Educational Case

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Educational Case: Infectious Diseases: Pathogenesis, Diagnosis, Treatment, and Prevention

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The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see http://journals.sagepub.com/doi/10.1177/2374289517715040.

Keywords
pathology competencies, diagnostic medicine, cytopathology, cytologic diagnosis, infectious diseases, cervix disease, herpes infection, trichomoniasis, candidiasis

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Primary Objective

Objective CYP1.3: Identifying Infectious Diseases. Describe the uses and limitations of cytology, with examples, in identifying common infectious diseases.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic CYP: Cytopathology; Learning Goal 1: Cytologic diagnosis.

Patient Presentation #1

A 32-year-old female, gravida 1 para 0, at 36 weeks of gestation, presented to the OB/GYN clinic complaining of itchiness and pain in the genital area. The patient denied a significant past medical history. Physical examination revealed several small vesicles in the vulvar and vaginal area. A cervical vaginal cytology (PAP) test was performed.

Diagnostic Cytologic Findings, Part 1

Microscopic examination of the SurePath Pap test showed cells with dense, intranuclear inclusions surrounded by a clear halo and multinucleated cells with nuclear molding and chromatin margination beneath the nuclear membrane imparting a clearing or "stained glass appearance" of the nuclei (Figure 1).

What Is Your Differential Diagnosis Based on the Clinical History and Cytologic Findings?

The differential diagnosis for large multinucleated cells on cervical Pap test includes reactive endocervical cells, herpes simplex virus (HSV) infection, syncytiotrophoblasts, low-grade squamous intraepithelial cells, neoplastic cells (such as carcinosarcoma, choriocarcinoma), and radiation- or chemotherapy-induced changes. Based on the cytological features,[1] the final diagnosis for this Pap was "cellular changes consistent with HSV infection."

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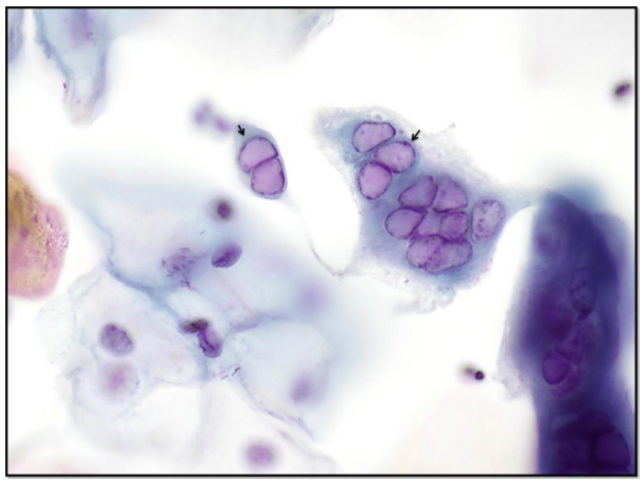

Figure 1. Slide shows a single and a multinucleated squamous cells with nuclear molding and chromatin margination (arrow) beneath the nuclear membrane imparting a clearing or "ground glass appearance" of the nuclei (PAP-stained, high power ×60 magnification).

Questions/Discussion Points, Part 1

What Is Genital Herpes Simplex Virus Infection?

Herpes simplex virus, commonly known as herpes, is a chronic, common, life-long viral infection caused by the 2 members of the herpesvirus family, *Herpesviridae*. Herpes simplex virus are categorized into 2 types: HSV-1 and HSV-2. Herpes simplex virus type 1 is mainly transmitted by oral-to-oral contact and causes infection in or around the mouth (oral herpes). Herpes simplex virus type 2 is almost exclusively sexually transmitted causing infection in the genital or anal area (genital herpes). Most cases of recurrent genital herpes are caused by HSV-2, and approximately 50 million people in the United States are infected with this type of genital herpes.[2,3]

Describe the Symptoms and Transmission of Genital Herpes Simplex Virus

Genital herpes infections often are asymptomatic or might present with mild symptoms that sometimes go unrecognized. Most infected people are unaware that they have the infection. When symptoms do occur, genital herpes is characterized by one or more genital or anal blisters or open sores (also called ulcers), pain during urination, and itching. In addition to genital ulcers, symptoms of recently acquired genital herpes infections often include fever, fatigue, body aches, and swollen lymph nodes. After an initial genital herpes infection with HSV-2, recurrent symptoms are common but often less severe than the first outbreak, and the frequency of outbreaks tends to decrease over time.

