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ORIGINAL PAPER

Role of in vivo confocal microscopy in the diagnosis of infectious keratitis

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Abstract

Purpose To investigate the utility of in vivo confocal microscopy (IVCM) in the diagnosis of infectious keratitis (IK).

Methods Retrospective chart review of 46 patients with a final diagnosis of IK were included in the study. All patients received IVCM corneal imaging using the Heidelberg Retinal Tomography III system. All available scans were randomized and analyzed in a masked fashion. Sensitivity and specificity of IVCM in diagnosing bacterial keratitis (BK), Acanthamoeba keratitis (AK), fungal keratitis (FK), and HSV viral keratitis (VK) were assessed.

Results The pooled sensitivity and specificity of IVCM in identifying atypical IK (AK and FK cases combined) were 85.3% (95% CI 68.2–94.5%) and 100% (95% CI 74.7–100%), respectively. The sensitivity and specificity of IVCM in identifying BK were 66.7% (95% CI 35.4–88.7%) and 89.2% (95% CI 73.4–96.5%), respectively. The sensitivity and specificity of IVCM in identifying VK were 100% (95% CI 46.3–100%) and 93.2% (95% CI 80.3–98.2%). Additionally, IVCM was able to make the correct diagnosis in 8 out of the 11 atypical keratitis cases misdiagnosed clinically. In the AK subgroup, IVCM was more accurate than clinical assessment (16 vs. 11). In the FK subgroup, IVCM were as accurate as clinical assessment, but did correct one misdiagnosed cases by identfying fungal hyphae.

Conclusion IVCM is an non-invasive imaging modality that can rapidly and accurately diagnose IK even for experienced corneal specialists. In complex cases of polymicrobial infection, IVCM may guide the correct clinical diagnosis and initiation of the appropriate treatment.

Keywords Infectious keratitis · In vivo confocal microscopy · Fungal keratitis · Acanthamoeba keratitis · Sensitivity and specificity · Mixed-organism infection

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Introduction

Infectious Keratitis is an important cause of blindness worldwide [1, 2]. Diagnosis of infectious keratitis has relied mainly on physicians' clinical experience in addition to microbiological analysis of corneal biopsy

[1, 3, 4], the gold standard being isolation of the organisms by culture. The rate of positive culture in cases of infectious keratitis has varied widely between 40 and 70% [5] due to a variety of limiting factors [6–8]. The positive predictive value of physicians' clinical experience when diagnosing infectious keratitis had also been shown to be variable depending on the etiology of the infection [9].

In vivo confocal microscopy (IVCM) is a non-invasive imaging modality that can allow direct visualization of potential causative pathogens in real time [10, 11]. Many investigators have previously demonstrated good sensitivity and specificity of IVCM in detecting Acanthamoeba cysts and fungal elements [12–15]. A prospective study assessing the accuracy of HRT3 IVCM in microbial keratitis reported high sensitivity and specificity of HRT3/RCM IVCM in detecting fungal and Acanthamoeba keratitis [13].

In this current study, we aim to assess the specificity and sensitivity of HRT3/RCM IVCM in detecting various infectious keratitis entities including Acanthamoeba keratitis (AK), fungal keratitis (FK), bacterial keratitis (BK), and HSV viral keratitis (VK) seen by the cornea service of a tertiary referral-based ophthalmic center. Initial clinical assessment, microbial culture results, IVCM diagnoses as well as final diagnoses were compared and contrasted.

Materials and methods

Study design

Institutional review board/ethics committee approval was obtained. This study complied with the Health Insurance Portability and Accountability Act and adhered to the tenets of the Declaration of Helsinki. Prospective enrolment included patients who were evaluated at the Doheny Eye Center (DEC) UCLA with initial presentation between August 2014 and December 2016, diagnosed with infectious keratitis (IK) including BK, FK, AK, and VK, and underwent corneal imaging with IVCM. An informed consent for the IVCM examination was obtained from all subjects. Exclusion criteria includes (1) concurrent ocular trauma, (2) concurrent endophthalmitis, and (3) autoimmune ocular conditions including ocular GVHD disease.

