The Brief Case: Disseminated Microsporidiosis with Intestinal Cryptosporidium Coinfection in a Patient with Kaposi’s Sarcoma and Castleman Disease Presenting with Acute Kidney Injury

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CASE

This is a case report of a 35-year-old male with a 12-year history of HIV/AIDS who was poorly compliant with highly active antiretroviral therapy (HAART). His past medical history included oral thrush, intestinal Cryptosporidium infection, cutaneous Kaposi’s sarcoma, and lymphadenopathy due to concomitant Kaposi’s sarcoma (detected 2 months back) and multicentric Castleman disease (detected 2 months back).

On this admission, he presented with watery diarrhea, fever up to 103°F, and tachycardia up to 170 beats per minute. His creatinine at that time was 4.5 mg/dl, and soon thereafter, bibasilar lung opacities were noted. His creatinine peaked at 6.3 mg/dl. His baseline creatinine was 0.8 to 1.0 mg/dl. During his current admission, BK virus was detected in urine with a level of 68,509 IU/ml and Cryptosporidium spp. by PCR in stool. A sputum specimen was submitted to microbiology, and he was given cefepime and atovaquone for Pneumocystis pneumonia prophylaxis. A sputum Gram stain showed intracellular Gram-positive spores with a belt-like stripe in the center (Fig. 1A), and calcofluor white stain showed intracellular fluorescent spores (Fig. 1B). On this basis, he was diagnosed with microsporidia-like microorganisms, and albendazole was administered.

He was transferred to the intensive care unit due to declining renal function. Acute kidney injury was initially considered to be prerenal due to nausea and vomiting; however, fractional excretion of sodium was 7.9%, which suggested an intrinsic renal failure. A renal biopsy was performed, and this was notable for intratubular and interstitial lymphohistiocyte aggregates (Fig. 1C). Microsporidia-like organisms were highlighted by Gram stain, Lillie-Twort Gram stain (Fig. 1D to F), and Grocott’s methenamine silver stain (Fig. 1G). Immunohistochemistry was reactive for Encephalitozoon spp. (Fig. 1H). On electron microscopy, the organisms were predominantly degenerated, consisting of 1- to 2-μm spores in different stages of development (Fig. 2A). Rare viable microorganisms containing seven coils of polar tubules were identified, confirming presence of microsporidium (Fig. 2B). Subsequently, Encephalitozoon intestinalis species was confirmed by PCR and DNA sequencing (1).

He received atovaquone and steroids for suspected PCP pneumonia. He received azithromycin for 6 weeks for Cryptosporidium diarrhea. In addition, for disseminated microsporidia, he was started on albendazole for 3 weeks. His treatment for Kaposi’s sarcoma and multicentric Castleman disease was temporarily discontinued until renal function improved. After initiation of treatment, his fever subsided. Renal function

Citation


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For answers to the self-assessment questions and take-home points, see https://doi.org/10.1128/JCM.02336-20 in this issue.  

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FIG 1 (A) Gram stain of sputum showing Gram-positive spores (arrow) with belt-like stripe; ×1,000 magnification, oil immersion. (B) Sputum calcofluor white stain showing multiple fluorescent ovoid spores (arrow) of microsporidia; ×400 magnification. (C) Kidney biopsy specimen showing lymphohistiocytic infiltration in the interstitium (arrow, histiocyte). Hematoxylin and eosin (H&E) stain; ×400 magnification. (D) Lillie-Twort Gram stain of kidney biopsy specimen showing small ovoid Gram-positive organisms (arrow) in the tubules; ×400 magnification. (E) Gram stain of kidney biopsy specimen showing ovoid Gram-positive organisms (arrow); ×400 magnification. (F) Gram stain of kidney biopsy specimen at higher magnification showing Gram-positive spores (arrow); ×1,000 magnification. (G) Gomori methenamine silver (GMS) stain showing small ovoid organisms in the tubules; ×400 magnification. (H) Kidney biopsy specimen showing immunostaining of abundant microsporidia (arrow) in the tubules and interstitium; ×400 magnification.
gradually improved, with a final measured creatinine of 3.2 mg/dl, and the patient was ultimately discharged from the hospital.

