Diagnosis of vaginitis: time to improve and move on

Diagnóstico de vaginite: hora de melhorar e seguir

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"Miss Elvira's bedroom smells like used clothes and women: women don't smell like perfume, they smell like stale fish."
The hive, Camilo José Cela.

Vulvovaginitis is the ultimate example of Potter Stewart's "I know it when I see it": everybody knows what it is, but no one can find an adequate and encompassing definition.

Despite this obvious limitation, one thing is factual: vulvovaginitis is the reason for at least 2% of office appointments among women, representing a huge amount of time and money spent[1]. The human suffering (physical and psychological) associated is uncountable and comes in different dimensions: the recurrence of symptoms, severe conditions misdiagnosed or undiagnosed (even malignant and premalignant ones), and increased risk of developing gynecological and obstetrical complications. The list of complications is extensive, including: pelvic inflammatory disease, ectopic pregnancy, infertility, sexually transmitted infections (HIV, HPV, herpes), progression of cervical intraepithelial lesion, postoperative infection (pelvic surgery, C-section), preterm birth, premature rupture of membranes, neonatal sepsis, etc. These risks seem to be independent of the presence of symptoms (which is the seed for the controversial concept of screening vaginitis)[2,3]. What have we done lately for the 965,000 deaths/year attributable to preterm birth? One every 33 seconds[4].

In the next three questions, we will try to do the exercise of evaluating whether or not what we are doing is enough, and what will be the future in the diagnosis of vaginitis.

IS THE CLASSIC TRILOGY OF CANDIDIASIS/BACTERIAL VAGINOSIS/ TRICHOMONIASIS ENOUGH?

Until 1955, women with vaginitis had either trichomoniasis or *monilia* (candidiasis). All the remaining women (the majority) had "non-specific vaginitis".

Herman Gardner changed this scenario with his seminal paper: "*Haemophilus vaginalis* vaginitis: a newly defined specific infection previously classified non-specific vaginitis". The *H. vaginalis* would later be rechristened *Gardnerella vaginalis* and the condition, bacterial vaginosis (BV).

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Nowadays, "non-specific" vaginitis still can unacceptably represent up to 30% in some series[5]. If other conditions, such as desquamative inflammatory vaginitis (DIV) – and its lighter form, aerobic vaginitis –, cytolytic vaginosis, and lactobacillosis, besides the "classic" vaginitis are considered, that percentage decreases substantially[6]. How many cases of "resistant" or "recurrent" candidiasis are actually cytolytic vaginosis that was never appropriately evaluated[7]? "Non-specific vaginitis" is often a euphemism for our lack of knowledge, resistance to change, and insufficient diagnostic approach.

ARE WE DOING OUR BEST WITH WHAT WE HAVE AVAILABLE?

Guidelines and protocols for the diagnosis of vaginitis are similar everywhere: clinical history, gynecological examination, pH, whiff test, and wet mount microscopy (WMM). These methods are low-tech, easy, and cheap, but not used most of the time. In some cases, point-of-care tests, cultures (fungi), and nucleic acid amplification tests (NAATs) (*T. vaginalis*) may be used. A recent study in the USA showed that WMM was performed in only 17% of women with symptoms of vaginitis[8].

The diagnosis is often based on a "typical" history and the characteristics of the discharge, leading to missing or wrong diagnoses in half of the patients[9]. After all, it should be "I don't know it if I only see it".

On the other hand, we also excessively rely on culture exams, treating, for instance, any positive culture for *Candida* spp. or *G. vaginalis* — which colonize up to 10–20 and 60% of women, respectively[10].

Should we still rely on the Amsel criteria for the diagnosis of BV? Four criteria, with one of them being the presence of clue cells. If one has access to and training in WMM, the microscopic diagnosis of BV can be made without the need to resort to the Amsel criteria. However, most providers (including gynecologists — shame on us!) do not use the microscope and, thus, are limited to the other three criteria. Nevertheless, the "normal" mean pH is higher than previously believed and with substantial ethnic variations[11]. Therefore, the established cut-off value (4.5) has low specificity. Not to mention that it cannot be applied to postmenopausal women. In addition, the presence of discharge and fishy smell is subjective and possibly absent/unnoticed in asymptomatic women. Before questioning yourself what is the point of applying the Amsel criteria to asymptomatic women, we must remember the non-dropping rates of preterm birth and the nearly one-third of new cases of HIV in Africa attributable to BV[12]. Recent meta-analyses are excluding papers in which women were diagnosed with BV using the Amsel criteria[13].