In vivo confocal microscopy imaging acquisition

The confocal microscope uses a 670-nm red wavelength diode laser source and is equipped with a 63× objective immersion lens with a numerical aperture of 0.9 (Olympus, Tokyo, Japan). The obtained images represent a coronal section of the cornea of 400 × 400 μm, providing a scanned area of 160,000 μm^2/frame, at a determined corneal depth. Adjacent images were separated by approximately 1–4 μm, with a lateral resolution of 1 μm/pixel. One drop of hydroxypropyl methylcellulose 2.5% (Gen-Teal gel; Novartis Ophthalmics, East Hanover, NJ) was placed inside the disposable sterile Tomocap (Heidelberg Engineering GmbH). One drop of 0.5% proparacaine hydrochloride (Alcaine; Alcon, Fort Worth, TX) was instilled in both eyes for topical anesthesia, followed by a drop of hydroxypropyl methylcellulose 2.5%. Six to ten volume and sequence scans were taken at the corneal lesion area and surrounding areas, focusing mainly on the corneal epithelial layers, subepithelial area, subbasal nerve plexus area and stroma. Digital images were stored on the instrument's computer at 30 frames per second. IVCM imaging was performed by one of the three experienced confocal microscopists (TT, EB, and PH). The central corneal area (within a variable circumference that included the clinically determined ulcer or infiltrate) was scanned thoroughly first; then the four standard quadrants of peripheral cornea (inferior, superior, nasal and temporal, approximately 1–2 mm anterior to the limbus) were scanned by adjusting patients' gaze positions.

Confocal image analysis and grading

All of the confocal sequences from each eye were reviewed by two experienced confocal microscopists (TT and EB) in a masked and independent fashion. Criteria used to identify Acanthamoeba elements were the presence of Acanthamoeba cysts or trophozoites. Cysts are typically round or ovoid, double-walled, highly refractive bodies measuring 10–25 μm; trophozoites are 25–40 μm in diameter and appear as hyperreflective and ovoid structures [16–18] (Fig. 1, representive study images). The criteria used to identify fungal elements were the presence of highly reflective, branching, linear opacities, varying in size between 3 and 8 μm. These "filaments", usually not seen in

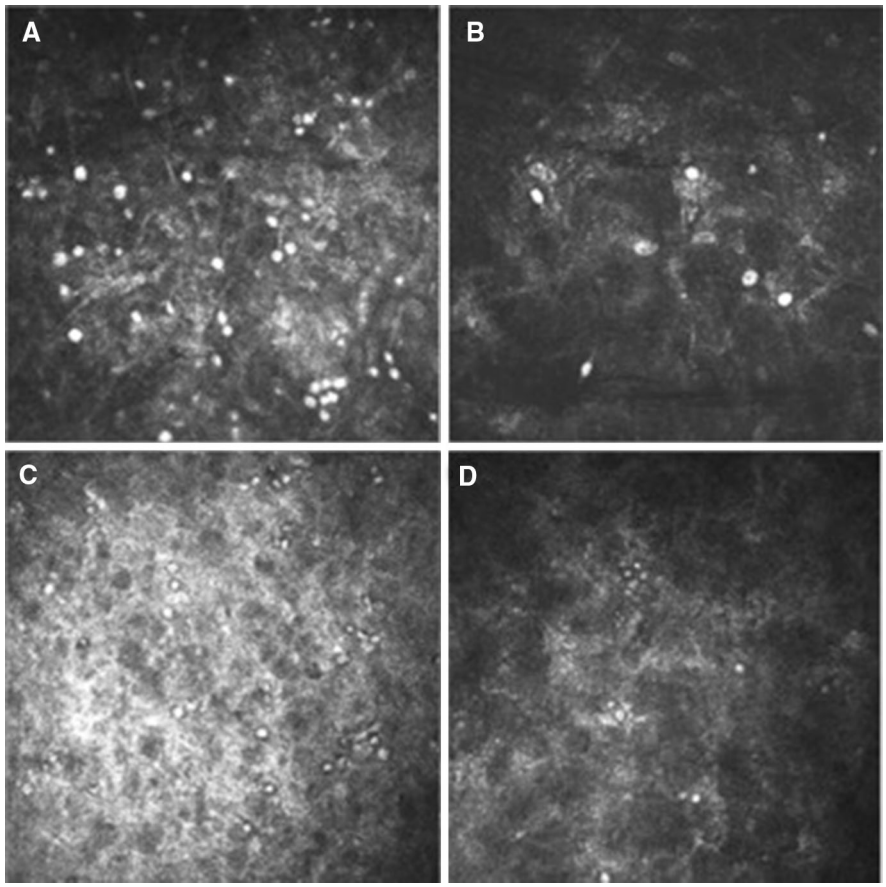

Fig. 1 Confocal microscopy images of Acanthamoeba Keratitis. **a**–**d** Representative images at 10-63 micron depth, showing scattered round or oval-shaped hyper-reflective structures representing Acanthamoeba cysts

