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Anal cytology, histopathology and anoscopy in an anal dysplasia screening program: is anal cytology enough?

Marco Silva, Armando Peixoto, José Alexandre Sarmento, Rosa Coelho and Guilherme Macedo
Department of Gastroenterology. Centro Hospitalar de São João. Porto Medical School. Porto, Portugal

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Correspondence: Marco Silva. Department of Gastroenterology. Centro Hospitalar de São João. Alameda Professor Hernâni Monteiro. 4200-319 Porto, Portugal
e-mail: marcocostasilva87@gmail.com

ABSTRACT

Background and aim: The human papilloma virus is the leading cause of anal squamous cell carcinoma. Cytological screening may reduce the associated morbidity and mortality. The aim of the study was to estimate the agreement between anal cytological examination, histopathology and anoscopic visual impression.

Methods: A prospective study of patients who underwent anal dysplasia screening between 2011 and 2015, in a proctology clinic of a tertiary referral center.

Results: During the study period, 141 patients (91% men, 87% with HIV infection) underwent 175 anal cytology tests. Of these, 33% were negative for intraepithelial lesions or malignancy (NILM), 22% were atypical squamous cells of uncertain significance (ASCUS), 33% were low-grade squamous intraepithelial lesion (LSIL) and 12% were high-grade squamous intraepithelial lesion (HSIL). With regard to anoscopic visual impression, 46% of patients had no lesions and excision/biopsy of the identified lesions was performed in the remaining patients. The weighted kappa-agreement
between abnormal cytological results and anoscopic visual impression was moderate (k = 0.48). The weighted kappa-agreement between simultaneous anal cytological examinations and anal histopathologic findings was low (kappa = 0.20). With regard to the histological examination of cases with HSIL or superficially invasive squamous cell carcinoma, 64% of patients had dysplasia of a lower grade according to the cytological analysis (6 ASCUS, 18 LSIL and 4 NILM).

**Discussion:** There was a poor correlation between anal cytology, histopathology and anoscopic visual impression and a high number of histological studies of HGD that were of a lower dysplastic degree according to the cytological examination. Therefore, anal cytology screening should not be used as the sole method of anal dysplasia screening.

**Key words:** Anal dysplasia. Anal cancer. Anoscopy. Screening. Reliability.

**INTRODUCTION**

Squamous cell carcinoma of the anus (SCCA) is relatively uncommon and accounts for 2% of gastrointestinal cancers. However, its incidence is increasing (1). SCCA and its precursor lesion, anal intraepithelial neoplasia (AIN) are strongly associated with human papillomavirus (HPV) infection and is the causative agent in up to 85% of cases (2).

There are 2 different biological types of HPV-infected squamous cells in the anal canal. A productive viral infection is characterized by a low-grade squamous intraepithelial lesion (LSIL) morphology and a transforming or neoplastic viral infection is characterized by a high-grade squamous intraepithelial lesion (HSIL) histomorphology. HSIL is regarded as a cancer precursor, whereas LSIL is not (3). Although the estimated progression rate of HSIL to squamous cell carcinoma are lower in the anus compared to the cervix, identifying anal HSIL could potentially lead to an early diagnosis and interventions aimed at reducing the morbidity and mortality associated with anal cancer (4).

Guidelines suggest that anal cancer screening may decrease the incidence of anal cancer (5), however there is currently no evidence of its effectiveness. Anal screening
has been proposed for high-risk patients such as HIV-positive men and women, men who have sex with men (MSM), women with a history of vulvar or cervical cancer, organ transplant recipients and patients with a history of anogenital condylomas (6).

The anal cancer screening protocol has been adapted from strategies for cervical cancer and include anorectal cytology (ARC) and visualization of the anal canal using standard anoscopy or high resolution anoscopy (HRA) (7). HRA is similar to colposcopy, the anal canal and transition zone are inspected under a high-resolution microscope with the addition of acetic acid and/or lugol iodine in order to identify areas of dysplasia which are subsequently biopsied. This procedure has been shown to be highly effective in the diagnosis of AIN (8-13).