The gold standard for the diagnosis of vaginitis can be time-consuming (Nugent score for BV, culture for *Candida* spp.) and/or expensive (NAATs for *T. vaginalis*). While waiting a few days for

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the return of the results may be acceptable for women with chronic or recurrent disease, those with an acute episode demand immediate treatment. WMM is a cheap and effective option that usually provides an immediate and relatively accurate diagnosis, besides allowing to assess multiple infections, presence of inflammation, and hormonal status.

The tools to do a better job have been within our reach for several decades.

IS THIS THE TIME TO MOVE TO MOLECULAR DIAGNOSIS?

The diagnosis of vaginitis is already in the molecular era: for the diagnosis of trichomoniasis, either the parasite is seen moving under the microscope, or a NAAT is needed (culture is not easily available commercially, does not have a better performance, and takes longer to provide results).

The molecular diagnosis of candidiasis has proven to be feasible and even to increase the sensitivity of cultures. Other possible advantages include: the diagnosis of low levels of fungi (important in recurrent/chronic candidiasis) — as an alternative to repeated sampling — and the identification of non-*albicans* species[14]. However, the method is not risk-free: detection of non-viable fungi is possible, and, similarly to what happens currently with cultures, it does not allow distinguishing between colonization and infection.

While the molecular diagnosis of *T. vaginalis* and *Candida* spp. is straightforward, that of BV is a greater challenge. BV is characterized by the depletion of lactobacilli and the proliferation of anaerobes. However, no single bacterium has been identified as a universal marker of BV; we have no bacterial "formula" to define it. Nevertheless, the method has shown to be feasible, using quantitative polymerase chain reaction (PCR) and targeting different species of lactobacilli and anaerobes (variable combinations, usually including *G. vaginalis*, *Atopobium vaginae*, and *Mobiluncus* spp.). These approaches have sensitivities higher than 90% and specificities close to 90%[14-16].

The molecular diagnosis of the three most common causes of vulvovaginitis is now available, based on the collection of one[14,16] or two[15] vaginal swabs, with a turnaround time of hours (which will be shorter with increasing use and performance of more runs in the laboratories).

Multiplex PCR is just the beginning of a revolution: the chance of coupling vaginitis diagnosis/screening with HPV and/or sexually transmitted infections, characterizing the profile of BV (*i.e.*, which bacteria are present), testing for antibiotic resistance, etc.

While next-generation sequencing (NGS) is being used in cutting-edge investigations in the field of vaginitis and microbiome, the information it provides is too complex to be used in clinical practice. However, it will be useful to define targets for PCR.

Next steps in multiplex PCR tests for vaginitis will include diagnosing, for instance, DIV and cytolytic vaginosis — but first these must be acknowledged by clinicians.

Indeed, it is time to move on; the future has arrived. Nonetheless, we should not dispose of the microscope yet, as it will allow the correct diagnosis in most cases, besides showing the broader picture of the vaginal milieu. Multiplex tests will find their place in settings in which, for some reason, WMM is not available or did not lead to a diagnosis and in screening scenarios (when automatization, high-throughput capacity, and self-sampling are needed). Ironically, PCR may have its strongest impact in low-income countries: a higher burden of complications related to dysbiosis (preterm labor, HIV) associated with a lack of health care professionals and facilities. WMM will still be the leading option in diagnosis, while the major role of PCR will be screening (pregnancy, high risk for HIV acquisition, HPV positive women, infertility, etc.)

These are the days of "big data", NGS, and microbiome. We await to jump into the future, but we neglect the basics. We could be doing much better than we are... In 1836, Alfred Donné looked into the microscope and published about the *animalculi* (*T. vaginalis*) he saw in genital discharge[17]. Why can we not do the same nearly 200 years later?

Would you treat Miss Elvira for BV without further testing? Are you sure it is BV? Can you exclude trichomoniasis?

"I know it when I see it" is only acceptable if you say it while sitting behind your microscope. "I know it when I hear it" is never acceptable.