isolation [15, 19] may appear to be "double walled" on IVCM and are typically uniform in width with an irregular pattern of branching (Fig. 2, representive study images). Furthermore, yeasts present with confocal microscopy examination as round, budding bodies with the length of 10–40 μm and a width of

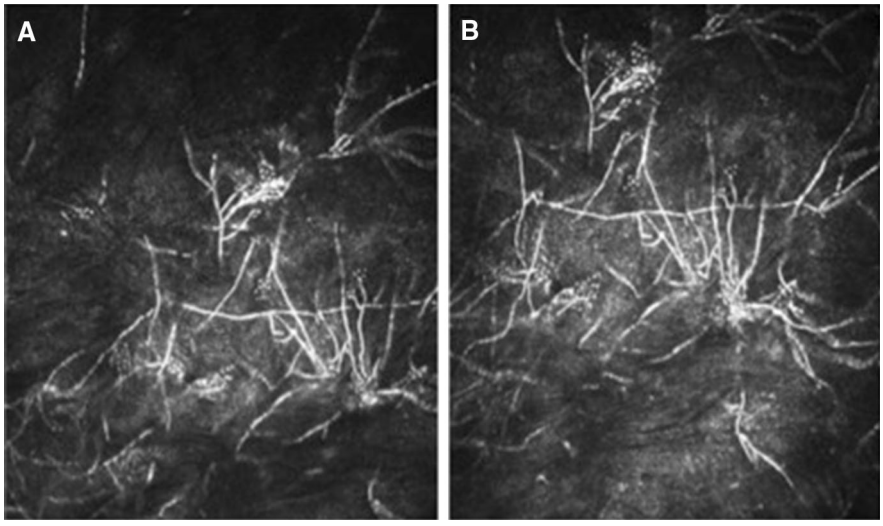

Fig. 2 Confocal microscopy images of Fungal Keratitis. **a**, **b** Representative images at 36-37 micron depth, showing groups of hyper-reflective linear structures with acute angle branching, typical of fungal hyphae

5–10 μm, possibly developing pseudohyphae (candida albicans) [11] or as small hyper-reflective round structures with a diameter of 3–5 μm (*Candida parapsilosis*) [20]. BK was defined as the lack of atypical organisms such as Acanthamoeba cysts or trophozoites, fungal filaments or yeasts, and the presence of abundant dendritic cells (DC), neutrophils and lymphocytes [21], out of proportion to severity of those seen in normal eyes (Fig. 3, representive study images). VK was defined as the lack of atypical organisms, presence of some DC within the basal epithelial layer and sub-epithelial nerve plexus area, absence of sub-epithelial nerve plexus and hyper-reflective keratocytes in the anterior stroma [22] (Fig. 4, representive study images). When disagreement occured, a third grader (HP) was introduced to perform adjudication grading independently, and the final diagnosis was made when agreement was reached between any two of the three graders. One of the graders (TT) re-graded all study images one month after the initial grading for calculation of intra-grader agreemnt. All three graders are certified reading center graders, and have had 2–3 years of intensive experience with image acquisition and interpretation of confocal microscopy. We consider their level of experience comparable."

Statistical analysis

The intra-grader agreement and inter-graders agreement was calculated using the Kappa statistics. Sensitivity and specificity of the IVCM diagnosis of IK were determined conventionally using the two by two contingency table with the reference standard defined as final diagnosis and response to the appropriate treatment [22]. The final diagnosis was made by the primary corneal specialist, at the end of the clinical course, based on clinical presentation, available microbial analysis, and response to appropriate treatments. Comparison of percentage measurements was conducted using the Chi square test. All statistic analysis was performed using the JMP 13 software (SAS cooperation, Cary, NC).

Results

Study participants

A total of 49 eyes from 46 patients were included in the final analysis.