The aims of this study were: a) to estimate the agreement between simultaneous ARC and anoscopic visual impression; b) to estimate the agreement between simultaneous anal cytological examinations and anal histopathologic findings; and c) to determine the value of ARC as a screening test for AIN.

METHODS

A prospective observational cohort of patients that underwent screening for anal dysplasia at the proctology outpatient clinic of our center, between 2011 and 2015 were included in the study. Patients included in the screening program were: a) HIV-positive men and women; b) MSM (without HIV); c) women with a history of vulvar or cervical cancer; d) organ transplant recipients; and e) patients with a history of anogenital condylomas. All consecutive eligible patients were included and written informed consent for the procedure was obtained. The study was previously approved by the ethics committee review board of our institute (“Comissão de Ética para a Saúde do Centro Hospitalar de São João / Faculdade de Medicina da Universidade do Porto, Portugal”) and the study was conducted in accordance with the ethical principles of the Declaration of Helsinki and in compliance with good clinical practices.

Screening measures included a liquid-based ARC using ThinPrep® (Hologic Corporation, Bedford, MA, USA) and standard anoscopy with the addition of acetic acid to identify areas of dysplasia in all cases. Any suspicious lesion was biopsied during the same visit. A cytology examination with a minimal cellularity of 2,000-3,000 nucleated squamous
cells was considered as adequate, as outlined in the Bethesda system for Reporting Cervical Cytology of 2001 (14). A repeat ARC 1-2 years later was offered to the patients with an initial negative screening (NILM and absence of lesions on anoscopy) according to their risk factors.

ARC results were classified into 4 categories according to the Bethesda classification (15):

1. Negative for intraepithelial lesions or malignancy (NILM).
3. LSIL.
4. HSIL.

The histopathological exams were classified into 3 categories according to the lower anogenital squamous terminology (LAST) (3):

1. Normal.
2. LSIL.
3. HSIL.

Cytological and histopathological analysis were performed by different pathologists who were considered as experts in these fields. Pathologists were not blind to the ARC results and could consult other results if it was considered necessary.

The agreement between measurements was estimated by weighted kappa-statistics. Two types of agreement were evaluated:

1. Agreement between the ARC results and the visual impression of the anoscopist with regard to the presence of a lesion (NILM vs. no lesion and ASCUS, LSIL, or HSIL vs. presence of a lesion).
2. Agreement between simultaneous anal cytological examinations and anal histopathologic findings (NILM vs. normal, LSIL vs. LSIL and HSIL vs. HSIL/superficially invasive squamous cell carcinoma [SISCCA]).

ARC sensitivity was defined as a positive test (any cytologic abnormality) in the presence of disease (HSIL or SISCCA histology) and ARC specificity was defined as a negative test (negative cytology) in the absence of disease (normal or LSIL histology).

In addition, the ARC positive predictive value was defined as the proportion of cases with the disease (HSIL or SISCCA histology) among cases with a positive test result (any
abnormal cytology). The negative predictive value was defined as the proportion of disease free cases (normal or LSIL histology) among cases with a negative test result (negative cytology).

Statistical analysis was performed using the IBM SPSS Statistics 22® (IBM, Armonk, New York, USA) and a p < 0.05 was considered as statistically significant. Receiver operating characteristics (ROC) curves with regard to ARC diagnostic accuracy for AIN and the corresponding areas under the curve were calculated. Demographic and clinical characteristics were collected from electronic medical records for all individuals who underwent anal cancer screening. Continuous data, such as age and CD4+ lymphocyte count are presented as mean ± standard deviation (SD). With regard to qualitative variables, gender (male or female), SCCA risk factors (HIV-positive, MSM, history of vulvar or cervical cancer, organ transplant recipients and history of anogenital condylomas) and HIV risk factors (MSM, heterosexual transmission and history of intravenous drug use) are presented as total number and frequencies.

