# Bacterial vaginosis, aerobic vaginitis, vaginal inflammation and major Pap smear abnormalities

Pedro Vieira-Baptista[1], Joana Lima-Silva[1], Cândida Pinto[1], Conceição Saldanha[2], Jorge Beires[1], José Martinez-de-Oliveira[3,4], Gilbert Donders[5,6]

[1] Department of Gynecology and Obstretrics, Centro Hospitalar de São João, Porto, Portugal
[2] LAP - Laboratório de Anatomia Patológica, Unilabs, Porto, Portugal
[3] Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal
[4] Department of Children and Women's Health, Centro Hospitalar da Cova da Beira, Covilhã, Portugal
[5] Department of Obstetrics and Gynecology, University Antwerp, Antwerp, Belgium
[6] Femicare Clinical Research for Women, Tienen, Belgium

Corresponding author:

Pedro Vieira-Baptista
Centro Hospitalar de São João
Serviço de Ginecologia e Obstetrícia
Alameda Prof. Hernâni Monteiro
4200 Porto
Portugal

pedrovieirabaptista@gmail.com

+351 917718495

Abstract

Purpose: To evaluate the impact of the vaginal milieu on the presence of abnormal Pap smears and a positive HPV test.

Methods: Cross sectional study, conducted between June 2014 and May 2015, evaluating the vaginal discharge by fresh wet mount microscopy and comparing these data with Pap smear findings. Wet mount slides were scored for bacterial vaginosis (BV), aerobic vaginitis (AV), presence of Candida, and *Trichomonas vaginalis*. Cytologic evaluation was done on all Pap smears according to Bethesda criteria. Cobas© HPV (Roche) test was performed for Human Papilloma Virus (HPV) detection.

Results: 622 cases were evaluated. The mean age was 41.6±10.65 (21-75) years.
Eighty-three women (13.3%) had a cytology result worse than low-grade squamous intraepithelial lesion (LSIL). When comparing this group with the one with normal or minor (atypical squamous cells of undetermined significance (ASC-US) or LSIL) Pap smear abnormalities, there were no differences in the presence of Candida (32.5% *vs*. 33.2%, *p*=1.0), absence of lactobacilli (38.6% *vs*. 32.5%, *p*=0.32) or BV (20.5% *vs*. 13.2%, *p*=0.09). On the other hand, moderate/severe inflammation (msI) (41.0% *vs*. 28.8%, *p*=0,04), moderate/severe AV (msAV) (16.9% *vs*. 7.2%, *p*=0.009) and msAV/BV (37.3% *vs*. 20.0%, *p*=0.001) were more common in women with such major cervical abnormalities.

No significant association was found between deviations of the vaginal milieu and high risk HPV infection.

Conclusion: The presence of msI or msAV, but not BV, are independently associated with an increased risk of major cervical cytological abnormalities, but not with HPV infection.

Keywords: aerobic vaginitis, bacterial vaginosis, inflammation, Pap test, vaginal flora, wet mount, bacterial vaginosis, HPV

## Introduction

Aerobic vaginitis (AV) was first described by Donders et al. in 2002[1]. It is characterized by the presence of immature epithelial cells, a disturbed population of *Lactobacilli*, the presence of aerobic pathogens and inflammation (evaluated by the number of leucocytes and their toxic appearance). The pathogens involved are enteric bacteria (*Escherichia coli, Staphylococcus aureus,* group B streptococcus, and enterococci), and lack of oestrogen is also believed to play a role in its origin[2,3]. The extreme presentation of AV is comparative to what is known as 'desquamative inflammatory vaginitis' (DIV) [2,4]. It is postulated that AV is the aerobic counterpart of anaerobic bacterial vaginosis (BV). In some cases, mixed presentations (AV and BV) can be found, representing either a transient form or prolonged co-infection [3]. Indeed, the vaginal milieu cannot be seen as a static system, but rather as a complex dynamic system.

AV is still not widely known and so it is under diagnosed by many clinicians, and may even have been mistaken as BV in several former studies[5]. Indeed, in major studies discussing the role of a disturbed vaginal flora in preterm labour, AV hardly ever has been acknowledged as a potential risk factor[6,7], despite its clear pathogenic role[3,8–1112]. Hence, the lack of recognition of AV may explain some of the conflicting results found among studies involving BV.

