# Cytohistological discrepancy: case study of an anal-rectal cytology

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## ABSTRACT

Anal cancer is rare, but its incidence has been increasing, especially among the HIV-positive population, in whom HPV infection is more persistent. A high risk HPV infection is linked to the presence and progression of dysplasia that are precursors lesions of anal cancer. For this reason, HIV-positive individuals are considered a risk group and should be referred to screenings of anal lesions.

This case study refers a 53-year-old man, HIV-positive, with disparate cytological and histological diagnoses of anal-rectal samples, where the cytological assessment resulted in the attribution of lesions of a higher grade than the ones detected in the histology.

The present study is aimed at alerting readers for the importance of performing anal cancer screening tests, especially to risk groups individuals, and for the importance of assessing disparate cytological and histological results.

**Keywords:** anal-rectal cytology, low grade squamous intraepithelial lesion, high grade squamous intraepithelial lesion, human papillomavirus, human immunodeficiency virus.

## INTRODUCTION

Anal cancer is a rare disease, affecting 1 to 2 people in 100 000 in the general population[1]. Some risk factors increase the vulnerability of individuals to these lesions, namely the human immunodeficiency virus (HIV) infection, immunodepression and injectable drugs use[1]. The risk of developing anal cancer is higher among homosexual and bisexual men and HIV-positive individuals, the incidence ratio for this group being 70 in 100 000[1].

Anal-rectal cytology screening is already being performed in Portugal. Patients who are referred to these tests are mostly HIV-positive. The main objective of anal-rectal cytology is to detect lesions that may develop into more serious lesions, such as anal cancer. The cytomorphology of anal-rectal lesions associated with human papillomavirus infection (HPV) is similar to those lesions affecting the cervix, thus, the diagnostic criteria are the same and the applied terminology is also the Bethesda system[2].

An anal-rectal cytology aims to collecting a cytological sample representative of such location. The brush needs to reach the anal-rectal transition zone in order to collect an adequate sample, which will be used to perform conventional smears or liquid-based cytology slides. The liquid-based cytology promotes better results, since it reduces the sample's contamination by faecal matter and it eliminates fixation artifacts[3].

Prevention and early detection of anal cancer are very important, due to its impact on survival. The survival rate for patients with localized cancer is nearly 80%; however, the existence of metastasis decreases that percentage to 30%[3].

This article is about the clinical case of a man followed at the *São João* Hospital, EPE, with different cytological and histological diagnosis for precursor lesions of anal cancer. Reasons behind these disparities will be discussed hereafter.

## CLINICAL HISTORY

In June 2015, an HIV-positive 53-year-old man underwent an anal-rectal liquid-based cytology collection, which microscopic study resulted in a diagnosis of high grade squamous intraepithelial lesion (HSIL). In December of the same year, he was submitted to an anal-rectal biopsy; the diagnosis was focal, moderate grade anal intraepithelial neoplasia (AIN2).

In subsequent follow-up, namely in June 2016, a cytological and a histological samples were collected; the cytology study maintained the HSIL result, while histology examination resulted in a diagnosis of AIN1. In August, the patient was submitted to a biopsy that reinforced the AIN1 diagnosis.

The cytology here reported was performed in October 2016, which result maintained the HSIL diagnosis.

The following step was the detection and typification of HPV.

## CYTOLOGYCAL FINDINGS

The liquid-based anal-rectal cytology of June 2015 was considered satisfactory for evaluation; it had a clear background where it was possible to observe the transformation zone represented by cells of the anal-rectal junction and squamous cells of a deeper layer, organized in more individualized groups with an increase of the nuclear-cytoplasmic ratio, nuclear hyperchromasia, granular chromatin, anisokaryosis and pleomorphism (**Fig.1**). Such cellular changes are compatible with an HSIL result.