The mean age of the study participants was 54.6 ± 20.8 (standard deviation) years. There were 13 (27%) male and 36 (73%) female. 25 (51.0%) right eyes and 24 (40.8%) left eyes were included in the final analysis. 29 out of 49 (59.2%) eyes had a history of contact lens wear, 6 (12.2%) patients had history of corneal transplant.

By our final diagnosis, there were 10 (20.4%) BK, 21 (42.9%) AK, 10 (20.4) % FK, 4 (8.2%) VK, and 4 cases of combined keratitis including two AK + FK cases, one BK + VK case, and one BK + FK case.

Microbial analysis

Within the 24 cases of AK, 18 patients had cultures taken, with an acanthamoeba positive rate of 33% (6/18). Within the 12 cases of FK, 11 patients had culture results, with a fungal positive rate of 45.5% (5/11). Candida, Fusarium and Paecilomycese were the

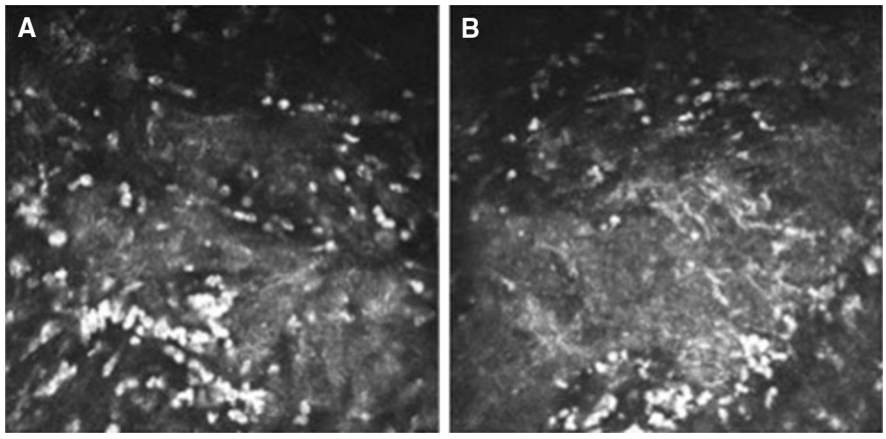

Fig. 3 Confocal microscopy images of Bacterial Keratitis. **a**, **b** Representative image at 86 micron depth, showing abundant polymorphic neutrophils and lack of atypical elements

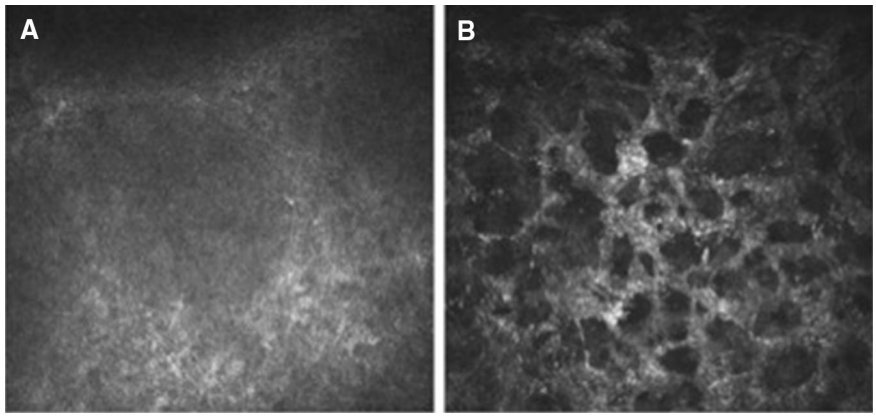

Fig. 4 Confocal microscopy images of HSV Keratitis. **a** Representative image at 25 micron depth, showing absence of corneal nerve endings. **b** Representative image at 60 um depth in the stroma showing the hyper-reflective keratocyte network, with no atypical organisms or no neutrophils present

fungal organisms isolated in culture positive cases. Within the 12 BK cases, 9 patients had culture results, with bacterial positive rate of 66.7% (6/9). Isolated organisms included various Staphylococcus organisms, P. acne, and Pseudomonas. Finaly, within the VK group, only one patient received viral PCR study, while the other 4 did not have any virology study.

