with a high dose of corticosteroids, and if it does not, other diagnoses should be considered.

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Diagnosis of Microsporidia Keratitis by Polymerase Chain Reaction

Report of a Case. A 33-year-old man positive for human immunodeficiency virus had a 6-month history of bilateral blurred vision, tearing, and photophobia, with associated conjunctival hyperemia. The patient had been previously treated with topical antibiotic drops, topical steroids, and topical nonsteroidal anti-inflammatory drops for several months without resolution of his symptoms. He was taking highly active antiretroviral therapy as well as trimethoprim-sulfamethoxazole prophylaxis; his CD4 cell count was 3. His medical history included weight loss and diarrhea. His ocular history was otherwise unremarkable. His uncorrected visual acuity was 20/70 OD and 20/50 OS with pinhole to 20/50 OD and 20/30 OS. The conjunctiva had 2+ hyperemia with a moderate papillary reaction bilaterally. The corneas exhibited diffusely distributed small, gray intracorneal epithelial lesions without associated stromal infiltrate (Figure 1). There was no anterior chamber reaction, and the posterior segments were unremarkable.

The patient underwent a conjunctival biopsy and cytologic smear examination. Gram stain, bacterial culture, fungal culture, chlamydial testing, and viral culture were negative. Cytologic examination was negative for microsporidia or typical viral or chlamydial inclusion bodies. A fine-needle corneal epithelial scraping was performed. The scraped sample was suspended in 50 µL of phosphate-buffered saline and boiled for 15 minutes. Polymerase chain reaction (PCR) for microsporidia was performed using a protocol adapted from Muller et al,1 which is capable of identifying several Enterocytozoon and Encephalitozoon species of microsporidia. Briefly, 5 µL of the sample was subjected to 35 cycles of thermocycling using forward primer V1 (5’-CACTGACATTCT-GCCTGAC-3’) and reverse primer PMP2 (5’-CCTCTCCCGAAC-CAAACCGTG-3’) with 1-minute denaturation at 94°C, 2-minute annealing at 60°C, and 3-minute extension at 72°C. A single ~270-base pair fragment was observed on agarose gel electrophoresis and ethidium bromide staining of the PCR-amplified patient sample, but not from the phosphate-buffered saline-only control sample (Figure 2A). The PCR product was directly sequenced. A BLAST search of the National Center for Biotechnology Information database revealed a near-perfect alignment with the ribosomal RNA small unit gene of Encephalitozoon hellum (Figure 2B). The patient was prescribed hourly 1% topical clotrimazole and 100 mg of oral itraconazole 2 times daily, and showed gradual improvement in his symptoms and clinical findings during a 2-week period.

Comment. Microsporidia are a group of at least 6 genera of intracellular protozoa that are frequent opportunistic pathogens in immunocompromised patients, particularly infecting the gut. Diagnosis is
challenging and has generally relied on direct visualization of the parasites on cytologic or histologic sections. Microsporidia keratitis has been reported in immunocompromised hosts but has been difficult to diagnose; the sensitivity of direct observation for detection of the pathogen is unknown. Although serologic testing is positive in patients with microsporidia keratitis, the specificity of this finding in patients with human immunodeficiency virus is also unknown. Our report demonstrates that the PCR, which is useful in the diagnosis of a number of ocular infectious conditions, may be successfully applied to the diagnosis of microsporidia keratitis and may have higher sensitivity than traditional cytologic and histologic detection methods. Its specificity is presently unknown. Further studies to determine the sensitivity, specificity, and positive and negative predictive values of PCR for microsporidia keratitis will define the full clinical utility of this test.

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