Diagnosis of Aspergillus Keratitis In Vivo with Confocal Microscopy

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Confocal microscopy provides a new, noninvasive way of imaging the human cornea in vivo. One of its most important clinical uses is the diagnosis and management of infectious keratitis. The authors used tandem scanning confocal microscopy to image the corneas in two culture-proven cases of Aspergillus keratitis. Fungal hyphae were imaged as high-contrast filaments 6 μm in diameter from 60 to 400 μm in length. Confocal microscopy may be a fast and safe diagnostic tool in determining the presence of fungal hyphae in vivo within the human cornea.

Key Words: Confocal microscopy—Cornea—Fungal keratitis.

The confocal microscope is a new, noninvasive technique for imaging the cornea. It provides superior resolution and contrast compared with conventional slit-lamp microscopy and enables us to image a single plane within the cornea without interference from scattered light above and below the focal plane (1). The confocal microscope has been used in vivo to image infectious keratitis in animal and, more recently, human corneas. Imaging Aspergillus keratitis in a rabbit eye was recently reported by Chew et al. (2). There has been no reported use of the confocal microscope in imaging keratomycosis in human corneas. We present here two cases of Aspergillus keratitis, in which the confocal microscope produced images of fungal elements.

The TSC microscope, model 165A, and objective lens used in this study were manufactured by the Tandem Scanning Corporation (Reston, VA, U.S.A.). This confocal microscope was recently approved for clinical use by the U.S. Food and Drug Administration. An image-intensifier camera (Dage-MTI, Inc., Michigan City, IN, U.S.A.) was used to capture the images in real time, 30 frames/s. These were recorded on Super-VHS tape by using a Panasonic Super four-head videocassette recorder. No image processing was used to enhance the images. Individual images were captured from videotape for publication. Background information regarding this instrument and details of how it is used clinically are described elsewhere (3).

CASE REPORTS

Patient 1

The patient was a 56-year-old white man who was referred to the University of Iowa on October 25, 1993. A week before, he had felt dust in his left eye from a "moldy corn bin." He was treated with 0.3% tobramycin. After initial improvement for 2 days, the symptoms worsened. Corneal cultures grew Aspergillus, and he was referred to the Cornea Service at the University of Iowa for treatment.

On examination, his best corrected visual acuity was 20/16 in the right eye and 20/160 in the left eye. Slit-lamp examination of the right eye was essentially normal. The slit-lamp examination of the left eye was notable for 3+ injection of the conjunctiva. There was a dense, white, anterior stromal infiltrate in the inferocentral cornea, measuring 2×1.5 mm, which was slightly elevated and was associated with an epithelial defect (Fig. 1). The infiltrate was surrounded by stromal haze with a small anterior stromal scar inferonasally. Biomicroscopy of the anterior chamber revealed 2+ cell and no flare. Intraocular pressures and fundus examinations were normal in both eyes.

Confocal microscopy of the left inferocentral cor-
FIG. 1. Slit-lamp photograph of left cornea of patient 1. Note the slightly elevated, dense white anterior stromal infiltrate.

nea in the area of the infiltrate showed a dense mass of interlocking white lines, ~6 μm in width and 200–400 μm in length (Fig. 2). These were presumed to be filaments of Aspergillus. Treatment was begun with topical natamycin (5%) every hour while awake and every 2 h while asleep. He was also treated with topical scopolamine, voltaren, and oral ketoconazole, 200 mg twice daily. Four days later, the stromal infiltrates and epithelial defect were decreased in size. Repeated confocal microscopy revealed multiple short white filaments in the stroma. The longer white filaments were not present. The natamycin was subsequently decreased to every 2 h while awake.

Twelve days later, the patient's visual acuity had improved to 20/50, with a pinhole acuity of 20/30, and the epithelial defect was slightly smaller. Confocal microscopy revealed a number of bright white ovoid masses, 5–10 μm in diameter, at the base of the corneal ulceration. No filamentous organisms were seen. Natamycin was decreased from every 3 h to every 6 h.

Nine days later, there was only a trace epithelial defect, and the infiltrate was decreased in size. Confocal microscopy then revealed that the bright white ovoid masses had disappeared. The topical natamycin was discontinued, and the oral ketoconazole was decreased to 200 mg/day.

Patient 2

The patient was a 32-year-old white man who was treated with high-dose oral prednisone for scleritis in the left eye for 4 months. He was referred to the Cornea Clinic at the University of Iowa on December 13, 1993, for evaluation of his corneal thinning and perilimbal infiltrate. On examination, his visual acuity was 20/20 in the right eye and light perception in the left eye. Slit-lamp and fundus examinations of the right eye were essentially normal. The slit-lamp examination of the left eye was notable for severe conjunctival injection. There was a stromal infiltrate in the peripheral cornea for ~360°. For 5 clock hours, there was an 80% peripheral stromal thinning with an intact epithelium in the area of the infiltrate. Centrally, the cornea showed diffuse haze. There was 4+ cell and flare, which permitted only a very limited view of the iris. There appeared to be a fluffy white mass on the surface of the anterior iris. The intraocular pressure was 30. There was no red reflex or view of the fundus. An ultrasound examination showed thickening and infiltration of the posterior sclera and diffuse vitreous cells.

FIG. 2. Confocal microscopy of left corneal stroma of patient 1. Note the mass of interlocking white lines ~6 μm in width and 200–400 μm in length. These lines are presumed to be filaments of Aspergillus.
Confocal microscopy revealed a number of white lines 200–300 μm in length and 6 μm in width in the corneal stroma (Fig. 3). A corneal scraping, examined with hematoxylin–eosin stain, showed multiple septate hyphae with branches at 45° angles (Fig. 4). A vitreous aspiration was performed, and 0.01 mg of amphotericin B was given intravitreally. The patient was treated with natamycin (5%) drops every 30 min and 40 mg a day of intravenous amphotericin B. Corneal cultures grew α streptococci and Aspergillus flavus. Cultures from episcleral and conjunctival biopsies and vitreous fluid also grew A. flavus. Despite intensive treatment, the condition of the eye further deteriorated, necessitating enucleation.