AV, being present in 2-12%[13–16] of women, has been implied as a possible cause of serious adverse gynaecological and obstetric outcomes, such as an increased risk to acquire sexually transmitted diseases (including Human Immunodeficiency Virus)[17], premature rupture of membranes, preterm labour and ascending chorioamnionitis[18].

Jahic et al[19], in 2013, added to this list an increased risk of having a LSIL Pap smear result. These authors concluded that the association was independent of being high-risk HPV (HR-HPV) positive, and that treating AV would decrease the number of abnormal Pap test results.

Role of the persistent infection by one of the high risk HPVs in the development of cervical cancer is well established[20]. Although the infection is common, few patients develop high-grade lesions or invasive cancer. As the progression risk is still poorly understood, several factors need to be investigated, including smoking, contraception, presence of sexually transmitted diseases, as well as factors from the vaginal milieu [21].

Clarke MA et al. have shown that a high pH, indicative of an abnormal vaginal flora, is a risk factor for HPV infection and abnormal Pap tests (LSIL) [22]. This relation was more marked in women under 35 and over 65 years old and was assumed to be associated with BV.

However, an elevated pH is not always indicative of BV. The relation between increased vaginal pH and vaginal abnormalities, the lactobacillary flora profiles and AV is better accomplished by using wet mount, rather than Gram stain[23].

The aim of this paper is to investigate the correlation between the vaginal flora in wet mounts and major Pap test abnormalities.

## Material and methods

Between June 2014 and May 2015 we performed microscopic examination of vaginal discharge collected from 622 consecutive patients, who attended the clinic with an indication for cervical Pap test (cross sectional study). After the insertion of an unmoistened speculum, discharge from the lateral upper third of the vaginal wall was collected with a spatula and spread onto a glass slide. The slides were then allowed to dry and later rehydrated with a drop of saline[24] . All samples were evaluated blindly by one of the authors (PVB), according to Femicare's criteria[8,25]. It included: lactobacillary grade evaluation (Grade 0 – no lactobacilli or other bacteria; grade I – lactobacillary flora, no other bacteria; grade IIa – lactobacilli dominating flora, but with other bacteria; grade IIb – lactobacilli present, but other bacteria dominating; grade III – absent lactobacilli, other bacteria present); presence of "clue cells"; leucocytes (≤10/high power field, >10/high power field and ≤10/epithelial cell, >10/epithelial cell); proportion of toxic leucocytes (none or sporadic, ≤50% of leukocytes, >50% of leukocytes); background flora (unremarkable or cytolysis, small coliform bacilli, cocci or chains, anaerobic); proportion of parabasal epitheliocytes (none or <1%, ≤10%, >10%); presence of *Candida* spp. (spores/bastospores, hyphae/pseudohyphae); presence of structures compatible with *Trichomonas vaginalis*.

Moderate or severe inflammation (msI) was defined as more than 10 leukocytes per high power field, and more than 10 leukocytes per epithelial cell respectively); moderate or severe aerobic vaginitis (msAV) was defined as an AV score of more than 4, according to the original definition of Donders et

all [8]. The parameters evaluated to determine the AV score were: lactobacillary grade, number of leucocytes, proportion of toxic leucocytes, background flora and proportion of parabasal cells. Atrophic vaginitis was defined by the presence of parabasal cells, as predominant cell type, and leucocytes or, in some cases, a paucity of cells, usually with lack of lactobacilli.

All Pap tests (liquid based cytology, ThinPrep medium, Hologic, Bedford, MA) were read at the institution's laboratory according to the Bethesda criteria[26]. Cobas© HPV (Roche Molecular Systems Inc., Pleasanton, CA) test was performed for all patients, according to the institution's protocol.

Most women (85.5%) were recruited from the Cervical Pathology Unit and the remaining from the Family Planning Services.

