\(^+\) identifies a high number of patients that do not actually have CIN 2\(^+\).

Moderate SE (72%) and SP (79%) of ASC-H\(^+\) to identify glandular neoplasias mean that ASC-H\(^+\) involves lower rates of false-positives (28%) and false-negatives (21%). Analyzed individually, SE of cytology for the detection of glandular neoplasias means that absence of ASC-H\(^+\) identifies a high number of patients that do not actually have glandular neoplasias.

### Table 1 Sociodemographic and behavioral profile of 1,571 participants

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range</td>
<td>15–85</td>
<td></td>
</tr>
<tr>
<td>Mean (sd)</td>
<td>31.7 ± 10.8</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Single</td>
<td>817</td>
<td>52.0</td>
</tr>
<tr>
<td>Married</td>
<td>752</td>
<td>47.9</td>
</tr>
<tr>
<td>Age at first intercourse</td>
<td>Years</td>
<td>9–47</td>
</tr>
<tr>
<td>Mean (sd)</td>
<td>18.9 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>Lifetime sexual partners</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>≤ 2</td>
<td>624</td>
<td>39.7</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>756</td>
<td>48.1</td>
</tr>
<tr>
<td>Full-term pregnancy</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>≤ 1</td>
<td>1,107</td>
<td>70.5</td>
</tr>
<tr>
<td>&gt; 1</td>
<td>453</td>
<td>28.8</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Past and current smoker</td>
<td>148</td>
<td>9.4</td>
</tr>
<tr>
<td>Never smoker</td>
<td>1,073</td>
<td>68.3</td>
</tr>
</tbody>
</table>

**Abbreviation:** SD, standard deviation. Missing data: *2; b128; c192; d11; e350.

### Table 2 Cytological, colposcopic, and final diagnoses in 1,571 participants

<table>
<thead>
<tr>
<th>Colposcopy referral</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCUS/LSIL</td>
<td>796</td>
<td>50.7</td>
</tr>
<tr>
<td>ASC-H/HSIL</td>
<td>532</td>
<td>34.0</td>
</tr>
<tr>
<td>AGC</td>
<td>29</td>
<td>1.8</td>
</tr>
<tr>
<td>Adenocarcinoma in situ</td>
<td>22</td>
<td>1.4</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>16</td>
<td>1.0</td>
</tr>
<tr>
<td>Adenocarcinoma in situ</td>
<td>16</td>
<td>1.0</td>
</tr>
<tr>
<td>Other skip</td>
<td>153</td>
<td>9.7</td>
</tr>
<tr>
<td>Colposcopy findings</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Normal</td>
<td>9</td>
<td>0.5</td>
</tr>
<tr>
<td>Minor</td>
<td>618</td>
<td>39.3</td>
</tr>
<tr>
<td>Major</td>
<td>908</td>
<td>57.8</td>
</tr>
<tr>
<td>Suspicious for invasion</td>
<td>26</td>
<td>1.6</td>
</tr>
<tr>
<td>Final diagnosis</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>≤ CIN 1</td>
<td>303</td>
<td>19.3</td>
</tr>
<tr>
<td>CIN 2/CIN 3</td>
<td>1,124</td>
<td>71.6</td>
</tr>
<tr>
<td>Microinvasive squamous carcinoma</td>
<td>13</td>
<td>0.8</td>
</tr>
<tr>
<td>Squamous carcinoma</td>
<td>17</td>
<td>1.1</td>
</tr>
<tr>
<td>Adenocarcinoma in situ</td>
<td>78</td>
<td>5.0</td>
</tr>
<tr>
<td>Microinvasive adenocarcinoma</td>
<td>8</td>
<td>0.5</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>28</td>
<td>1.8</td>
</tr>
</tbody>
</table>

**Abbreviations:** AGC, atypical glandular cells; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion. a*cervical polip, cervical bleeding, unknown cytological finding, etc. b*missing data:10.
neoplasias has been reported in a wide range of values, such
as 43.1%\textsuperscript{14} and 91.2%\textsuperscript{15}. These results contrast with the
findings of this study, since most patients with glandular
neoplasias were found using ASC-H\textsuperscript{+} (72%) cytology, and
most patients without cytological reports of ASC-H\textsuperscript{+} (NPV
88%) were truly free of glandular neoplasias. For this reason,
when ASC-H\textsuperscript{+} is found, whether squamous neoplasias have
already been identified or not, it would be safer to exclude
the possibility of coexistent glandular neoplasias.

Sensitivity and SP are inherent properties of a test and do
not change. However, the predictive values depend on the
prevalence of the disease in the study sample.\textsuperscript{13} Therefore,
the PPV will proportionally increase according to the preva-
ience of the disease in the studied group. In the present
study, on one hand, the high prevalence of squamous neo-
plasias implied a high PPV of ASC-H\textsuperscript{+} for CIN 2\textsuperscript{+} detection,
because most participants with ASC-H\textsuperscript{+} actually had CIN 2\textsuperscript{+}.
On the other hand, the low prevalence of glandular neo-
plasias implied a low PPV of ASC-H\textsuperscript{+} ability to predict AIS\textsuperscript{+}.
Hence, most positive results of ASC-H\textsuperscript{+} do not correspond to
patients with glandular neoplasias, but rather to patients
with squamous neoplasias.