DISCUSSION

Mycotic keratitis is a slowly progressive infectious keratitis that is difficult to diagnose and is often refractory to treatment. The most frequent settings are ocular trauma from plant material and immunosuppression. Once the organisms gain entrance into the corneal stroma, they slowly proliferate, destroying surrounding tissue with the elaboration of toxins. Inflammatory cells surround the area of necrotic kerocytes and appear clinically as a stromal infiltrate. Peripheral fungal colonies appear as satellite lesions. In contrast to bacteria, fungi can easily penetrate through Descemet’s membrane and gain entrance into the anterior chamber (4).

Because fungal keratitis is relatively rare and clinically resembles more common causes of infectious keratitis, it is frequently not suspected until treatment for bacterial or viral keratitis or both has failed. Even with a high index of suspicion, the diagnosis can be difficult. Identifying fungal elements on corneal scraping is usually sufficient for diagnosis. However, in cases of fungal keratitis, only 65–75% of smears, stained with Gram and Giemsa stains, are positive for fungal hyphae (5,6). For a definitive diagnosis to be established, a positive culture is usually required. Although most fungal cultures can yield positive results within 2–3 days, some take ≤3 weeks to grow. In the case of deep stromal infections, a corneal biopsy or anterior chamber tap may be necessary. In the clinical setting, corneal biopsy is often performed weeks after unsuccessful treatment of presumed bacterial keratitis. This delay in diagnosis and treatment can result in avoidable loss of sight (7,8). Uncontrolled mycotic keratitis can cause corneal perforation and spread to the sclera, both of which threaten the integrity of the globe.

The treatment of fungal keratitis is difficult and not always successful. Only a very limited number of topical antifungal agents have been approved for the eye. Therapy often needs to be continued for several weeks before it is effective (7). Response to treatment may be slow, which increases the importance of being confident of the diagnosis.

The most common agents causing fungal keratitis are Aspergillus, Fusarium, and Candida. Candida albicans is a yeast composed of round, budding bodies that may develop pseudohyphae. Aspergillus is a mold that consists of septate hyphae with dichotomous branches, which are oriented at 45° and are 5–10 μm in diameter (9). Histopathologically, they are quite similar to Fusarium, also a septate

**FIG. 3.** Confocal microscopy of corneal stroma of patient 2. Note branching lines similar in size and shape to those seen in Fig. 2.
filamentous fungus with hyphae that branch at 90° angles.

In both cases of culture-proven Aspergillus keratitis, confocal microscopy showed similar images. An area of interlocking white lines, each line ~6 μm in width and 200–400 μm in length, was visualized in the corneal stroma of both patients. This corresponds to Aspergillus hyphae which, as mentioned, are 5–10 μm in diameter and of varying lengths. The filaments imaged can be contrasted with basal epithelial corneal nerves (Fig. 5). The relatively moderate branching of corneal basal epithelial nerves seen in confocal microscopy differs from the images of fungal filaments, which have much more profuse and chaotic branching. Stromal nerves are much larger in diameter (25–50 μm) and deeper in the stroma. Partial treatment appeared to reduce the filaments to round masses 5–10 μm in diameter. These could be confused with inflammatory cells, which are also small (5–7 μm) but less highly reflective.

Our first patient was diagnosed with mycotic keratitis early in the course of the infection and showed a good response to antifungal therapy. The second patient, whose infection was most likely due to immunosuppression after prednisone administration, was diagnosed much later in the disease process and required enucleation for advanced fungal panophthalmitis. These results are consistent with what is well known about ocular fungal infections: for treatment to be successful, it must be initiated early in the course of the disease.
Confocal microscopy was helpful not only in diagnosis but also in the management of fungal keratitis. The findings on confocal microscopy: The gradual break-up and then disappearance of filamentous forms closely paralleled the improvement observed clinically and confirmed the rate at which to reduce the antifungal therapy. The patients tolerated the confocal microscopy procedure well. The first patient experienced decreased vision in the examined eye for ~10 min, probably from bleaching out of the retina, as occurs in indirect ophthalmology. Except for this, the patients experienced no discomfort or adverse effects from the examination. No change in the visual acuity of the second patient could be discerned. This is consistent with prior reports that demonstrate the clinical safety of this instrument (1,3).

The confocal images of human intracorneal Aspergillus presented here are the first to be reported. A prior study reported the imaging of Aspergillus in rabbit eyes (2). A recent study reported the imaging of Acanthamoeba in the living human cornea (1). The benefits that the confocal microscope provides in the diagnosis and management of Aspergillus keratitis are similar to those of Acanthamoeba keratitis.

As in the case with Acanthamoeba, the confocal microscope provides a significant advantage in diagnosing and managing Aspergillus keratitis because of its ability to image identifying elements in the cornea in vivo. It is able to achieve this because of its increased magnification (×400) and enhanced resolution (lateral resolution is 1–3 μm) versus the more limited resolution of the slit lamp (lateral resolution is 10–20 μm). In addition, confocal microscopic images with their optical sectioning have significantly improved contrast that obviates the need for staining for visualization.

A high degree of patient cooperation is required for successful imaging; even small eye movements blur the images. It is, therefore, not a reasonable diagnostic option in children, very highly debilitated patients, or in perforated corneas. The report of these two cases suggests that the confocal microscope is a fast, safe, and sensitive diagnostic tool in determining the presence of fungal keratitis.

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REFERENCES