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Prospective Study of the Diagnostic Accuracy of the In Vivo Laser Scanning Confocal Microscope for Severe Microbial Keratitis

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Purpose: To determine the diagnostic accuracy of in vivo confocal microscopy (IVCM) for moderate to severe microbial keratitis (MK).

Design: Double-masked prospective cohort study.

Participants: Consecutive patients presenting to Aravind Eye Hospital, Madurai, India, between February 2012 and February 2013 with MK (diameter ≥3 mm, excluding descemetocele, perforation, or herpetic keratitis).

Methods: Following examination, the corneal ulcer was scanned by IVCM (HRT3/RCM, Heidelberg Engineering, Heidelberg, Germany). Images were graded for the presence or absence of fungal hyphae or *Acanthamoeba* cysts by the confocal microscopist who performed the scan (masked to microbial diagnosis) and 4 other experienced confocal graders (masked to clinical features and microbiology). The regrading of the shuffled image set was performed by 3 graders, 3 weeks later. Corneal-scrape samples were collected for microscopy and culture.

Main Outcome Measures: The main outcome measures were sensitivity, specificity, and positive and negative predictive values of IVCM compared with those of a reference standard of positive culture or light microscopy. Sensitivities and specificities for multiple graders were pooled and 95% confidence intervals calculated using a bivariate random-effects regression model.

Results: The study enrolled 239 patients with MK. Fungal infection was detected in 176 (74%) and *Acanthamoeba* in 17 (7%) by microbiological methods. IVCM had an overall pooled (5 graders) sensitivity of 85.7% (95% confidence interval [CI]: 82.2%–88.6%) and pooled specificity of 81.4% (95% CI: 76.0%–85.9%) for fungal filament detection. For *Acanthamoeba*, the pooled sensitivity was 88.2% (95% CI: 76.2%–94.6%) and pooled specificity was 98.2% (95% CI: 94.9%–99.3%). Intergrader agreement was good: κ was 0.88 for definite fungus; κ was 0.72 for definite *Acanthamoeba*. Intragrader repeatability was high for both definite fungus (κ: 0.88–0.95) and definite *Acanthamoeba* classification (κ: 0.63–0.90). IVCM images from 11 patients were considered by all 5 graders to have a specific organism present (10 fungus, 1 *Acanthamoeba*) but had negative results via culture and light microscopy.

Conclusions: Laser scanning IVCM performed with experienced confocal graders has high sensitivity, specificity, and test reproducibility for detecting fungal filaments and *Acanthamoeba* cysts in moderate to large corneal ulcers in India. This imaging modality was particularly useful for detecting organisms in deep ulcers in which culture and light microscopy results were negative. *Ophthalmology 2016;■:1–9 © 2016 by the American Academy of Ophthalmology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).*

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Severe microbial keratitis (MK) is an important cause of blindness worldwide.[1] In recent years, outbreaks of fungal and *Acanthamoeba* keratitis (AK) have brought to light the complexity of identifying a causative organism in these infections.[2] Although experienced cornea specialists can correctly identify fungal from bacterial keratitis based on clinical features alone in ≤66% of cases,[3] larger ulcers may present a diagnostic challenge, as tissue destruction may obscure classical features.[2] In these cases, microbiological techniques such as culture and light microscopy can aid in diagnosis but they do not offer a high diagnostic accuracy. Culture-positivity rates in MK vary widely, from 40% to

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73% in different settings, most likely because of the small size of corneal-scrape samples, prior antimicrobial treatment inhibiting microbial growth, and the fastidious nature of some organisms requiring special growth media (e.g., fungi and *Acanthamoeba*).[4–7] Direct visualization of fungal filaments or *Acanthamoeba* cysts in corneal scrapings using light microscopy can give a higher detection rate when compared with culture alone,[8] but it relies on the availability of trained, experienced observers who may not be present in some health care settings.