Evidences involving the performance of cytological ab-
normalities for predicting intraepithelial and invasive cervi-
cal neoplasias, whether squamous or glandular, were found
with SE ranging from 30 to 100%, and SP from 86.8 to
99.3%.\textsuperscript{16–29} This large divergence may be due to the diversity
of cut-off points chosen to consider the tests as positive or
negative, as well as to the use of different morphological
criteria to interpret cytological smears and classify abnormal
findings. Conversely, a study that used ASC-H\textsuperscript{+} as a cut-off
point to ascertain the performance for the detection of CIN 2\textsuperscript{+} reported SE of 67.9% and SP of 87%.\textsuperscript{30}

In this study, SE of major or suspicious for invasion
colposcopic findings was moderate (62%), while SP was low (59%). Although these values were different from each other, this difference was not statistically significant, since their confidence interval over-
lapped. The major or suspicious for invasion colposcopic
findings failed to identify a substantial number of patients
with CIN 2\textsuperscript{+}. Consequently, the absence of colposcopic
images showing major or suspicious for invasion findings
does not rule out a large number of healthy participants.

These results are in line with a previous study,\textsuperscript{3} and suggest
that guided biopsies are needed by women with cytological
abnormalities, even if they present subtle colposcopic find-
goings, that is, those colposcopic images classified as minor
findings by the IFCCPC. Taking multiple biopsies would also
be an acceptable strategy to improve the SE of colposcopy.\textsuperscript{31}

The SE of major or suspicious for invasion colposcopic
findings for the detection of AIS\textsuperscript{+} was high (86%), while the
SP and PPV were low (59% and 44%, respectively). This high SE
value found for the detection of AIS\textsuperscript{+} implies that the major or
suspicious for invasion colposcopic findings express low rates
of false-negatives for this singular histopathological type of
neoplasia. In view of these colposcopic images, whether
squamous neoplasias are suspected or not, the need to exclude
a possible coexistent glandular neoplasia still persists.

These results show that the SE values of colposcopic
findings were higher for the detection of squamous (62%)
and glandular (86%) neoplasias than the cytological findings of ASC-H+ (44% and 72% for squamous and glandular neoplasias, respectively). However, SP values of ASC-H+ (79% and 79%) were higher than those of colposcopic findings (59% and 59%) for the detection of these two main histopathological types of neoplasia. Moreover, the SE of major or suspicious for invasion colposcopic findings and ASC-H+ cytological findings were higher for glandular (86% and 72%) than for squamous neoplasias (62% and 44%).

The high SE of colposcopy for the detection of glandular neoplasias found in this study was probably due to the inclusion of new subtle images in the major colposcopic finding category. Such new images are similar to those identified by Wright et al10 as suggestive of glandular neoplasias and are not described in the International Colposcopic Classification of the IFCPC.9 They are herein named: obstructed dilated grouped glands, fused acetowhite vili with invaginated borders, and atypical vessels in cylindrical epithelium area (Fig. 1).

Considering that the PPV of ASC-H+ (88%) and major or suspicious for invasion colposcopic findings (85%) for the detection of CIN 2+ were high, the see and treat protocol, already endorsed by previous published evidences,32 is here corroborated as a secure option. The sequential use of two tests with high PPV prior to the see and treat protocol substantially decreases the probability of performing unnecessary excisional procedures in disease-free patients, mainly because both cytological and colposcopic findings must simultaneously be abnormal to indicate such management.

The possibility of finding an unexpected glandular neoplasia in a see and treat excision piece, estimated to happen in 52% of the patients with final diagnosis of AIS+ 33 would not contraindicate this approach. As a matter of fact, evidences have shown that outpatient excisional procedures are also suitable for the management of glandular neoplasias.33,34

**Conclusion**

The performance of cytological abnormalities is somehow different from that of colposcopic findings to predict the diagnoses of AIS+ and CIN 2+. Sensitivity of major or suspicious for invasion colposcopic findings for the diagnoses of CIN 2+ was moderate. The high PPV values found for ASC-H+ and major or suspicious for invasion colposcopic findings for the detection of CIN 2+ endorse the see and treat protocol. Sensitivity results of ASC-H+ and major or suspicious for invasion were higher for the diagnosis of glandular than squamous neoplasias. These results reinforce the role of cytology in sorting out women at risk who should have their diagnosis settled by colposcopy and histopathology.

**Contributions**

Fachetti-Machado G., Figueiredo-Alves R. R. and Moreira M. A. R. contributed with the project and interpretation of data, writing of the article, critical review of the intellectual content and final approval of the version to be published.

**Conflicts of Interest**

The authors have no conflicts of interest to declare.

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