



Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Acute Vaginitis

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Slide 1:

Hi, my name is Ingibjorg Hilmarsdottir. I'm a clinical microbiologist at Landspítali University Hospital of Reykjavik, and an assistant professor at the Medical School of the University of Iceland. In this lecture I will share with you some useful information about a common "feminine" problem which is acute vaginitis.

Slide 2:

I will start by mentioning the causes of vaginitis, and the clinical relevance of this health issue, and then I will focus on the three most common causes of vaginitis, and the available tests for point-of-care and the microbiology laboratory. And we will end with some take-home messages.

Slide 3:

Vaginitis can be acute or chronic. Acute vaginitis (10) is common, and will be the scope of this Pearl. However, I will briefly mention some causes of chronic or persistent vaginitis, which is rare. These include desquamative inflammatory vaginitis, also termed "aerobic vaginitis", herpes simplex, allergy, irritation, dermatological disorders, and postmenopausal atrophy (11). Now, the most common cause of acute vaginitis is bacterial vaginosis, which seems to be caused by the patient's endogenous bacterial flora. The second commonest cause is *Candida* vaginitis, which so often involves not only the vagina but also the vulva, that it is usually called *Candida* vulvovaginitis. Like bacterial vaginosis, it is caused by an endogenous organism, in this case a yeast. The third most common cause is, unlike the other two, a sexually transmitted disease caused by the protozoan, *Trichomonas vaginalis*. It is important to bear in mind that non-vaginal infections, such as *Chlamydia* infection, gonorrhoea, and even urinary tract infections, can present with vaginal discharge or dyspareunia, which means pain during sexual intercourse, and may be missed in the absence of an appropriate workup.

Slide 4:

Vaginitis is among the most common reasons for women's visits to a health care provider. Although commonly regarded as a benign disease, it has been associated with serious health issues such as negative pregnancy outcomes, post-surgical infections after gynecological surgeries, and enhanced acquisition of HIV, *Chlamydia*, and other sexually transmitted infections. Despite these potential complications, vaginitis is all too often mismanaged by doctors and patients, who tend to initiate a blind therapy without any diagnostic tests. Studies from the US indicate that about 50% of patients who see a physician for vaginitis are treated without appropriate testing (27), and that the majority of women who buy over-the-counter antifungal drugs do not have *Candida* infection (12).

Slide 5:

The keyword of this slide is work-up, because many studies have confirmed that symptoms and signs of vaginitis are non-specific and insufficient for making a diagnosis (2,16). Common symptoms are increased or changed vaginal discharge, burning, itching, dyspareunia, and dysuria.

A physical examination with the use of a speculum usually reveals a red and inflamed mucosa in *Candida* and *Trichomonas* vaginitis, but not in bacterial vaginosis. A few and simple point-of-care tests are often sufficient to make a correct diagnosis, but when they are not available or inconclusive, laboratory tests should be requested.

The cause of symptoms can be found in 50–80% of cases, depending on the extent of testing (16,23). And in more than half of acute vaginitis cases, the tests will reveal bacterial vaginosis, *Candida* vulvovaginitis, or *Trichomonas* vaginitis.

Patients with a known vaginal infection may have coinfections with other vaginal infections (12,22) or sexually transmitted diseases, and therefore, complementary tests may be required, depending on the patient history, symptoms, and signs.

Slide 6:

With this slide, we will start reviewing the three most common causes of acute vaginitis and how to diagnose them.

The most common cause is bacterial vaginosis. This infection is of uncertain etiology but is characterized by a disturbance of the vaginal flora (17). The normal flora is dominated by lactobacilli, which are gram-positive rods that produce lactic acid and maintain a pH of less than 4.5 in the vaginal fluid. In bacterial vaginosis, there is a greatly decreased number of lactobacilli, with a subsequent rise of pH to over 4.5, and an overgrowth of various anaerobic and fastidious bacteria such as *Atopobium vaginae*, *Gardnerella vaginalis*, *Bacteroides*, and many more.

Bacterial vaginosis is associated with increased risk of adverse pregnancy outcomes, post-hysterectomy infections, pelvic inflammatory disease, and acquiring HIV, HPV, Herpes, *Chlamydia*, gonorrhea, and *Trichomonas* (18).

Up to 50% of women who have bacterial vaginosis detected after work-up are asymptomatic. And because of potential pregnancy complications, there have been many studies on screening for and treating bacterial vaginosis in pregnancy. So far, no benefits of screening and treating have been found

in low-risk pregnancy, and conflicting results have been seen in high-risk pregnancy (26). As of today, it is not recommended to screen in pregnancy, but symptomatic pregnant women should be diagnosed and may be treated.