RESULTS

During the period of the study, 141 patients underwent 175 anal cytology tests with technically satisfactory samples (Table 1). 91% of cases were male and 9% were female. The mean age was 37 ± 14 years. According to the high-risk factors for SCCA, 87% patients were HIV-positive, 7% were MSM, 2% had a history of vulvar/cervical cancer, 2% had a history of anogenital condylomas and 2% were organ transplant recipients. With regard to the transmission risk factors of HIV in this population, 83% of cases were MSM, 5% had a history of intravenous drug abuse and transmission was related to heterosexual relations in 12% of cases. The median CD4+ lymphocyte count was 477 cells/mL (IQR: 286-657 cells/mL).

With regard to the distribution of the ARC results (n = 175), 33% were NILM, 22% ASCUS, 33% LSIL and 12% HSIL (Fig. 1). With regard to anoscopy, 80 (46%) patients had no detectable lesions on standard anoscopy (53% NILM, 22% ASCUS and 25% LSIL). Excision/biopsy of the identified lesions was performed in the remaining patients (Table 3) and 40 (42%) HSIL, 33 (35%) LSL and 4 (4%) SISCCA were found. Eighteen patients (19%) with suspicious lesions on anoscopy
had no dysplasia according to the histopathological examination.
The weighted kappa-agreement between the ARC results and the visual impression of
the anoscopist with regard to the presence of a lesion (NILM vs. no lesion and ASCUS,
LSIL, or HSIL vs. presence of a lesion) was moderate (kappa = 0.48, overall agreement
of 70%, p = 0.069). The weighted kappa-agreement between simultaneous anal
cytologic examinations and anal histopathologic findings (NILM vs. normal, LSIL vs. LSIL
and HSIL vs. HSIL/superficially invasive squamous cell carcinoma [SISCCA]) was low
(kappa = 0.20, overall agreement of 46%, p = 0.079). ARC had a moderate diagnostic
accuracy for AIN with an AUROC of 0.608 (p = 0.154) (Fig. 2). ARC had an overall
sensitivity of 90.9%, a specificity of 40.5%, a positive predictive value of 37.3% and a
negative predictive value of 92.9%.

DISCUSSION
The annual incidence of SCCA is increasing, particularly in high-risk populations (16).
SCCA has many similarities to the cervical counterpart (17), both diseases have a causal
association with high-risk HPV, are thought to originate at a mucosal transformation
zone and have a intraepithelial cancer precursor (HSIL) stage that can be detected by
exfoliative cytology (17,18).
Not all HPV types have been associated with SCCA (5). Persistent HPV infection with
high-risk types (oncogenic HPV strains 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and
66) is the cofactor that leads to the dysplastic changes of AIN that are seen before an
anal invasive carcinoma diagnosis is made (6,19). The aim of an anal screening
program should be the detection of HSIL, as this lesion may progress to carcinoma. To
date, there are no large randomized trials that have examined the benefits of an anal
screening program to improve survival rates (6). However, the available guidelines
suggest that anal cancer screening may be beneficial (5,20-22). In addition, people that
have anoreceptive intercourse and immunosuppressed individuals are at risk and may
benefit from screening (5,20-22).
It has been proposed that cytology-based anal cancer screening programs may lead to
a reduction in anal cancer incidence, as the etiology and pathogenesis of SCCA are
analogous to cervical disease (3). To the best of our knowledge, such programs have
been implemented in a limited number of centers, mostly in clinical settings that provide care to MSM with HIV (10,18,23).

ARC and HRA have been used to facilitate the detection of AIN and therefore allow for an early diagnosis and interventions aimed at reducing anal cancer rates in high-risk populations (3,24). Due to the effectiveness of cytology in relation to cervical cancer, ARC has been adopted as one of the first steps in screening for AIN. However, the operational and methodological variations and the limited sensitivity and specificity impairs its efficacy as a screening technique (3,8,25). Accuracy calculations of ARC are based on the imperfect HRA-guided biopsy as a ‘gold standard’, which is subject to sampling and measurement errors (26,27). In addition, the success of ARC in anal cancer screening should be based on serial testing over time (26).

