ultrasonographically mimicked a choroidal melanoma. The initial ultrasonographic characteristics suggestive of a melanoma include the collar-button shape, low internal reflectivity, and moderate vascularity. The reduction in lesion thickness and change from low-medium to medium internal reflectivity on A-scan examination across time argue against a melanoma. Whereas the collar-button shape is characteristic of a melanoma, other lesions such as choroidal metastases and disciform processes can have this configuration. We presume that the sizable preretinal fibrosis accounts for the vascularity seen on ultrasonography, and the membrane size explains the diffuse rather than expected focal nature of the vascularity. Although the long duration of the vitreous hemorrhage argues against a melanoma, the clinical picture was complicated by the use of warfarin. The dense vitreous hemorrhage seen initially as well as the changing characteristics on serial ultrasonographic examinations helped determine the ultimate diagnosis and guided the physicians in avoiding unwanted treatment.

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Histopathological Features of Conjunctival Sarcoid Nodules Using Noninvasive In Vivo Confocal Microscopy

Sarcoidosis is characterized by granulomatous inflammation, which may affect multiple organs or systems, most commonly including the lungs, skin, joints, and eye. In patients with typical ocular inflammatory disease, biopsy of conjunctival lesions, with finding of the classic noncaseating granulomas, may be helpful in confirming the diagnosis of sarcoidosis. In vivo confocal microscopy (IVCM) allows noninvasive in vivo examination of the eye at the cellular level. Until recently, IVCM has been used solely to describe cornea-related pathologic characteristics. We performed noninvasive IVCM on sarcoid granulomas of the conjunctiva to try and delineate the typical histopathological features seen in sarcoidosis. The images obtained by IVCM were then compared with a conjunctival biopsy specimen taken after IVCM imaging.
Report of a Case. A 61-year-old white man of Northern European extraction with skin, biopsy-proven sarcoidosis was referred to our clinic for immunosuppressive treatment of chronic, bilateral intermediate uveitis.

Ophthalmic examination revealed a visual acuity of 20/30 OU. Slitlamp biomicroscopic examination of the conjunctiva showed multiple yellowish conjunctival nodules in the inferior fornices bilaterally (Figure 1). The bulbar conjunctiva was normal in both eyes. Corneas were clear with no keratic precipitates, and anterior chamber inflammation was graded at trace cell on the right and 1+ cell on the left. Iries showed no nodule formation. He had 1 to 2+ vitreous cells and snowballs inferiorly in both eyes. Funduscopy revealed bilateral, punched-out choroidal lesions with normal maculae and optic nerves. The ophthalmic examination was consistent with the diagnosis of bilateral uveitis secondary to sarcoidosis. The patient started taking 15 mg of oral methotrexate once a week.

The patient consented to have IVCM on his conjunctival nodules followed by a conjunctival biopsy of his right eye. He was aware that these investigations were intended for research purposes rather than for diagnostic confirmation of sarcoidosis.

In Vivo Confocal Microscopy. The patient’s conjunctiva was examined with an ASL-1000 Scanning Confocal Microscope (Advanced Scanning Ltd, New Orleans, La) connected to a standard slitlamp frame. Topical anesthetic in the form of 0.5% proparacaine hydrochloride was instilled, and the applying objective was rested comfortably in the inferior fornix of the eye with the patient in upgaze. The area observed was about 500 × 300 µm at a magnification of approximately ×400 with a depth of field of approximately 12 µm. Both inferior fornices were examined using this technique. Images were captured using a digitally enhanced black-and-white camera (Kappa Optoelectronics Inc, Huizen, Germany). Video capture totaled 2 minutes for each eye. The videos were then viewed with Adobe Premiere version 6.5 (Adobe Systems Inc, San Jose, Calif), and single frames were captured and viewed individually.

The IVCM images of the conjunctival nodules taken from the right inferior fornix revealed a vast number of hyperreflective cells in the subepithelial layer and substantia propria, at times arranged in discrete nodules (Figure 2A), with morphologic features resembling inflammatory cells. Some areas of the conjunctival nodules consisted of discrete, circular, hyperreflective bodies. These appeared to represent multinucleated giant cells (MGCs), which may be seen in noncaseating sarcoid granulomas (Figure 2B).