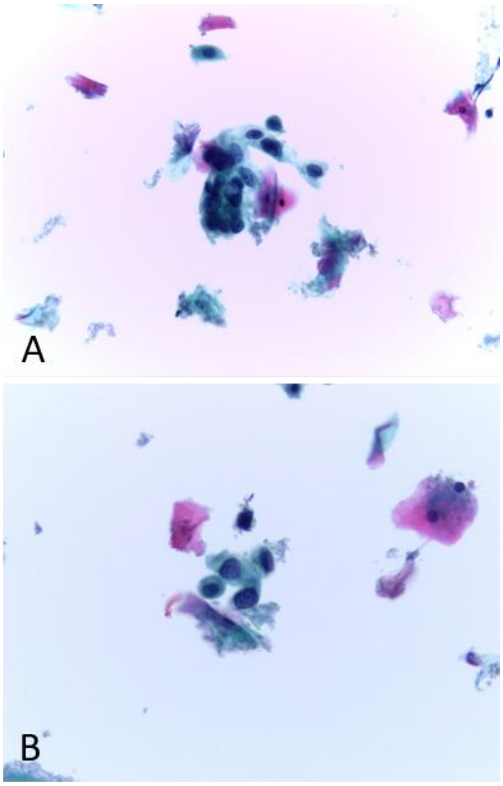


**Fig. 1** – Liquid-based cytology (*ThinPrep*®) – Parabasal cells with an increased nuclear-cytoplasmic ratio, nuclear hyperchromasia, granular chromatin, anisokaryosis and pleomorphism – HSIL (**A** e **B**). *Papanicolaou* stain. Magnification: 400x.

The microscopic evaluation of the anal-rectal cytology collected in October 2016 revealed a sample satisfactory for evaluation with transformation zone representation (**Fig. 2**). There, it was possible to observe a clean background with squamous cells exhibiting an increased nuclear-cytoplasmic ratio, nuclear hyperchromasia, irregular nuclear membrane, granular chromatin, anisokaryosis and pleomorphism, cytological features that support an HSIL result (**Fig.2A-D**).

## HISTOPATHOLOGICAL FINDINGS

The histology evaluation of the anal and perianal area sampled in December 2015 resulted in the same diagnosis of the anal-rectal cytology performed before (June 2015), where it was possible to observe a stratified squamous epithelium with koilocytosis and low grade squamous intraepithelial lesion (AIN1) (**Fig.3A** and **B**), as well as a focal high grade squamous intraepithelial lesion (AIN2) of the anal canal (**Fig.3A**).

However, the result of the anal canal histology performed in October 2016 didn't match the result of the anal cytology performed in the same day. The former exhibited a mucosa enveloped by a stratified squamous epithelium, with cytoarchitectural changes typical of HPV infection, with no evidence of dysplasia or malignancy, thus resulting in an AIN1 diagnosis.

## HPV DETECTION AND TYPIFICATION

In 2016, simultaneously to the cytological study, HPV detection and typification by the Cobas® test was performed. This technique the typification of HPV 16 and 18, as well a joint-detection of "other" high-risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). In the present case, the result of the Cobas® test was positive for the presence of "other" high-risk HPV types but negative for types 16 and 18.

## DISCUSSION AND CONCLUSION

Cytology of the anal canal enable the detection of precursor lesions (AIN1, AIN2 and AIN3) as well as carcinomas[4-6]. Despite the low prevalence of precursor lesions of anal cancer, some studies have pointed to its gradual increase, especially among men who have sexual relationships with other men[4-6].

An anal canal cytology sample may contain intermediate and superficial nucleated squamous epithelial cells, anucleated squamous cells, metaplastic cells or rectal columnar cells - which represent the anal-rectal transition zone located in the pectineal line[2]. Reparative changes are less frequent in anal canal cytologies than in cervicovaginal samples, whereas keratinizing lesions are most common[2]. Besides, the differential diagnosis between HSIL and invasive carcinoma can be