After explanation of the study and obtaining informed consent a clinical history, including contraceptive methods, hormonal treatments, hormonal status, vulvovaginal symptoms, smoking habits, comorbidities, history of cervical disease and conization were recorded. In patients with symptoms (e.g. pruritus, burning or discharge), vulvar dermatoses were excluded by thorough clinical examination, performed by clinicians with expertise in this area, before attributing these symptoms to "vaginitis".

Exclusion criteria were age less than 21 years old, pregnancy, total hysterectomy, vaginal bleeding, and vaginal medication within the last 48 hours or refusal to participate in the study.

Whenever an immediate diagnosis was necessary (symptomatic vaginitis) two slides were taken: one for immediate diagnosis and an additional one to be read blindly together with the other slides. All diagnosed vaginitis were treated according to the *lege artis*.

According to the Bethesda classification "major Pap smear abnormality" (MPSA) was defined as any cellular abnormality more severe than LSIL: LSIL cannot exclude high grade intraepithelial neoplasia (LSIL-H)[26], atypical glandular cells (AGC), atypical squamous cells cannot exclude high grade intraepithelial lesion (ASC-H), high grade intraepithelial lesion (HSIL), adenocarcinoma *in-situ* (AIS), carcinoma). At the time of the study, the now no longer recommend subtype "LSIL-H"[26] was still being used at our institution and was included in the major abnormalities group. The cases with cytology showing NILM, ASC-US or LSIL composed the control group.

According to the study protocol, colposcopy driven biopsies were taken at the physician's discretion. The available biopsy results (punch biopsies and excisional procedures) were also evaluated. These

results were available for 190 (30.5%) of women and were classified as normal, cervical intraepithelial neoplasia (CIN) 1, CIN2, CIN3, carcinoma, adenocarcinoma *in situ* or adenocarcinoma.

The study was approved by the Ethics Committee of the Centro Hospitalar de São João (Number 266-2014).

Statistical analysis was performed using Microsoft® Excel® 2011 (Microsoft Corporation©, Redmond, Washington) and IBM® SPSS® 20.0 (IBM Corporation©, Armonk, NY). The Student's T test was used for continuous variables and the Fisher's exact test was used for categorical ones. A $p$-value <0.05 was considered statistically significant.

## Results

The mean age was 41.6±10.65 (21-75) years (n=622). There were 83 (13.3%) women with MPSA (case group) (**Table 1**), most of whom (95.2% [79/83]) were enrolled at the Cervical Pathology Unit

The characteristics of the two groups are summarized in **Table 2**. There were no differences between the control and study group in terms of age, contraceptive use, menopause status, use of hormonal replacement therapy or the presence of symptoms suggestive of vaginitis.

Patients in the study group were significantly more likely to be smokers, but less likely to have had a previous cervical excisional procedure. In the control group, a significant number of patients (24.5% [132/539]) had already been treated with previous conization, and were in the follow-up phase now.

There was no association between AV and smoking (6.0% [5/83] AV in smokers *vs*. 7.4% [24/326] in non-smokers, $p$=0.813). On the other hand, the percentage of BV among smokers was somewhat higher, but without reaching statistical significance (21.7% [18/83] *vs*. 13.8% [45/326], $p$=0.088).

The presence of msAV was significantly more common in the MPSA group than in the control group (16.9% *vs*. 7.2%, $p$=0.009) and the same was true for moderate/severe inflammation (41.0% *vs*. 28.8%, $p$=0.029). Also, if women with aerobic vaginitis and/or bacterial vaginosis were considered jointly (msAV/BV), these abnormal flora conditions were more frequent in women with MPSA than in

women with LSIL, ASC-US or NILM (37.3% *vs*. 20.0%, *p*=0.001). The prevalence of single BV, however, was somewhat higher in the study group, but without statistical significance (20.5% *vs*. 13.2%. *p*=0.090, **Table 3**).

Lactobacilli were absent (LBG 0 and III) in 12.3% (51/415) of women with MPSA and in 15.5% (32/207) of control women (*p*=0.3).

89 (47.1%) of the 189 women with msI had Candida*,* 48 (25.4%) had msAV, 22 (11.6%) showed *Trichomonas vaginalis,* and 16 (8.5%) had atrophic vaginitis.

Considering the cases of Candida and stratifying it according to the presence of inflammation, still there was no difference in the risk of MPSA (13.5% [12/89] vs. 12.8% [15/117], *p*=1.0).