Herpes simplex virus type 1 and 2 are transmitted by contact with an infected person. Herpes simplex virus type 2 is mainly transmitted during sex, through contact with genital surfaces, skin, sores, or fluids. Herpes simplex virus type 2 is periodically shed in the human genital tract, and most sexual transmissions occur during periods of asymptomatic shedding. The majority of HSV-1 infections spread easily via contact with sores, saliva, and surfaces in or around the mouth causing oral herpes infection. However, HSV-1 can also be transmitted to the genital area through oral–genital contact to cause genital herpes.[3]

Both viruses may also be transmitted vertically during childbirth. However, the risk of infection transmission is minimal if the mother has no symptoms or exposed blisters during delivery. The risk is considerable when the mother is infected with the virus for the first time during late pregnancy.

How Is Herpes Simplex Virus Infection Diagnosed?

The patient's prognosis and the type of counseling needed depend on the type of genital herpes (HSV-1 or HSV-2) causing the infection; therefore, the clinical diagnosis of genital herpes should be confirmed by type-specific laboratory testing. Both type-specific virologic and type-specific serologic tests for HSV should be available in clinical settings that provide care to persons with or at risk for sexually transmitted diseases (STDs).[2] The Centers for Disease Control and Prevention recommends that persons with genital herpes should be tested for HIV infection as well.[2]

The following tests are used for HSV diagnosis:

- *Viral culture.* This test involves taking a tissue sample or scraping of the sores for examination in the laboratory. The sensitivity of viral culture is low, especially for recurrent lesions, and declines rapidly as lesions begin to heal.
- *Polymerase chain reaction test.* Polymerase chain reaction is used to copy the DNA from a sample of blood, tissue from a sore, or spinal fluid. The DNA can then be tested to establish the presence of HSV and determine which type of HSV. Polymerase chain reaction is the test of choice for diagnosing HSV infections affecting central nervous system (CNS) and systemic infections.
- *Blood test.* Both type-specific and type-common antibodies to HSV develop during the first several weeks after infection and persist indefinitely. The blood sample is analyzed via enzyme-linked immunosorbent assay which detects IgM and type-specific IgG.

Although the cytologic detection of cellular changes associated with HSV infection is an insensitive and nonspecific method of diagnosing genital lesions (ie, Tzanck preparation), the cytologic changes of infected cells are recognized in PAP cervical smears.

In 1989, the Bethesda System (TBS) was introduced to standardize the reporting cervical cytology PAP results and to incorporate the insights into human papillomavirus (HPV) biology and cervical disease association. The current 2014

TBS, like its predecessors, recommends the use of a specific format for the cytology cervical report.[1]

The cytologic features of the HSV-infected squamous cells are variable. The infected cells can show accumulation of viral particles within the nuclei, resulting in an eosinophilic inclusion, known as Cowdry inclusions, which are classified as type A (inclusion in the nucleus) and type B (inclusion in the cytoplasm), which are seen in cytomegalovirus infection. The cells can also show lysis of the chromatin giving a glassy appearance to the nuclei. The infected cells aggregate forming a **M**ulti-nucleated cell with nuclear **M**olding and chromatin **M**argination (3 M's of herpes) features easily recognized under the microscope[1,4]; however, some other lesions or changes may mimic HSV infection on cytologic examination, including reactive or reparative changes, air drying artifact, multinucleated giant cells, poor cell preservation, radiation effect, among others.[4] Under the current Bethesda system for reporting PAP results, for the cytologic changes caused by HSV infection, it is recommended to interpret it as "cellular changes consistent with HSV.[1]

Which Are the Possible Complications of Herpes Infection?