Bacterial vaginosis is treated with metronidazole, clindamycin or tinidazole (28). It should be noted that relapses of bacterial vaginosis are common.

Slide 7:

Now, let's move on to the second most common vaginal infection, which is *Candida* vulvovaginitis (25). *C. albicans* is the causative species in about 85% of cases, and *C. glabrata* or other *Candida* spp. are responsible for about 15% of cases. Most patients have symptoms and signs of inflammatory vaginitis, such as itching and burning and a red inflamed vaginal mucosa. The pH of the vaginal fluid is below 4.5, unless coinfections with bacterial vaginosis or *Trichomonas* are present.

It is important to distinguish asymptomatic colonization with *Candida* from symptomatic infection. *Candida* species, most often *C. albicans*, are part of the normal commensal flora in about 20% of asymptomatic women. The mere detection of *Candida* from a vaginal swab or fluid is therefore not sufficient to make a diagnosis of *Candida* vulvovaginitis and should not lead to therapy in the absence of vaginal symptoms.

About 75% of women have ≥ 1 simple episode during a lifetime, and these can be managed with short course antifungal therapy. A small percentage of women have either frequently recurring or severe infections that necessitate a more meticulous work-up and oral therapy of long duration.

Slide 8: Classification of Lactic Acidosis

The third most common cause of vaginitis is *Trichomonas vaginalis*, which is a flagellate protozoan that has a "corkscrew" motility when seen in a wet mount microscopy (4,28). *Trichomonas* causes cervicitis, vaginitis, and urethritis in women and urethritis in men. Women may have symptoms and signs of inflammatory vaginitis, but like bacterial vaginosis, *Trichomonas* infection is often asymptomatic. The pH of the vaginal fluid is above 4.5 and most often > 5 .

Trichomonas infection is the most prevalent curable STI in the world, and is found in 3-34% of women of reproductive age. The infection is associated with increased risk of preterm birth, pelvic inflammatory disease, and increased HIV transmission. However, since women who are screened and treated during pregnancy may have a worse pregnancy outcome than those who are not, systematic screening of asymptomatic pregnant women is not recommended. Pregnant symptomatic women can be treated though.

Trichomonas infection is treated with metronidazole or tinidazole, and as for other STI, partners of infected women should also receive therapy.

Slide 9:

On this slide, we have a table showing the various point-of-care tests available for the diagnosis of the three most common vaginitis causes. The first one on the list is wet mount microscopy of vaginal fluid. For this test, the vaginal fluid is mixed with a drop of saline, and examined in regular light microscope, or a phase contrast microscope. The test can reveal clue cells, which suggests bacterial vaginosis, yeast cells, or motile trichomonads. *Trichomonas* loses its motility quickly outside the body and the wet mount must therefore be examined immediately after sampling (4). When wet mount microscopy is used as a part of Amsel's method (5,16) for the detection of bacterial vaginosis, no further tests for that infection are required. Amsel's method consists of four simple criteria, and bacterial vaginosis is considered present when 3 out the 4 criteria are met. The criteria are:

1. clue cells seen in wet mount microscopy,
2. vaginal pH over 4.5,
3. a homogenous grey-white discharge seen on physical examination, and
4. a positive whiff test, which stands for the production of a fishy odor when KOH is placed on a drop of vaginal fluid (17).

Wet mount microscopy is not a sensitive test for *Candida* (1,2,16,25) and *Trichomonas* (2,4,16) infections, so if no diagnosis is made after the microscopy, then more sensitive tests need to be done at point-of-care or requested from the laboratory.

Other tests in the table are simple and user-friendly commercial point-of care-tests that are specific for bacterial vaginosis (5,10,15), *Candida* infection (7,9) or *Trichomonas* infection (20). The test for bacterial vaginosis seems to be comparable to Amsel's method in terms of diagnostic accuracy, and the ones for *Candida* vulvovaginitis and *Trichomonas* vaginitis seem promising. The availability of these tests varies between countries.

Slide 10:

Here we see photographs from wet mount microscopy showing *Candida* hyphae and budding yeast cells on the left hand side, and *Trichomonas* trophozoites on the right hand side. *Trichomonas* trophozoites are pear-shaped and highly mobile. However, once outside the body, they round up and quickly lose their motility, and are likely to be missed on microscopical examination.