In women with inflammation, if more than 50% of the leucocytes were toxic, the rate of MPSA was 23.3% (17/73), compared to 12.0% (66/549) in women with lower numbers of toxic leukocytes (*p*= 0.016).

The rate of AV was 7.9% (29/367) in LSIL and 8.8% (8/91) in NILM (*p*=0.8).

The HPV test results were available in 609 (97.9%) women. There was no relation between the presence of HR-HPV and msAV, inflammation or BV (**Table 4**).

Amongst women with HR-HPV, msAV was significantly more common if they had also MPSA, as compared to HR-HPV positive women with a Pap smear LSIL or less (17.3% [13/75] *vs*. 7.7% [15/194], *p*=0.025). Women with HR-HPV had a non-significant higher chance to have BV than HR-HPV women with low grade or normal cytology (21.6% [16/74] *vs*. 12.4% [24/194], *p*=0.083).

Of women who had additional information about pathology results of cervical biopsies, CIN2+ was present in 77.8% (7/9) of those with msAV and in 74.5% (35/47) of those without msAV (*p*=0.2). Of all women, CIN2+ was present in 40% (10/25) of those with msAV and in 30.1% (50/166) of women without msAV (*p*=0.4).

Discussion

AV is a highly prevalent condition, with specific criteria that can be easily recognized through wet-mount microscopy[24].

BV has been extensively debated in the literature as a risk factor for cervical dysplasia[27] and cancer[28]. On the contrary, little is known about the role of AV. When interpreting the data relative to BV, it must be kept in mind that AV was not recognized or ignored in most of these older studies, while in others it might even have been mistaken as BV[29].

The role of (chronic) inflammation in the development of precancerous lesions has been gaining increasing attention in the recent years[30–32]. According to the literature, the interleukin (IL) profile of AV (increased IL-1b, IL-6 and IL-8) is similar to what is found in women with cervical intraepithelial neoplasia[33–36]. In contrast, in BV, IL-6 and IL-8 are not expressed[37].

Our data has shown a relation between AV, AV and/or BV, but not isolated BV, and a Pap test with major abnormalities. There was also an association between msI and a Pap test with major abnormalities. The majority of the cases with msI were not in the context of AV, pointing to the role of the inflammatory response as such, and its own relevance, rather than the presence of infectious agents – which in some cases can be commensals of the vagina. Already in 2001, Castle et al. [36] found a non-significant relation between the presence of inflammation and high-grade CIN (OR, 1.9; 95% CI, 0.91–4.1) on Pap smears.

The annotation “inflammation” can have different meanings: it can be more or less exuberant and there can be significant differences in terms of the presence of toxic leucocytes. Indeed, we found not only that inflammation is linked to major abnormal Pap smear results, but also that the presence of toxic leukocytes do had a stronger association with more severe cervical cytologic abnormalities .

The thesis that a chronic inflammation in the vagina decreases the innate immune response in the cervix, allowing for persistence of HPV infection and increasing the progression to high grade lesions deserves further study. This theory would fit with the observation that in some series anti-inflammatory drugs were associated with a decreased risk of cervical cancer [38,39].

Despite the fact that a low pH and the presence of *Lactobacilli* are considered an important part of the defence system of the vagina[40] and that it might be also true for the cervix[41], we could not establish an association between *Lactobacilli* absence and an abnormal Pap test result. This may be explained by

the fact that the absence of lactobacilli can be due to a variety of reasons, including AV, BV, Trichomonas infection, severely inflamed candidosis, etc., but also due to hormonal influences like after menopause or during the postpartum period[42–44]. Hence, the absence of *Lactobacilli* in the vagina cannot always be considered pathological[45].

We could not confirm the results of Jahic et al. [19], who found AV to be more common in women with LSIL, when compared to women with NILM. In our study the rate of AV was similar in women with LSIL and NILM. A possible explanation for these different findings may be that Pap tests in that study could be collected for a period of up to 6 months before the wet mount smear was taken. Since both cervical dysplasia and vaginal flora are dynamic, it may indicate that in women with minor Pap smear abnormalities, like LSIL, regression of AV may be more likely than in women with more severe cytological abnormalities, but this is purely hypothetical. Finally, in Jahic's study, no information was given about the AV prevalence in women with major cytological abnormalities.