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REFERENCES

1. Lipsky MS, Waters T, Sharp LK. Impact of vaginal antifungal products on utilization of health care services: Evidence from physician visits. J Am Board Fam Med. 2000;13(3):178-82. https://doi.org/10.3122/15572625-13-3-178
2. Chesson HW, Blandford JM, Pinkerton SD. Estimates of the Annual Number and Cost of New HIV Infections Among Women Attributable to Trichomoniasis in the United States. Sex Transm Dis. 2004;31(9):547-51. https://doi.org/10.1097/01.olq.0000137900.63660.98
3. Owusu-Edusei K, Chesson HW, Gift TL, Tao G, Mahajan R, Ocfemia MCB, et al. The Estimated Direct Medical Cost of Selected Sexually Transmitted Infections in the United States, 2008. Sex Transm Dis. 2013;40(3):197-201. https://doi.org/10.1097/OLQ.0b013e318285c6d2
4. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, et al. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015;385(9966):430-40. https://doi.org/10.1016/S0140-6736(14)61698-6
5. Anderson MR, Klink K, Cohrssen A. Evaluation of vaginal complaints. JAMA. 2004;291(11):1368-79. https://doi.org/10.1001/jama.291.11.1368

6. Vieira-Baptista P, Bornstein J. (Candidiasis, Bacterial Vaginosis, Trichomoniasis and Other Vaginal Conditions Affecting the Vulva. In: Bornstein J, ed. Vulvar disease - breaking the myths. Cham: Springer; 2019.
7. Yang S, Zhang Y, Liu Y, Wang J, Chen S, Li S. Clinical Significance and Characteristic Clinical Differences of Cytolytic Vaginosis in Recurrent Vulvovaginitis. Gynecol Obstet Invest. 2017;82(2):137-43. https://doi.org/10.1159/000446945
8. Hillier SL, Austin M, Macio I, Meyn LA, Badway D, Beigi R. Diagnosis and Treatment of Vaginal Discharge Syndromes in Community Practice Settings. Clin Infect Dis. 2020;ciaa260. https://doi.org/10.1093/cid/ciaa260
9. Lowe N, Neal J, Ryan-Wenger N. Accuracy of the Clinical Diagnosis of Vaginitis Compared to a DNA Probe Laboratory Standard. Obstet Gynecol. 2009;113(1):89-95. https://doi.org/10.1097/AOG.0b013e3181909f63
10. Stockdale CK. A Positive Culture Result for Gardnerella Is Not Diagnostic of Bacterial Vaginosis. J Lower Genit Tract Dis. 2016;20(4):281-2. https://doi.org/10.1097/LGT.0000000000000237
11. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SSK, McCulle SL, et al. Vaginal microbiome of reproductive-age women. PNAS. 2011;108(Suppl. 1):4680-7. https://doi.org/10.1073/pnas.1002611107
12. van de Wijgert JHHM, Morrison CS, Brown J, Kwok C, Van Der Pol B, Chipato T, et al. Disentangling Contributions of Reproductive Tract Infections to HIV Acquisition in African Women. Sex Transm Dis. 2009;36(6):357-64. https://doi.org/10.1097/OLQ.0b013e3181a4f695
13. Brusselaers N, Shrestha S, van de Wijgert J, Verstraelen H. Vaginal dysbiosis and the risk of human papillomavirus and cervical cancer: systematic review and meta-analysis. Am J Obstet Gynecol. 2019;221(1):9-18.e8. https://doi.org/10.1016/j.ajog.2018.12.011
14. Vieira-Baptista P, Silva AR, Costa M, et al. Clinical validation of a new molecular test (Seegene® AllplexTM Vaginitis) for the diagnosis of vaginitis. 2020 accepted for publication at the British Jpurnal of Obstetrics and Gynecology.
15. Schwebke JR, Taylor SN, Ackerman R, Schlaberg R, Quigley NB, Gaydos CA, et al. Clinical Validation of the Aptima Bacterial Vaginosis and Aptima Candida/Trichomonas Vaginitis Assays: Results from a Prospective Multicenter Clinical Study. J Clin Microbiol. 2020;58(2):e01643-19. https://doi.org/10.1128/JCM.01643-19
16. Sherrard J. Evaluation of the BD MAX™ Vaginal Panel for the detection of vaginal infections in a sexual health service in the UK. Int J STD & AIDS. 2019;30(4):411-4. https://doi.org/10.1177/0956462418815284
17. Donné A. Animalculi observed in purulent fluids and secretions of genital organs from Men and Women. CR Acad Sci. 1836;3(385).