Severe disease. In immunocompromised people, such as those with advanced HIV infection, HSV-1 can have more severe symptoms and more frequent recurrences. Rarely, HSV-1 infection can also lead to more severe complications such as encephalitis or keratitis (eye infection).

Genital herpes in pregnancy and neonatal herpes. Neonatal herpes can occur when an infant is exposed to HSV in the genital tract during delivery. This is a rare condition, occurring in an estimated 10 out of every 100 000 births globally, and the risk of HSV transmission to the neonate from an infected mother is high (30%-50%) among women who acquire genital herpes near the time of delivery and low (<1%) among women with prenatal histories of recurrent herpes or who acquire genital HSV during the first half of pregnancy.[2] Neonatal HSV infection can be divided into 3 clinical groups: (1) skin, eyes and mouth disease (SEM) is a localized infection affecting the skin, eyes, or mouth; (2) CNS disease is defined as encephalitis with or without SEM disease, and (3) disseminated disease involves infection in multiple organ systems and can include hepatitis, pneumonitis, and disseminated intravascular coagulation. Cutaneous lesions may be seen in all types and disseminated disease may occur with or without the presence of CNS disease.[3]

Prevention of neonatal herpes depends both on preventing acquisition of genital HSV infection during late pregnancy and avoiding exposure of the neonate to herpetic lesions and viral shedding during delivery. Women without symptoms or signs of genital herpes or its prodrome can deliver vaginally. Although cesarean delivery does not completely eliminate the risk for HSV transmission to the neonate, women with recurrent genital herpetic lesions at the onset of labor should deliver by cesarean delivery to reduce the risk for neonatal HSV infection.[2] Acyclovir can be administered orally to pregnant women with first-episode genital herpes or recurrent herpes and should be administered intravenously to pregnant women with severe HSV infection. All infants who have neonatal herpes should be promptly evaluated and treated with systemic acyclovir.

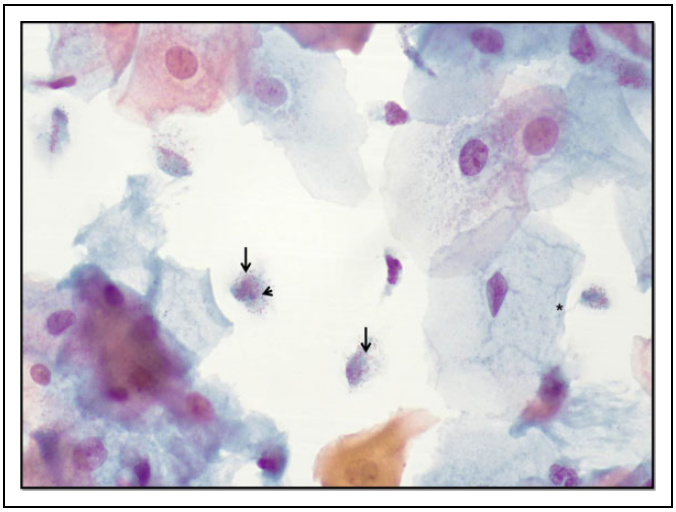

Figure 2. Slide shows several oval-shaped organisms (arrow) with eccentric dark nuclei (arrow head) and gray cytoplasm with red granules. A flagella (asterisk) is also seen. Benign squamous cells are present in the background. These organisms are consistent with *Trichomonas vaginalis* (PAP-stained, high power ×60 magnification).

What Is the Treatment for Herpes Infection?

Antivirals, such as acyclovir, famciclovir, and valacyclovir, are the most effective medications available for people infected with HSV. These can help to reduce the severity and frequency of symptoms, but cannot cure the infection.[2,3]

In our case, the patient was treated with oral acyclovir with improvement of the symptoms. Soon after, she delivered a healthy female baby. Her HIV testing was negative.

Patient Presentation #2

A 67-year-old male with a past medical history of urolithiasis and blood in his urine (gross hematuria) presented to his primary care physician for follow-up. Urine was collected and sent for cytologic examination.