We performed IVCM on 11 control subjects to determine whether the bodies found on IVCM in our patient with sarcoidosis were not an element of normal conjunctiva. The control group consisted of 6 women and 5 men aged 18 to 64 years. None of the controls had an established diagnosis of sarcoidosis. We did not find any lesions that were consistent with or similar to the bodies found on IVCM in our patient with sarcoidosis.

Conjunctival Biopsy and Histopathological Features. Twenty minutes after IVCM was completed, a biopsy of the conjunctiva of the inferior fornix of the right eye was performed. The specimen was fixed in 10% formalin, embedded in paraffin, and sectioned. To our knowledge, the appearance of MGCs on IVCM has never been described. Therefore, to determine that the bodies seen on IVCM were in fact MGCs, we performed IVCM on unstained 3-µm histological sections of the biopsy specimen. Figure 3A reveals the morphologic features of actual MGCs as seen by IVCM (Confoscan; Nidek Co Ltd, Gamagori, Japan). The cytoplasm is hyperreflective with a surrounding hyporeflective ring. The nuclei in the MGCs are more hyperreflective than the cytoplasm. The MGCs corresponded in size to the IVCM images. After staining the sections with hematoxylin-eosin, microscopic examination disclosed multiple noncaseating granulomas in the substantia propria. These findings were consistent with the diagnosis of sarcoidosis. Some granulomas contained MGCs (Figure 3B). Comparison of the IVCM images (Figures 2A and 2B) with the histological sections viewed with both IVCM (Figure 3A) and conventional light microscopy (Figure 3B) seems to confirm that the bodies seen on IVCM are MGCs.

Comment. In vivo confocal microscopy has previously been used to describe corneal anatomy and pathologic characteristics in the living human. Our group recently described the original use of IVCM to

Figure 1. Conjunctival nodules evident in the right inferior fornix of a 61-year-old man with a long-standing diagnosis of sarcoidosis.

Figure 2. In vivo confocal microscope (IVCM) images taken of the conjunctival nodules in the right inferior fornix of our patient before biopsy. A, The IVCM appearance of a sarcoid granuloma at the subepithelial level showing the multiple hyperreflective nuclei of inflammatory cells. B, Deeper sections showing separate multinucleated giant cells (arrows).

Figure 2A reveals...
determine the morphologic features of keratic precipitates during intraocular inflammation. To our knowledge, the use of IVCM to describe conjunctival pathological characteristics has not previously been reported. The human conjunctiva is an excellent tissue to examine using the confocal microscope because it is semitranslucent and the underlying vessels and cellular structures are easily accessible.

The histopathological features of conjunctival sarcoid granulomas are well described and often used to confirm the diagnosis of sarcoidosis. Conjunctival biopsy can be used as a diagnostic tool when the diagnosis of sarcoidosis is suspected. Nichols et al. showed that in patients with biopsy-proven sarcoidosis from other sites conjunctival biopsy results were positive in 55%. Karcioğlu and Brear reported positive conjunctival biopsy results in 71% of patients in this same group. Interestingly, they also found a 29% positive biopsy rate in patients with suspected sarcoidosis.

There is some difficulty when comparing IVCM images and stained histopathological slides. We therefore used IVCM on unstained, sectioned tissue to delineate the focal appearance of MGCs. This allowed us to directly compare the images captured on IVCM with the histopathological features seen on light microscopy.

Our case illustrates that a non-invasive procedure, IVCM, can visualize the markers of granulomatous inflammation in a patient with a known diagnosis of sarcoidosis, allowing MGCs and granulomas to be imaged without harm to the patient. In this case, we performed a conjunctival biopsy to confirm and establish the validity of these initial and novel IVCM images. Further study is required, especially in patients with known sarcoidosis without grossly visible conjunctival nodules. We surmise that the confocal microscope, as with conjunctival biopsy, may be able to detect MGCs and granulomas in these patients before the nodules become clinically apparent. With further validation, we believe that IVCM someday may be used similarly to histopathological diagnosis as a useful clinical adjunct to confirm the diagnosis of sarcoidosis in individuals with a suspicious history and examination results, without the morbidity of an invasive procedure.

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