All patients with AIN should be followed-up and undergo a periodic control of any suspicious lesions, either by a simple inspection (standard anoscopy) and biopsy of suspicious lesions or via HRA in expert centers (1). There is considerable debate with regard to the optimal protocol for the management and surveillance of these lesions (8). Currently, there are two competing strategies for the treatment of AIN including "expectant management". This term is a contradiction as this strategy includes surveillance with regular examinations and a simple inspection as well as active treatment of identified lesions. Although, HRA is recommended in some patients with AIN (8). However, HRA has not been universally adopted as the standard for screening or treatment (8,28,29). This procedure is not standardized and variations in the HRA procedure have been reported (4). In addition, the HRA procedure is complex, technically more difficult than colposcopy and entails a steep learning curve (1,17).

There are convoluted mucosal folds of the anal canal which may limit observations with HRA and therefore, positive cytological findings with no histological HSIL are more likely to represent a false-negative HRA rather than a false-positive cytology (17,30-33). HRA is also associated with an increased morbidity due to repeated procedures and focal destruction of the anal canal mucosa (8). Moreover, the usefulness of HRA as a screening test is impaired by the logistical needs associated with its use (34).

Recently, Crawshaw et al. showed that there was no progression to SCCA regardless of the treatment protocol (HRA vs. standard anoscopy) as long as the patient was
compliant with therapy (8). Like the cervical Pap test, ARC has never been studied in a randomized clinical trial between cytological screening and expectant management (34). In addition, there are very few studies of the performance of ARC tests for the detection of histologically confirmed HSIL (17). Cytology seems to underestimate the grade of dysplasia compared to the corresponding biopsy sample and ARC tests routinely report low-grade atypia in lesions that are subsequently identified as HSIL via histological examination (35). In this study, 7% of NILM, 15% of ASCUS and 29% of LSIL cases were found to have HSIL on biopsy. These results are comparable to previously reported data of ASCUS and LSIL, 17-46% for ASC-US and 13-56% for LSIL (35).

With regard to the patients with HSIL/SISCCA on histological examination, 28 (64%) patients had lower grade lesions on cytological examination (6 ASCUS, 18 LSIL and 4 NILM). An inaccurate prediction of the grade of dysplasia on cytology was also reported by Palefsky et al., of the 147 histological HSIL included in their study, only 27% showed HSIL on cytology (18). Therefore, these authors recommend that anoscopy and biopsy should be performed in the case of an abnormal cytology.

Several factors may be responsible for the discrepancies observed between anal cytology and biopsy results. The sample collection method (liquid-based or conventional smears), specimen adequacy (it is essential that the transformation zone is sampled) and cellularity may play a role (35). In order to decrease the risk of these possible limitations, a liquid-based cytology was used in this study as the background is much cleaner and allows atypical cells to be detected more readily. The Bethesda System recommendation was followed for cytology examinations with regard to the requisite of a minimal cellularity of 2,000-3,000 nucleated squamous cells in order for a specimen to be considered adequate (35).

Recent reviews have also highlighted the wide variations in the reported sensitivity and specificity of cytology for the detection of histologically confirmed anal HSIL (3,24,36,37). The false negative cytology rate for MSM can reach 23% for HIV-negative patients and 45% for HIV-positive patients (38). In this study, the overall false negative rate was 9.1%, of the 57 NILM cytology tests, 26% had suspicious lesions according to the anosscopic visual impression and 9 (60%) had AIN on histopathological examination (4 HSIL and 5 LSIL). The false negative rate was similar to the Betancourt et al study
that reported a false negative rate of 7%, although this study used HRA (35). This high rate of missed lesions in high-risk patients have led to suggestions that ARC tests are inadequate as a sole method of screening (34). It has been suggested that ARC should be paired with a direct visual modality such as HRA in order to be considered as an appropriate screening test (25,34). Unfortunately, as previously discussed, the usefulness of HRA as a screening test is limited due to the logistical needs and the complexity of its use. We have shown that standard anoscopy, which is a much simple and cheaper procedure than HRA, could detect HGD lesions and consequently decrease the incidence of SCCA in high-risk population. In this context, we suggest that anoscopic screening should be offered to all patients.

