Vaginitis/Vaginosis: Diagnostic Updates to Improve Cure Rates and Patient Outcomes

Introduction

Most women will have one or more vaginal infections during their lifetime. These infections are typically characterized by discharge, itching, or odor. Nearly 90% of all cases of vaginitis are attributable to 3 causes:[1]

1. *Trichomonas vaginalis* (trichomoniasis)
    a. Caused by trichomonads, a flagellated protozoan
2. Vaginal candidiasis (also termed vulvovaginal candidiasis or VVC)
    a. 85% due to yeast infection with *Candida albicans*; 5% to 10% caused by *Candida glabrata* or *Candida parapsilosis,* with other *Candida* species to a lesser extent
3. Bacterial vaginosis (BV; non-inflammatory)
    a. Typically due to overgrowth by *Gardnerella vaginalis, Mobiluncus* species*, Mycoplasma hominis, Peptostreptococcus* species, and other anaerobes

Vaginal symptoms/vaginitis account for millions of women's healthcare visits annually.[1,2] Despite technological advances, standard diagnostic testing still often consists of history and physical examination, pH determinations, whiff test, and wet prep microscopy. Application of culture in Diamond's media, Gram stain with Nugent's criteria, and agar culture were reported generally to not be available in many office settings. In the absence of office testing, nearly 30% of symptomatic women remain undiagnosed after clinical evaluation. Even more troubling are the nearly 25% of patients placed on a pharmacologic therapy that is not based on validated diagnostic testing. This clinical failure puts these women at risk for acquiring additional sexually transmitted infections (STIs) as well as being at an increased risk for adverse pregnancy outcomes.[1,3-9] New testing platforms have made the diagnosis of trichomoniasis caused by *Trichomonas vaginalis* (which is the primary focus of this CME journal article), vulvovaginal candidiasis, and bacterial vaginosis more accurate and rapid, so timely treatment can be offered without guesswork. But of course, that happens only if the new testing platforms are widely accepted into daily clinical practice.

***Trichomonas vaginalis* (Trichomoniasis)**

Trichomoniasis is caused by *Trichomonas vaginalis* with an estimated 5 million women infected annually in the U.S.[3] This STI is diagnosed in 4% to 35% of women with symptoms of vaginosis or vaginitis.[4] Trichomoniasis is associated with an increased risk of co-infection with human immunodeficiency virus (HIV), adverse pregnancy outcomes including premature rupture of the membranes, preterm labor and delivery, and low birth weight.[5-9] In men, *Trichomonas vaginalis* infection may cause urethritis that is commonly asymptomatic.

New developments in diagnostic testing have greatly improved the ability to detect this infection. This section of the article will address these new diagnostic tests, the integration of current STI screening and treatment guidelines for *Trichomonas vaginalis* into one's practice, and the application of one's knowledge of the epidemiology of trichomoniasis to the counseling and management of patients.

Epidemiology

A recent assessment estimated an annual incidence of 7.4 million new cases of trichomoniasis in the U.S.[10] The WHO has estimated that *Trichomonas vaginalis* infection accounts for almost half of all curable infections worldwide.[11] A survey of adolescent women in the U.S. estimated the overall prevalence to be 3.1%, with a much higher prevalence of 13.3% among African-American women.[12] Infection with *Trichomonas vaginalis* is also common in older women in the U.S. with one study demonstrating a prevalence of 11.3% and 13.0% among women ages 40-49 and ≥50 years of age, respectively. [13]

The reported prevalence of urethral infection with *Trichomonas vaginalis* in males has varied depending on the population studied and the diagnostic techniques used. Among men attending a sexually transmitted disease (STD) clinic in Birmingham Alabama, *Trichomonas vaginalis* was detected by PCR in 17% of men who were attending the clinic for a new-problem visit or screening. There was no significant difference in the detection of the organism between men with and without urethral symptoms (20% and 14.5%, respectively). Among men with non-gonococcal urethritis, nearly as many had *Trichomonas vaginalis* detected as had chlamydia (19.9% and 25.2%, respectively).[14] In a multi-center study of a new diagnostic test for *Trichomonas vaginalis* that was conducted in a wide range of clinical settings, the overall prevalence in men was 2.7%.[15]

Clinical Features

The incubation period of trichomoniasis is unknown. However, in vitro studies suggest an incubation period of 4 to 28 days in women.[16] Symptoms in women include vaginal discharge, pruritus, and irritation. Signs of infection include vaginal discharge (42%), odor (50%), and edema or erythema (22% to 37%). The discharge is classically described as frothy but is actually frothy in only about 10% of patients. The color of the discharge may vary. Colpitis macularis (strawberry cervix) is a specific clinical sign for this infection but is detected with reliability only by colposcopy and rarely during routine examination.[17] Other complaints may include dysuria and lower abdominal pain; the etiology of the latter is unclear. The urethra is also infected in the majority of women.[18]