Diagnostic Cytologic Findings, Part 2

Microscopic examination of the slide revealed benign urothelial and squamous cells in a background of acute inflammation and red blood cells. Incidentally, scattered, small, oval-shaped "cells" with eccentric dark nuclei and gray cytoplasm with rare red cytoplasmic granules were also seen (Figure 2).

What Is Your Differential Diagnosis Based on the Clinical History and Cytologic Findings?

The differential diagnosis for these small "cells" includes degenerated inflammatory cells, cellular debris, cellular fragments, and parasites. The cytologic findings were consistent with *Trichomonas* organisms.

Questions/Discussion Points, Part 2

What Is Trichomoniasis and How Is It Transmitted?

Trichomonas vaginalis (commonly known as "trich") is an anaerobic, flagellated protozoan parasite and the causative agent of the STD trichomoniasis. Trichomoniasis is the most prevalent nonviral STD in the United States, affecting an estimated 3.7 million people.[5]

Trichomoniasis is typically found in sexually active patients. Transmission occurs predominantly via sexual intercourse. In women, the most commonly infected part of the body is the lower genital tract (vulva, vagina, cervix, or urethra). In men, the most commonly infected body part is the urethra. It has not been isolated from oral sites, and rectal prevalence appears to be low in men who have sex with men.[5]

What Are the Signs and Symptoms of Trichomoniasis?

About 70% to 85% of infected people have minimal symptoms or are asymptomatic.[5] When trichomoniasis does cause symptoms, they can range from mild irritation to severe inflammation. Some people are symptomatic within 5 to 28 days after infection. Symptoms can come and go, and untreated infections might last for months to years.[5,6]

Some infected men have symptoms of urethritis (urethral discharge) and pain during urination, and some infected women have vaginal discharge that might be diffuse, malodorous, or yellow-green with or without vulvar irritation. Other symptoms includes itching, discomfort during sexual intercourse or urination, and cervicitis in women, which is characterized by purulent discharge and easily induced endocervical bleeding, also known as "strawberry" cervix (due to capillary dilation as a result of the inflammatory response).

What Are the Complications of Trichomoniasis?

Trichomonas vaginalis infection is associated with 2- to 3-fold increased risk for HIV acquisition. It also increases the susceptibility to other viruses, including herpes and HPV. In pregnant women, *T vaginalis* infection has been associated with an increased risk of low birth weight, preterm delivery, and intrauterine infection.[5]

Neonatal trichomoniasis has been described.[6] Respiratory or genital infection in the newborn may also occur. In men, complications of untreated trichomoniasis include prostatitis, epididymitis, urethral stricture disease, and infertility, potentially resulting from decreased sperm motility and viability.

How Is Trichomoniasis Diagnosed?

It is not possible to diagnose trichomoniasis based on symptoms alone. In women, vaginal trichomoniasis has historically been diagnosed by wet mount microscopy, which is performed by placing a small amount of vaginal discharge on a microscope slide and mixing with a few drops of saline solution. The slide is then examined under a microscope at low or medium power and a "corkscrew" motility is observed (parasite moving through the field). It is the most common method for *T vaginalis* diagnosis because of convenience and relatively low cost.[5,6]

Culture was considered the gold standard method for diagnosing *T vaginalis* infection before molecular detection methods became available. *Trichomonas vaginalis* may be accurately identified on Pap smear by morphology alone, but this test yields low sensitivity (50%-80%) and false-positive results are also common with this technique.[5] Cellular debris and degenerated inflammatory cells may be mistaken for trichomonads on cytologic examination.[4]

The use of highly sensitive and specific methods using molecular techniques for detecting antigens, DNA, or RNA are currently recommended for diagnosing *T vaginalis*.[5]

What Is the Treatment for Trichomoniasis?

Trichomoniasis can be treated with nitroimidazole, which is the only class of antimicrobial medications known to be effective against *T vaginalis* infections. Of these drugs, metronidazole and tinidazole have been cleared by the Food and Drug Administration (FDA) for the oral or parenteral treatment of trichomoniasis. It is not recommended to drink alcohol within 24 hours after taking this medication.