DISCUSSION

Microsporidia are a large group of obligate intracellular parasitic pathogens producing heat-resistant spores which protect the organisms from the environment. They have a unique mode of infection in which a coiled polar tubule is ejected on germination that facilitates transmitting the infective sporoplasm into the host. Over the past 150 years, the taxonomy of microsporidia has been changed multiple times. Initially, it was considered a yeast-like fungus and subsequently classified as Sporozoa. As the organism lacks eukaryotic features such as mitochondria, it was reclassified as Archezoa. Later, genetic studies disclosed genes encoding mitochondrial proteins, including heat shock proteins, providing support for mitochondriate ancestry (2–4). After further molecular characterization, they are now considered related to fungi (5–7).

There are more than 1,400 species of microsporidia which can infect vertebrates as well as invertebrate animals. Approximately 15 species are pathogenic to humans, the most common of which is Enterocytozoon bieneusi, followed by Encephalitozoon species. They produce spores ranging from 1 to 4 μm and infect predominantly by ingestion and involvement of the gastrointestinal tract, but disseminated infections can occur in immunocompromised patients.

Microsporidia can be detected by light microscopy using various staining techniques. These include Chromotrope 2R, quick-hot Gram chromotrope technique, Gram stain, trichrome blue, acid-fast stain, Warthin-Starry stain, and modified trichrome stains. On Gram stain, the mature spores are Gram positive, whereas the immature spores are Gram negative, and it is possible for them to be misinterpreted as bacilli. On modified trichrome, the spore stains pink with a clear interior and a diagonal stripe. Calcofluor white may be utilized as well. It stains the cell wall containing chitin and is detected at a wavelength of 395 to 415 nm using a fluorescence microscope. The staining, however, is nonspecific. Identification by transmission electron micrography is considered the gold standard where internal structures can be identified. Characteristic features are noted, such as number of coils of the polar tubule and presence of vacuoles within infected cells containing these fungi in different stages of development. Other methods of detection include PCR and immunohistochemistry.

Common Encephalitozoon species include E. intestinalis, E. hellem, and E. cuniculi. When in contact with the host cell, spores germinate and extrude the polar tubule whereby the sporoplasm is injected into the host cell. The sporoplasm undergoes binary fission to develop into meronts. Meronts undergo sporogony to develop into sporonts followed by sporoblasts and spores. All three of these species produce spores in vacuoles called parasitophorous vacuoles. However, in E. intestinalis, the sporoblasts

FIG 2 (A) Electron microscopy of renal biopsy specimen showing a parasitophorous vacuole with degenerated microsporidia in different stages of development separated by septae. (B) Electron microscopy of renal biopsy specimen showing seven coils of polar tubule in microsporidium.
are separated from each other by fibrillar matrix, giving it the characteristic septated vacuole, which can be visualized by electron microscopy and which gives rise to its initial name, *Septata intestinalis*.

This case report highlights the importance of microsporidia in the differential diagnosis of an immunosuppressed patient presenting with fever and acute renal failure and illustrates the appearance of these microorganisms in multiple preparations, as well as that *E. intestinalis* can infect the kidneys. This is a rare case presenting with multiple coinfections in a patient with microsporidiosis (in kidney and lung), cryptosporidiosis, BK virus infection, and human herpesvirus 8 (HHV-8)-associated multicentric Castleman disease and Kaposi’s sarcoma.

**SELF-ASSESSMENT QUESTIONS**

1. What is the infective form of microsporidia?
   - a. Meront
   - b. Sporont
   - c. Sporoblast
   - d. Spore
2. What is the gold standard test for identification of microsporidia?
   - a. Electron microscopy
   - b. Gram stain
   - c. PCR test
   - d. Serum IgG levels
3. What is the morphology of microsporidia on Gram stain?
   - a. Gram-positive diplococci
   - b. Gram-negative bacilli
   - c. Gram-variable spore with band-like stripe
   - d. True hyphae

**REFERENCES**