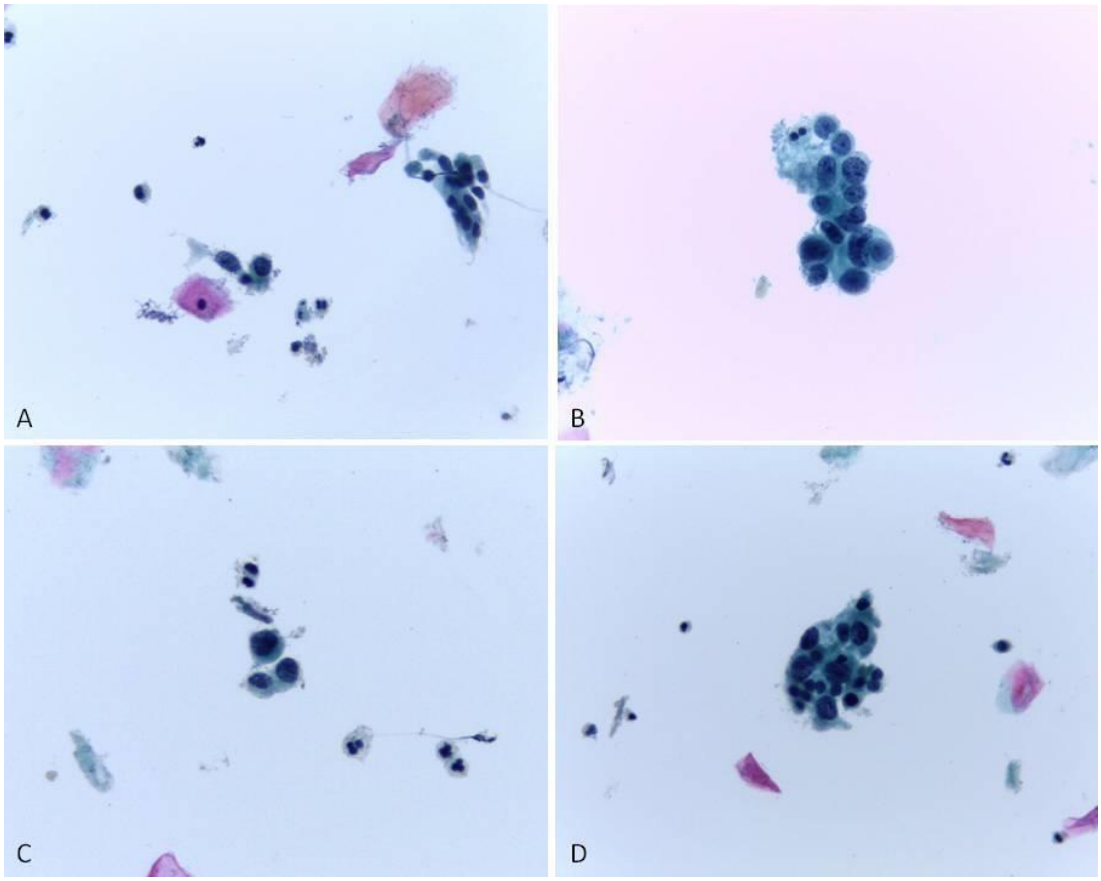


**Fig. 2** – Liquid-based cytology (*ThinPrep*®). Squamous cells with an increased nuclear-cytoplasmic ratio, nuclear hyperchromasia, irregular nuclear membrane, granular chromatin, anisokaryosis and pleomorphism (**A-D**). **Fig.2A** shows a group of rectal columnar cells representative of the anal-rectal transformation zone. *Papanicolaou* stain. Magnification: 400x.

hardened by the fact that faecal matter is often difficult to differentiate from the necrotic waste associated with tumour diathesis[2].

The literature reviewed showed different values for the specificity and sensitivity of the anal cytology, due to the heterogeneity of the assessed criteria[7]. However, values round 32-59% for specificity. and 69-93% for sensitivity[4].

In this case study, there is a discrepancy between the cytological result and the final histological diagnosis. This difference might be due to several factors, including the lack of background literature on anal cytology study support, the recent experience on these samples evaluation, as well as to other factors related to the collection methods.