Although it is tempting to explain our findings by immune system interactions, it is also possible that the presence of AV leads to a misreading of the Pap smear and an over diagnosis of MPSA. Indeed, the presence of inflammation, atrophy or reparative phenomena – all of which can be found in AV - can, perhaps influence the Pap test interpretation, rendering it merely a confounder instead of being a risk factor.

AV, BV, elevated vaginal pH and the presence of inflammation can also be markers of sexual activity or of specific habits, like smoking, that are known to increase the risk of having an abnormal Pap test. Women participating in this study were not interrogated about their sexual habits.

Smoking, as expected, was associated with having a Pap test with a major abnormality. Smoking is known to be associated with cervical dysplasia, cancer and also with BV, making it difficult to interpret the independent role of each of the factors, as BV can be a marker of smoking. However, for AV, we could not find such a relation with smoking.

Despite the small numbers of biopsies, the rate of CIN2+ in patients with a Pap test worse than LSIL was similar in both women with and without AV, thus ruling out the hypothesis that increased

prevalence of AV in women with abnormal cytology could be due to "false positives". This can be a phenomenon similar to the increased risk of false positive ASC-US Pap smear in women with *T. vaginalis* infection[46].

However, we did not find an association between the presence of *T. vaginalis* and an abnormal Pap test.

Also we found no relation between the presence of microscopic candidosis and high grade lesions of the cervix, not even after correcting for the presence of concurrent inflammation.

Although reporting the presence of inflammation and microorganisms in the Pap test may lead to unnecessary treatment and interventions[47], our data seems to indicate that reporting vaginal infectious conditions and inflammation on Pap smear analyses should be done systematically. For AV, however, Pap reading are not properly validated to provide a full diagnosis, but inflammatory cells can be easily recognized and graded[48,49], at least on conventional Pap smears. Hence, specific Pap test criteria for the diagnosis of AV, inflammation and BV should be developed and advocated, also for the new, liquid based cytology testing.

The weak points of this study may include the fact the most women were enrolled at a Cervical Pathology Unit, which enabled to have an enriched sample of abnormal Pap tests. A significant part of these women had a history of cervical disease and a high percentage of previous excisional procedures of the cervix, which may have distorted the 'natural' prevalence of lesions and HR HPV in the studied women. Indeed, in many women with original high-grade lesions, not only the lesion, but also the HR HPV disappears at later follow up.

Despite comparing the results of a cervical and a vaginal collection (Pap test and wet-mount, respectively), the effects of the vaginal milieu are without any doubt also exerted on the cervix. Furthermore, there is a strong concordance between the microbioma found in the cervix and the vagina[50].

Another disadvantage may be that we most likely underreported the presence of trichomoniasis, due to delayed reading of the wet mounts and on candidosis, as as according to some authors, sensitivity on wet mounts may be less than with cultures [51,52].

Finally the analysis based on histological biopsy results can be biased, as no systematic biopsies were performed.

## Conclusion

AV is a significant cause of vaginitis-like symptoms, which no longer can be ignored or neglected. Among other important adverse gynaecological and obstetrical outcomes, it may also be involved in the progression to cervical intraepithelial neoplasia and cancer in HR HPV positive women. This can be due to its chronic inflammatory features, leading to depression of the innate immune response mechanisms. The interleukin profile that has been associated with AV is coherent with that found in HR-HPV infected patients progressing to cervical dysplasia and cancer.

There was no association between the presence of AV and that of HR-HPV infection, so AV probably is not related to an increased acquisition risk of the later. Hence, more likely AV and inflammation can have a permissive role in the persistence of HR-HPV and in progression to high grade lesions once established.

Currently it is not clear whether AV is a marker or a risk factor, but our data suggests that inflammation plays a key role. Remarkably, not all types of "inflammation" are equally dangerous, as, opposed to AV, inflammation associated with Candida was not associated with an increased risk.

BV, which is not associated with inflammation, and was in our study also not significantly associated with abnormal Pap test results.

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