Because of the high rate of reinfection among women treated for trichomoniasis ($\sim$17%), retesting for T vaginalis is recommended for all sexually active women within 3 months following initial treatment regardless of whether they believe their sex partners were treated.[5] Concurrent treatment of all sex partners is critical for symptomatic relief, microbiologic cure, and prevention of transmission and reinfections. Current partners should be referred for presumptive therapy to avoid reinfection.

Although metronidazole crosses the placenta, data suggest that it poses a low risk to pregnant women. No evidence of teratogenicity or mutagenic effects in infants has been found in multiple studies of pregnant women. Women can be treated with 2 g metronidazole in a single dose at any stage of pregnancy. The patient and his partner were treated with oral metronidazole.

Patient Presentation #3

An 11-month-old girl with 1-month history of emesis, gagging, and choking with textured foods was brought to her pediatrician by her parents and, after examination, was admitted for management. The prior month, she was briefly admitted to the

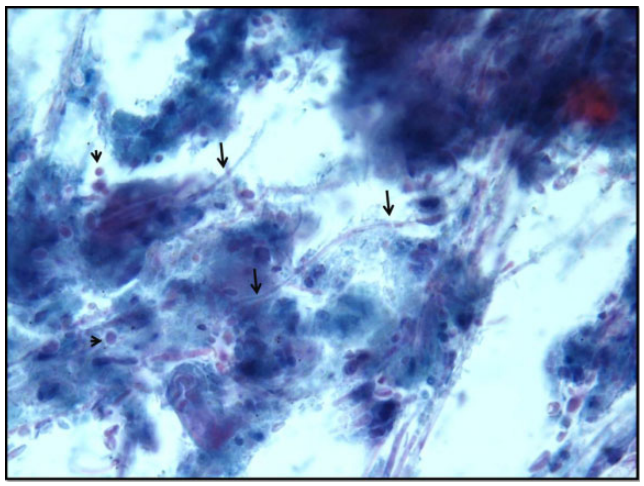

Figure 3. Microscopic examination of the smears reveled esophageal squamous mucosa admixed with acute inflammatory cells and fungal hyphae (arrow) and spores (arrow head), morphologically consistent with *Candida* species (PAP-stained, high power ×60 magnification).

hospital for a respiratory syncytial virus bronchiolitis and was given oral prednisone 10 mg twice a day for 5 days. On current admission, the baby underwent an esophagogastroduodenoscopy. The middle to distal esophagus was covered with yellow-white plaques scattered over the mucosa. The mucosa was hyperemic and friable, and the plaques could not be washed off. The lesions bled easily at the site of attachment, where they were brushed for cytology, Gram stain, and fungal culture. Biopsies were also taken.

Diagnostic Cytologic Findings, Part 3

Microscopic examination of the cytology brushing smears reveled esophageal squamous cells admixed with acute inflammatory cells. Fungal pseudohyphae and spores, morphologically consistent with *Candida* species, were also present (Figure 3). The Grocott-Gomori's methenamine silver (GMS) stain on smears and culture also showed *Candida* organisms. The biopsy results were consistent with chronic esophagitis suggestive of reflux, with *Candida*-like organisms. She was treated with oral fluconazole and omeprazole with improvement of her symptoms.

Questions/Discussion Points, Part 3

What Is Candidiasis?

Candidiasis is a fungal infection caused by the yeast *Candida*. More than 20 types of *Candida* can cause infection, with *Candida albicans* being the most common. *Candida* yeasts are generally present in healthy humans, frequently as part of the human body normal oral and intestinal flora, and particularly on the skin, but it can become pathogenic if the host's luminal flora or immune defenses are altered.[7,8]

Factors that increase the susceptibility of the host to *Candida* infection are various malignancies, immunodeficiency states, diabetes, stress, antibiotic treatments, nutrient deficiency, endocrinopathies, long-term use of steroids, and other immunosuppressive drugs.

What Is the Clinical Presentation of Candidiasis?