CONCLUSION
The low correlation between anal cytology, histopathology and anoscopic visual impression and the high number of histological examinations with HSL with a lower grade lesion on cytological examination (including NILM anal cytology), suggest that anal cytology screening should not be used alone for anal dysplasia screening. In this context, we suggest that anoscopic screening should be offered to all patients, even when anal cytology results are normal.

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**Fig. 1.** The distribution of anal cytology results.

**Fig. 2.** ROC curve of ARC diagnostic accuracy for AIN (AUROC of 0.608, \( p = 0.154 \)).
**Table 1.** Baseline demographic and clinical characteristics of the patient cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>141</td>
</tr>
<tr>
<td>Cytology (n)</td>
<td>175</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td>91</td>
</tr>
<tr>
<td>Female (%)</td>
<td>9</td>
</tr>
<tr>
<td>Age (mean ± SD, years)</td>
<td>37 ± 14</td>
</tr>
<tr>
<td><strong>SCCA risk factor</strong></td>
<td></td>
</tr>
<tr>
<td>HIV-positive (%)</td>
<td>87</td>
</tr>
<tr>
<td>MSM (%)</td>
<td>7</td>
</tr>
<tr>
<td>Vulvar/cervical cancer (%)</td>
<td>2</td>
</tr>
<tr>
<td>Anogenital condylomas (%)</td>
<td>2</td>
</tr>
<tr>
<td>Organ transplant recipients (%)</td>
<td>2</td>
</tr>
<tr>
<td><strong>HIV risk factor</strong></td>
<td></td>
</tr>
<tr>
<td>MSM (%)</td>
<td>83</td>
</tr>
<tr>
<td>Heterosexual transmission (%)</td>
<td>12</td>
</tr>
<tr>
<td>Intravenous drug abuse (%)</td>
<td>5</td>
</tr>
<tr>
<td><strong>ARC result</strong></td>
<td></td>
</tr>
<tr>
<td>NILM (%)</td>
<td>33</td>
</tr>
<tr>
<td>ASC-US (%)</td>
<td>22</td>
</tr>
<tr>
<td>LSIL (%)</td>
<td>33</td>
</tr>
<tr>
<td>HSIL (%)</td>
<td>12</td>
</tr>
</tbody>
</table>

SCCA: Squamous cell carcinoma of the anus; MSM: Men who have sex with men; HIV: Human Immunodeficiency Virus; ARC: Anorectal cytology; ASC-US: Atypical squamous cells of uncertain significance; NILM: Negative for intraepithelial lesion or malignancy; LSIL: Low-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial.
Table 2. Agreement between the ARC results and the visual impression of the anoscopist with regard to the presence of a lesion

<table>
<thead>
<tr>
<th>ARC</th>
<th>Standard anoscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lesion n (%)</td>
</tr>
<tr>
<td>NILM</td>
<td>15 (26)</td>
</tr>
<tr>
<td>ASC-US</td>
<td>23 (56)</td>
</tr>
<tr>
<td>LSIL</td>
<td>38 (66)</td>
</tr>
<tr>
<td>HSIL</td>
<td>19 (100)</td>
</tr>
</tbody>
</table>

ARC: Anorectal cytology; ASC-US: Atypical squamous cells of uncertain significance; NILM: Negative for intraepithelial lesion or malignancy; LSIL: Low-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial.

Table 3. Agreement between anal cytology and concurrent biopsy

<table>
<thead>
<tr>
<th>ARC</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal, n (%)</td>
</tr>
<tr>
<td>NILM</td>
<td>6 (40)</td>
</tr>
<tr>
<td>ASC-US</td>
<td>3 (13)</td>
</tr>
<tr>
<td>LSIL</td>
<td>9 (24)</td>
</tr>
<tr>
<td>HSIL</td>
<td>0</td>
</tr>
</tbody>
</table>

ARC: Anorectal cytology; ASC-US: Atypical squamous cells of uncertain significance; NILM: Negative for intraepithelial lesion or malignancy; LSIL: Low-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial; SISCCA: Superficially
invasive squamous cell carcinoma.