Nearly half of all women with *Trichomonas vaginalis* infections are asymptomatic.[19] Therefore, if these women are not screened, the diagnosis will be missed. In men, recent data suggests that *Trichomonas vaginalis* infection is a more common cause of non-gonococcal urethritis (NGU) than previously recognized.[14,20] NGU infection should be considered a diagnostic possibility in men who fail initial therapy for this condition. Although rare, *Trichomonas vaginalis* infection in men may also cause epididymitis, prostatitis, and superficial penile ulcerations.[21]

Diagnosis and Screening

The diagnosis of trichomoniasis in females can be accomplished at the time of care via direct microscopic examination of the vaginal fluid. However, even for skilled diagnosticians, the overall sensitivity of this test is only 60% and may be less in asymptomatic women.[22] The presence of motile trichomonads is diagnostic. The pH of the vaginal fluid will be >4.5 in the majority of patients but can be normal (≤4.5). In these cases, the trichomonads are often sparser as they prefer a more alkaline pH. Neutrophils as well as altered vaginal bacterial flora are often seen. Interestingly, Wiesenfeld and colleagues found that even these simple bedside tests were not utilized to determine a diagnosis of vaginitis in a women's health referral center. The absence of bedside testing resulted in over half of the women receiving inappropriate therapy.[23] Reliance only upon signs and symptoms for the diagnosis of vaginitis has been shown to be unreliable.[24] Until recently, culture media was the gold standard for diagnosis but was not widely available to clinicians.[25] Other commercially available tests include an RNA probe semi-automated system, which also detects candidiasis and bacterial vaginosis, and a dipstick ELISA. Both of these tests are currently licensed only for vaginal specimens, can be used as point-of-care tests, and have sensitivities of about 80%.[26,27] They may be a good choice if microscopy is not available.

The gold standard for diagnosing trichomoniasis in both men and women is now nucleic acid amplification testing (NAAT). Sensitivities and specificities for these tests are each in the 98% to 99% range. For women, various specimen types may be used, including vaginal, endocervical, ThinPrep PAP (Hologic; San Diego, CA), and urine.[2,28] For men, only one NAAT has been FDA-cleared for use with urine.[15] However, the other commercially available tests may be run by laboratories in an analyte-specific reagent (ASR) format once internally validated by the laboratory.

As originally described, in the CDC 2015 guidelines [1] the Aptima *Trichomonas vaginalis* assay (Hologic; San Diego, CA), a NAAT, was specifically identified as a test cleared by the FDA for the detection of *Trichomonas vaginalis*. This NAAT assay detected 3- to 5-fold more *Trichomonas vaginalis* infections than did wet mount microscopy.[29] Other tests mentioned included the BD ProbeTec *Trichomonas vaginalis* Qx Amplified DNA Assay (Becton Dickinson; Franklin Lakes, NJ), the OSOM Trichomonas Rapid Test (Sekisui Diagnostics; Framingham, MA), and the BD Affirm VPIII Microbial Identification Test (Becton Dickinson; Franklin Lakes, NJ), which is a DNA hybridization probe test. Of note, in 2016, the FDA provided market authorization for the BD MAX™ Vaginal Panel to detect microorganisms responsible for bacterial vaginosis, trichomoniasis, and vulvulvaginal candidiasis in a single test, representing the first multiplex, real-time PCR assay. More recently, the FDA has approved a CV/TV panel for Candida and T. vaginalis as well as a BV NAATs test manufactured by Hologic. It is anticipated that updated CDC guidelines, which are expected to be released in 2020, will describe in greater detail the existing assays.

The CDC 2015 guidelines[1] also stated that *"although data are lacking on whether screening and treatment for asymptomatic trichomoniasis in high-prevalence settings or persons at high risk can reduce any adverse health events and health disparities or reduce community burden of infection...decisions about screening [asymptomatic women] might be informed by local epidemiology of* ***Trichomonas vaginalis*** *infection."* CDC guidelines suggest that when a highly sensitive test such as NAAT is not immediately feasible, a testing algorithm should be employed first using a wet mount followed by NAAT when feasible if the wet mount test is negative.[1,28-31]

Current CDC STD Treatment Guidelines recommend screening for trichomonas in all HIV-infected women at entry to care and then annually due to high rates of infection in this population.[1,30] The CDC Guidelines also state that screening may be considered for other at-risk populations, such as STD clinic attendees and incarcerated individuals.[1,28] Routine screening with NAATs in both men and women has been shown to greatly enhance the identification of infected individuals at STD clinics.[32] Re-screening for all infected women is recommended at 3 months due to high rates of reinfection.[1]