Slide 11:

On this slide, we have a table showing laboratory tests for bacterial vaginosis, *Candida* and *Trichomonas* infections. The first one is based on DNA probe hybridization that combines the detection of all three infections in one test. It seems to be more sensitive for the detection of bacterial vaginosis (6,13) than the other two infections (3,6,21).

Further down we see two tests for bacterial vaginosis, one is a Gram stain of vaginal fluid and the other one a PCR method. The Gram stain method is based on Nugent's criteria (24) that I will explain on the next two slides, and like the Amsel's method, it is considered the gold standard for the diagnosis of bacterial vaginosis. PCR methods for bacterial vaginosis have been developed in-house but are not commercially available (8). Please note that culture is NOT appropriate for the diagnosis of bacterial vaginosis.

Yeast culture is the gold standard for the diagnosis of *Candida* vulvovaginitis (25) but a positive culture needs to be interpreted in light of clinical symptoms, since *Candida* can be part of the vagina's normal flora.

And finally, we have two laboratory tests for the diagnosis of *Trichomonas* vaginitis, a culture which has been considered as the gold standard (14), and a more recent and more rapid nucleic acid amplification, which has shown excellent diagnostic accuracy (3,4).

Slide 12:

The next two slides show the principles of the Nugent's method for the diagnosis of bacterial vaginosis. After a regular Gram stain, the vaginal fluid is examined for three different bacterial morphotypes, namely the Gram positive and regular morphotypes of *Lactobacillus* species, the gram variable and gram negative slender morphotypes of *Gardnerella* and *Bacteroides*, and finally, the gram negative and curved morphotypes of *Mobiluncus* species. Clue cells may be seen in Gram-stained vaginal fluid, but are not included in the diagnostic criteria of the Nugent's method. The upper picture shows a *Lactobacillus* dominant microflora, and the lower one shows the morphotypes associated with bacterial vaginosis, and a clue cell.

Slide 13:

On this slide, we see how each of these three different morphotypes are quantified and scored in the Nugent method. The three scores are added up, as you see in step two, and the sum yields one of three potential results, that is: presence of bacterial vaginosis, absence of bacterial vaginosis, or an intermediate status which is still of unknown significance. To give an example, the interpretation for a specimen with numerous lactobacilli, and scarce *Gardnerella*, *Bacteroides*, or *Mobiluncus* morphotypes would be "absence of bacterial vaginosis."

Slide 14:

On this slide, I set up a suggested work-up plan for vaginitis complaints, and took into account the various point-of-care options, which differ greatly between health care providers. A good start, at point-of-care, is to measure the pH of the vaginal fluid. A pH under 4.5 almost rules out bacterial vaginosis and *Trichomonas* vaginitis, and specific tests for *Candida* vulvovaginitis could be done at point-of-care or requested at the laboratory. A pH over 4.5 suggests bacterial vaginosis or *Trichomonas* vaginitis and warrants further testing. It should be noted that a high pH is also seen in the rare desquamative inflammatory vaginitis.

When a wet mount microscopy is available, along with a whiff test, and a pH indicator strip, the health care provider should be able to diagnose 30-40% of women who have vaginitis complaints. But often the simple point-of-care tests are either inconclusive or not available and in these cases, the health care provider should consider requesting laboratory tests in order to avoid mismanagement, such as inappropriate therapy or undiagnosed sexually transmitted infections.

On this slide I also included a schematic picture showing that for the diagnosis of bacterial vaginosis, *Candida*, and *Trichomonas* infections, the specimens should be obtained from the upper part of the vagina. The middle vagina should be sampled for pH measure, in order to avoid the more alkaline mucus

from the cervix. And last, but not least, it is worth noting that specimens that are submitted for group B streptococcus screening in pregnancy are not appropriate for the workup of vaginitis, since they should be taken from the lower vagina and the rectum.

Slide 15:

This pearl is coming to an end, and the take-home messages would be:

1. Most women have vaginitis at some point in their lives.
2. Vaginitis has been associated with serious health problems.
3. Symptoms and signs alone are unreliable for diagnosis.
4. “Blind” treatment is unacceptable in clinical practice.
5. Diagnosis of vaginitis can be done with simple or complex tests.
6. Laboratories need to offer tests for the top three causes of vaginitis and for best results they could work with local health care providers to create an algorithm for the diagnosis of this common health issue.

Thank you for listening and I hope you now know more about vaginitis.

Slide 16: References