Signs and symptoms of candidiasis vary depending on the area affected. Most candidal infections result in minimal complications such as redness, itching, and discomfort, though complications may be severe or even fatal if left untreated in certain populations.

The clinical presentation can be broadly divided into cutaneous candidiasis, mucosal candidiasis, and systemic candidiasis. In immunocompetent persons, candidiasis is usually a localized infection of the skin, fingernails or toenails (onychomycosis), or mucosal membranes. The most common manifestation of candidal infection in infants is diaper dermatitis. Candida organisms can also cause intertrigo in older individuals. The oral infection or oropharyngeal candidiasis, commonly called "thrush," frequently occurs in infants and toddlers. Infection in the esophagus is called esophageal candidiasis or Candida esophagitis, and the most common presenting complaints are difficulty and pain with swallowing, with one-third of patients also having oral thrush. Infection of the vulva or vagina also referred as "yeast infections" or "vulvovaginal candidiasis" affects nearly 75% of women, usually causing mild symptoms, such as itching, burning, soreness, irritation, and a whitish or whitish-gray cottage cheese-like discharge.[7]

Candida organisms can cause severe systemic infections in immunocompromised patients, such as patients with AIDS, premature babies, critically ill patients, or patients with cancer. In these patients, *Candida* yeast enters the bloodstream and spreads to internal organs, such as CNS, kidneys, liver, bones and joints, among others.

How Is Candidiasis Diagnosed?

Depending on the clinical presentation, several diagnostic tests are available and include the following:

- Mucocutaneous candidiasis—Using a wet mount, scrapings or smears obtained from skin, nails, or oral or vaginal mucosa are examined under the microscope; a potassium hydroxide (KOH) smear, GMS stain, or methylene blue is useful for direct demonstration of fungal organisms.
- Cutaneous candidiasis—Using a wet mount, scrapings or smears obtained from skin or nails can be examined under the microscope; KOH smears are also useful.
- Genitourinary candidiasis—A urinalysis and urine fungal cultures are useful. A sample of vaginal discharge can be examined under the microscope or sent to a laboratory for a fungal culture.

- Gastrointestinal candidiasis—Endoscopy with or without biopsy and brushings. Samples are submitted for microscopic examination, GMS stain, and culture.
- Systemic candidiasis—Usually blood cultures.

Candida is a common finding on PAP cervical smears and identification does not necessarily indicate infection (commensal in vaginal mucosa). Noncellular substances such as fibrin and contaminants such as carpet fibers or other fungi can be also mistaken with *Candida* under microscopic examination. The Bethesda system recommends reporting these fungi as "fungal organism morphologically consistent with *Candida spp*".[1]

How Is Candidiasis Treated?

Candidiasis is generally treated with antifungal medications; these include clotrimazole, nystatin, fluconazole, voriconazole, amphotericin B, and echinocandins. The management also depends on the clinical presentation.[7]

- Cutaneous candidiasis—Most localized cutaneous candidiasis infections can be treated with any number of topical antifungal agents.
- Mucocutaneous candidiasis—For oral cases, topical antifungals are used. For severe infections, treatment with oral agents is commonly used.
- Esophageal candidiasis—Treatment requires systemic therapy with fluconazole.
- Systemic candidiasis—Treatment requires systemic intravenous therapy.

Teaching Points

- Some infections are easily recognized with cytologic examination of PAP and other body site specimens.
- Herpes infections, oral and genital, are common chronic infections due to the herpes virus (HSV-1 and HSV-2), characterized by itching and blisters/sores on the mouth and/or genital area.
- Genital HSV in pregnant women can be complicated with neonatal herpes.
- Trichomoniasis, caused by the *T vaginalis* parasite, is one of the most common STD infections in the United States, and is usually asymptomatic.
- *Candida spp* is a fungus (yeast), part of the human body normal mucocutaneous and intestinal flora, but it can become pathogenic if the host's luminal flora or immune defenses are altered.
- Although HSV, *Candida spp* and *Trichomonas* infections can be identified with cytologic examination, false-positive results are common, sometimes requiring alternative confirmatory tests.

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