Treatment

The recommended therapy for trichomoniasis is a single, 2g oral dose of metronidazole or tinidazole. However, Kissinger and colleagues suggest 7-day dosing may be better, particularly in certain patient populations such as HIV-infected women.[33] Sexual partners should be routinely treated.[1] Tinidazole has superior pharmacokinetics against *Trichomonas vaginalis* but is more expensive than metronidazole.[35] Metronidazole intravaginal gel has limited efficacy and should not be used.[1] Failure to treat the male sexual partners is likely the most common cause of recurrent disease in women.[34,35] Women with asymptomatic infection should be treated. Although there continues to be some controversy about the safety of metronidazole in pregnancy, there has never been a documented case of fetal malformation attributed to its use even during the first trimester.[36] There are no data about the safety of tinidazole in pregnancy.[37]

Resistance to metronidazole has been reported, and tinidazole with more favorable pharmacokinetics may be the drug of choice when resistance is encountered. Paromomycin cream has also been used in combination with high dose oral tinidazole with some success.[37]

Complications

As mentioned, *Trichomonas vaginalis* has been implicated as a cause of preterm delivery.[7,37] Trichomoniasis has also been associated with vaginal cuff cellulitis following abdominal hysterectomy.[38] Acquisition and transmission of HIV has been associated with trichomoniasis. The associations between HIV and trichomoniasis may relate to increased shedding of HIV as a result of the local inflammation produced by the STI and/or increased susceptibility to HIV as a result of the macro- or microscopic breaks in mucosal barriers caused by inflammation.[38]

Given the higher prevalence and incidence of trichomoniasis than most other treatable STDs, most studies to date suggest the fraction of HIV acquisitions due to trichomoniasis may eclipse the relative contribution of other STIs.[39] Transmission of HIV is also enhanced by infection with *Trichomonas vaginalis*. HIV viral loads in the seminal fluid of men with urethritis were significantly higher in men with trichomoniasis than in those with symptomatic urethritis due to an unidentified cause.[40] Treatment of trichomonas urethritis reduced the levels of HIV to levels similar to uninfected controls.[41]

Vulvovaginal Candidiasis (VVC)

VVC is present in 17% to 39% of women with symptoms.[4] Nearly three-quarters of women develop VVC during their lifetime. About 45% of women suffer a second occurrence, and approximately 5% will have

[frequently] intractable recurrent candidiasis.[5] Although *Candida albicans* accounts for 80% to 90% of such infections, VVC has also been associated with *Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis.* Rare associations with *Candida kefir*, *Candida krusei*, *Candida pseudotropicalis*, *Candida lusitaniae*, and/or *Candida rugose* have been reported.[42] Typical symptoms of VVC include pruritus, vaginal soreness, dyspareunia, external dysuria, and abnormal vaginal discharge. None of these symptoms are specific for VVC.[1,42]

Laboratory diagnosis of VVC/yeast infection has classically been performed by wet preparation of vaginal discharge to assess for budding yeast, hyphae, or pseudohyphae, or by culture assessment.[1,2] These approaches are often time-consuming and not readily available in most offices.

NAATs testing is now the gold standard for the diagnosis of VVC as well. As previously mentioned, two diagnostic panels have received FDA-clearance (BD Max vaginal Panel and the Aptima CV/TV panel). These newer tests also differentiate *C. glabrata* from other Candida species. However, as *Candida* may colonize the vagina, it is important to distinguish colonization from true infection based on compatible symptoms when using these newer, highly sensitive diagnostic tests.

VVC can be classified as either uncomplicated or complicated:[1]

Uncomplicated VVC disease is:

1. Sporadic and infrequent, *and*
2. Mild to moderate, *and*
3. Likely *Candida albicans*, *and*
4. Typically diagnosed in a non-immunocompromised woman

Complicated VVC disease is:

1. Recurrent, or
2. Severe, or
3. Nonalbicans candidiasis, or
4. Typically diagnosed in women with diabetes, immunocompromising condition, debilitation, or on immunosuppressive therapy

Approximately 10% to 20% of women will have complicated VVC that requires special diagnostic and therapeutic considerations.[43] A short-course of topical formulations for 1 to 3 days or single dose oral fluconazole (150 mg) are usually effective in treating uncomplicated VVC. The azole drugs are more effective than nystatin. Azole treatment results in the relief of symptoms and negative cultures in 80% to 90% of patients completing therapy. Recurrent VVC and complicated VVC require more complex therapeutic regimens. Readers are encouraged to review the current CDC treatment recommendations for a more exhaustive listing.[1]

Bacterial Vaginosis (BV)