In regard to the assessment of anal cytology samples, a histological diagnosis of moderate dysplasia (AIN2) corresponds to a cytological result of HSIL (which includes lesions of moderate and severe dysplasia). However, despite the moderate dysplasia observed only in a December 2015 biopsy, the presence of HSIL is evident in all the anal canal cytology samples performed. The collection of both cytological and histological samples can be performed at relatively different places, in function of the instruments that are used. Therefore, the biopsy could not have been as deep as the cytology, whereas the brush used to collect cytological sample might have reach a deeper part of the anal canal.

The increased incidence of anal lesions among men is associated with the HPV infection[4-6,8,9]. Its prevalence is even higher among immunocompromised individuals, due to the HIV infection, or in immunodepressed

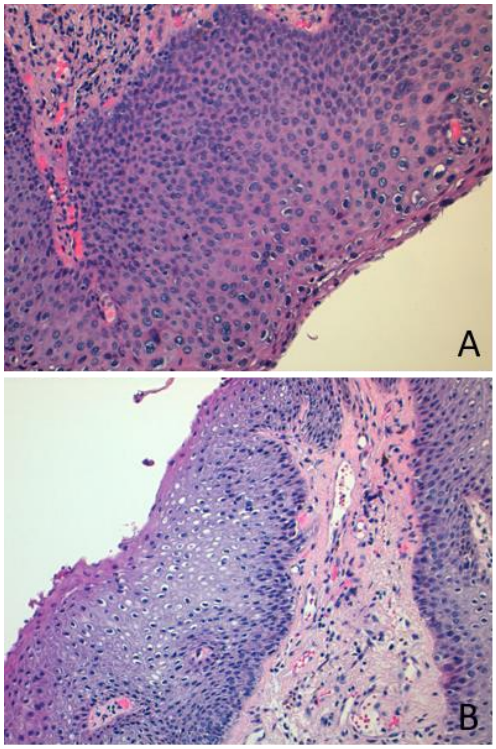


**Fig. 3** – Histological representation of the anal canal and perianal region biopsy – evidence of infection by HPV, AIN1 (**A** and **B**) and focal AIN2 (**A**). Hematoxylin-eosin stain, Magnification: 200x.

transplanted ones, which in consequence, will increase anal intraepithelial lesions of the anal canal incidence[4-6,8,9]. Therefore, these individuals are advised to undergo an anal cytology screening program, which is now a common practice in some Portuguese hospitals, such as the *São João* Hospital, EPE.

Infection by several high-risk HPV types is associated with the presence and progression of dysplastic lesions[9]. In HIV-positive individuals with AIN2 or AIN3, HPV infection is more common for high risk types, especially type 16, 18, 58 and 45. On those with AIN1 lesions, the infection can occur for high-risk or low-risk HPV types, mainly types 6, 11, 16, 39 and 42[5].

The *Cobas*® test enables the detection and typification of high-risk HPV only. However, low-risk HPV types (such as type 6 and type 11, which are more frequently associated with AIN1) can also be a root for anal cancer. These viruses contribute to the development of precursor lesions, whose persistence may result in the progression to more severe lesions.

HIV-infected individuals are at risk of developing anal cancer, even when they are subjected to active retroviral therapies, since the treatment does not exclude the risk of dysplastic changes[4,9]. This might explain consecutive low grade lesions in the anal canal of some patients, which, if not treated effectively, might transform into more severe lesions. The standard treatment for anal cancer is a combination of radiation therapy and chemotherapy, although some significant side effects might occur[3]. Other treatments addressed to small tumours are resection surgeries (which preserve the sphincter), such as cryotherapy, laser, infrared coagulation and fulguration, or topical therapies, such as Imiquimod, trichloroacetic acid and 5-fluorouracil[3]. In some rare cases, an abdominoperineal resection (which involves removal of the anus and rectum) might be necessary, which requires a colostomy[3].

In conclusion: HPV infection is one of the main factors for the development of anal cancer, thus the need to reinforce prevention and screening methods among high risk groups, namely through vaccination and regular cytologies of the anal canal[5,10].

The quality of these screening tests depends mainly on the collection; since this is a relatively new procedure, hence lacking in background literature, training aimed at improving cytological screenings becomes essential.

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