In vivo confocal microscopy (IVCM) is a noninvasive imaging technique that allows direct visualization of pathogens within the patient's cornea.[9] The 2 imaging modalities in current clinical use are the scanning slit IVCM (ConfoScan, Nidek Technologies, Fremont, CA) and the laser scanning IVCM (HRT3 with Rostock Corneal Module [RCM], Heidelberg Engineering, Heidelberg, Germany). The ConfoScan has a resolution of 1 micron laterally and up to 24 microns axially; the HRT3/RCM also has a lateral resolution of 1 micron but higher axial resolution of 7.6 microns.[10] Although many have reported the ability of both of these confocal microscopes to detect fungal filaments and *Acanthamoeba* cysts in human MK in vivo (summarized in Labbe et al[9]), only 2 studies have prospectively assessed the diagnostic accuracy of IVCM compared with standard microbiological techniques of culture with or without light microscopy.[11,12] Kanavi et al found that with a single IVCM grader, the ConfoScan 3.0 IVCM had a sensitivity of 100% for detection of *Acanthamoeba* and specificity of 84% compared with culture as the reference standard. For fungal filaments, the sensitivity was also high (94%) but the specificity lower (78%). The authors do not state whether the IVCM grader was masked to data from a clinical assessment of the patient. Vaddavalli et al also used the ConfoScan 3.0 with 2 IVCM graders who were masked to both the microbiological diagnosis and clinical assessment.[12] They found a sensitivity of 80% and specificity of 100% for the detection of *Acanthamoeba* cysts. For fungal filament detection, they found a sensitivity of 89.2% and specificity of 92.7%. In addition, a good interobserver agreement (κ 0.6) was found for the 2 graders. Hau et al have previously demonstrated that the diagnostic accuracy of IVCM for the diagnosis of MK is also affected by the experience of the IVCM grader.[13] As such, there is a need to determine the extent of variability between graders in the clinical setting. Resolution of the IVCM imaging system may also affect the ability of graders to detect pathogens, but to date there have been no formal prospective studies using the higher resolution HRT3 IVCM in the detection of MK.

In this study, we aimed to determine the diagnostic accuracy of HRT3 IVCM in moderate to severe MK in South India using 5 experienced confocal graders (masked to microbiological diagnosis). We also assessed intergrader and intragrader agreement.

Methods

This study was approved by the institutional review board of Aravind Eye Hospital, Tamil Nadu, India; the Indian Council for Medical Research; and the Ethics Committee of the London School of Hygiene and Tropical Medicine. Prior to enrollment in the study, all patients gave written informed consent; study participants who were illiterate gave informed consent with a witnessed thumbprint on the study consent form, as approved by the above ethics committees. This study adhered to the tenets of the Declaration of Helsinki and was conducted in accordance with the Standards for Reporting of Diagnostic Accuracy studies (STARD)[14]—see the STARD checklist in Supplementary Table S1, available at aaojournal.org.

Study Participants

This study was based in the Cornea Clinic at Aravind Eye Hospital, Madurai, Tamil Nadu, India. Consecutive patients presenting to the clinic between February 2012 and February 2013 were assessed for eligibility and prospectively enrolled into the study if they were found eligible. The inclusion criteria were age ≥18 years and the presence of a large corneal ulcer, defined as a stromal infiltrate ≥3 mm at the longest diameter, with an overlying epithelial defect and signs of acute inflammation. All eligible patients underwent slit lamp examination by an ophthalmologist (cornea specialist), and relevant clinical history and examination findings were recorded in the standardized study form. We excluded any patients with a descemetocele or >80% corneal thinning in the affected eye as assessed on slit lamp examination (i.e., in whom we could not safely applanate the IVCM onto the cornea for imaging), those considered to have herpetic stromal keratitis on clinical grounds (i.e., either a prior history of the disease or the presence of clinical features associated with herpetic disease), or if Snellen visual acuity was worse than 6/60 in the unaffected eye.

IVCM Imaging

The affected eye was anesthetized using 0.5% proparacaine eye drops (Aurocaine, Aurolab, Madurai, India), and volume scans of the corneal ulcer were obtained using the HRT3 IVCM (Heidelberg Engineering, Heidelberg, Germany) with RCM (63× magnification objective lens, Nikon, Tokyo, Japan), by an ophthalmologist trained in performing IVCM and following a standard procedure described elsewhere.[13] Briefly, volume scans were obtained in the center of the ulcer, and at the 12-, 3-, 6-, and 9-o'clock positions of the peripheral ulcer margins. Volume scans were taken from the surface of the ulcer and manually refocused several times to take progressively deeper overlapping scan sets covering as much of the full depth of the ulcer as possible.

Immediately after IVCM imaging, the patient underwent scraping of the ulcer base and leading margin for microscopy and culture. The confocal microscopist who performed IVCM imaging was masked to the microbiological diagnosis but had examined the ulcer at the slit lamp prior to performing IVCM. At the time of image acquisition, this grader (grader 5) was asked to grade the IVCM images for the presence or absence of fungal filaments or *Acanthamoeba* cysts; if the grader was suspicious but not confidently certain of a presence, then the image was graded as the possible presence of filaments or cysts.

Microbiological Diagnosis

Immediately after IVCM had been performed and grading recorded, the base and leading edge of the corneal ulcer were scraped using a flame-sterilized Kimura spatula. Scrapings were immediately placed onto 2 glass slides for light microscopy and on agar plates for culture: blood agar (BA), potato dextrose agar (PDA), and nonnutrient agar seeded with *Escherichia coli* in the laboratory if AK was clinically suspected. Standard microbiological methods were followed to detect any pathogen.[15] In brief, slides were stained with 10% potassium hydroxide or gram or Giemsa stain to aid in the visualization of fungal filaments, bacteria, or *Acanthamoeba* cysts,