BV is the most common finding in up to half of women with vaginal symptoms.[4] BV is characterized by the replacement of normal *Lactobacillus* flora with large concentrations of predominantly *Gardnerella vaginalis, Mobiluncus* species*, Mycoplasma hominis,* and other BV-associated anaerobic bacteria. Treatment of BV may reduce the risk of acquiring other STIs[5], which include *Chlamydia trachomatis, Neisseria gonorrhoeae*, *Trichomonas vaginalis*, HIV, and herpes simplex type 2.[1,44-47]

Symptoms are absent in approximately 50% of women with BV infection. While BV is not typically associated with soreness, itching, or irritation, there may be a classical "fishy" odor and vaginal discharge. A diagnosis of BV can be made if 3 out of 4 of "Amsel's criteria" are met:[1]

- A strong fishy odor on adding alkali to vaginal fluid (positive amine test)
- Clue cells (vaginal epithelial cells heavily coated with bacilli) on microscopy
- A thin, white, and homogenous discharge
- Vaginal pH >4.5

Again, NAAT testing is now the gold standard for the diagnosis of BV. Different NAATs base the diagnosis of BV on algorithms of BV-associated bacteria. No two algorithms are the same, but the tests appear to be equivalent to one another. Other less sensitive tests, including Affirm VPIII (Becton Dickinson, Franklin Lakes, NJ), which is a DNA hybridization probe testing for high concentrations of *Gardnerella vaginalis,* and the OSOM BV Blue test (Sekisui Diagnostics, Framingham, MA) which detects vaginal fluid sialidase activity, may be used.

Currently, the CDC recommends treatment only for symptomatic women with BV.[1]

Recommended treatment regimens include:

- Metronidazole 500 mg orally twice a day for 7 days
- Metronidazole gel 0.75%, one full applicator (5 g) intravaginally, once a day for 5 days
- Clindamycin cream 2%, one full applicator (5 g) intravaginally at bedtime for 7 days

Alternative regimens include:

- Tinidazole 2 g orally once daily for 2 days
- Tinidazole 1 g orally once daily for 5 days
- Clindamycin 300 mg orally twice daily for 7 days
- Clindamycin ovules 100 mg intravaginally once at bedtime for 3 days

Recently, single 2g dose of secnidazole has been recently approved for BV and will likely be included in the anticipated 2020 guidelines (see: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209363s000lbl.pdf). Follow-up is unnecessary if symptoms resolve, but recurrence is common.

Summary

Trichomoniasis is the most common curable STD worldwide. Treatment is usually achieved with metronidazole. In the CDC 2015 guidelines, the Aptima *Trichomonas vaginalis* assay (Hologic; San Diego, CA), a NAAT, was identified as a test cleared by the FDA for the detection of *Trichomonas vaginalis*; the assay detected 3- to 5-fold more *Trichomonas vaginalis* infections than did wet mount microscopy. Since then, other NAATs have been developed and approved, such as the BD ProbeTec *Trichomonas vaginalis* Qx Amplified DNA Assay and the BD Max Vaginal Panel (Becton Dickinson; Franklin Lakes, NJ). CDC 2015 guidelines also suggest that if highly sensitive testing, such as NAAT, is not immediately feasible, a testing algorithm should be employed using wet mount first, followed by NAAT when feasible if wet mount is negative.

VVC is present in 17% to 39% of women with symptoms. Nearly three-quarters of women develop VVC during their lifetime; about 45% suffer a second occurrence, and approximately 5% will have [frequently] intractable recurrent candidiasis. Laboratory diagnosis is often time-consuming and not readily available in most offices. As described previously, in 2016, FDA provided market authorization for the BD MAX™ Vaginal Panel to detect microorganisms responsible for bacterial vaginosis, trichomoniasis, and vulvovaginal candidiasis in a single test, representing the first multiplex, real-time PCR assay. Moreover, on May 29, 2019 FDA cleared the Aptima BV and Aptima CV/TV molecular assays that may well usher in a new era of comprehensive and objective diagnostic testing for vaginitis.[48] A short course of topical formulations for 1 to 3 days or single dose oral fluconazole are usually effective in treating uncomplicated VVC, with azole drugs being more effective than nystatin.

BV is the most common finding in women with vaginal symptoms, affecting between one-quarter and one-half of symptomatic women. Treating women may reduce the risk of acquiring other STIs, including *Chlamydia trachomatis, Neisseria gonorrhoeae*, *Trichomonas vaginalis*, HIV, and herpes simplex type 2. There are a number of established treatment regimens for BV using metronidazole, clindamycin, tinidazole, and secnidazole. NAAT testing is quickly becoming the gold standard diagnostic test for BV.

Due to the high prevalence of these three STIs, clinicians need to be more aware of them and be knowledgeable of their signs and symptoms in addition to the means to diagnose and treat them effectively.

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