Confocal analysis

IVCM scanning identified 12 cases of (24.5%) BK, 20 cases of (40.8%) AK, 7 cases of (14.3%) FK, 8 cases of (16.3%) VK, and 2 (4.1%) cases of AK and FK co-infection. The pooled sensitivity and specificity of IVCM in identifying atypical infectious keratitis (all AK and FK cases combined) were 85.3% (95% CI 68.2–94.5%) and 100% (95% CI 74.7–100%), respectively. The sensitivity and specificity of IVCM in identifying AK were 91.7% (95% CI 71.5–98.5%) and 100% (95% CI 83.4–100%), respectively. The sensitivity and specificity of IVCM in identifying FK were 66.7% (95% CI 35.4–88.7%) and 100% (95% CI 88.3–100%), respectively. The sensitivity and specificity of BK were also calculated to be 66.7% (95% CI 35.4–88.7%) and 89.2% (95% CI 73.4–96.5%), respectively. The sensitivity and specificity of VK were found to be 100% (95% CI 46.3–100%) and 93.2% (95% CI 80.3–98.2%).

The intra-observer agreement was excellent (Kappa = 0.94, SD = 0.04, $p < 0.001$). The inter-observer agreement in grading the scans was also substantial (Kappa = 0.68, SD 0.08, $p < 0.001$). The accuracy of each grader was assessed independently by comparing their confocal grading with the final diagnosis. Grader 1 matched the final diagnosis in 39 (79.6%) cases (95% CI 66.4–88.6%). Grader 2 matched the final diagnosis in 38 (77.6%) cases (95% CI 64.1–87.0%).

For our secondary analysis, initial clinical diagnosis, culture diagnosis, and confocal diagnosis were compared and contrasted in each subgroup. In the final diagnosis-defined AK group, 6 cases were culture positive, 12 cases were culture negative, and the remaining 6 had no available culture results. Out of the 18 non-culture proven (either culture negative or culture unavailable) cases, 16 cases were confocal positive for AK, with 2 remaining cases being confocal negative. In contrast, amongst these 18 cases, 11 had the initial clinical diagnosis by a corneal specialist as AK, while 7 (29.2%) had initial clinical diagnosis other than AK. In all 24 cases of final diagnosis-defined AK, corneal specialists at DEC initially identified 17 cases of those as AK, missing the remaining 7 cases, with 5 as BK, 1 as FK, and 1 as VK. In the final diagnosis-defined FK group, 5 cases were culture proven positive, and 7 were non-culture proven. 4 out of the 7non-culture proven cases were correctly diagnosed as FK by confocal findings. Additionally, 4 out of these 7 non-culture proven cases had initial clinical diagnosis other than FK. For 1 out of the 4 incorrect initial clinical diagnosis that was misdiagnosed as AK, confocal analysis identified fungal hyphae and altered the final diagnosis. In all 10 cases of final diagnosis-defined FK (not including cases of coinfection), corneal specialists initially diagnosed 6 cases as FK, 2 cases as BK, and 2 cases as VK. In all 10 cases of final diagnosis-defined BK (not including cases of coinfection), no atypical organisms, such as double walled cysts typical of AK or hyphae typical of FK, were identified. In contrast, in the one case of combined BK and AK co-infection,

Acanthamoeba cysts were identified in the IVCM images. In these same 10 cases of final diagnosis-defined BK, corneal specialists clinically diagnosed all 10 cases as BK.

The 4 multi-organism cases were diagnosed by corneal specialists as AK in 1 case, BK in 2 cases, and FK in 1 case. When initial clinical diagnosis was inconsistent with the final diagnosis, which included 11 out of the 49 cases, IVCM was able to make the correct diagnosis in 8 out of the 11 cases. In other words, IVCM was able to alter the incorrect initial diagnosis to match the final diagnosis in these 8 cases.

Discussion

Corneal ulcers, when large in size and present with atypical or mixed clinical characteristics, can be diagnostically challenging [23]. Dahlgren et al. had previously demonstrated relatively low positive predictive value of clinician's diagnosis of atypical infections. Given the relatively low sensitivity of microbial culture results espeically in cases of atypical organisms, delays in diagnosis and treatment are common [6]. This can lead to significant loss of vision or even loss of the eye [6].

IVCM is a noninvasive imaging modality which allows direct visualization of fungal and Acanthamoeba elements in vivo [24, 25]. Consequently, it can rapidly provide clinically relevant information prior to the return of microbiological data. Previous studies utilizing the ConfoScan IVCM have demonstrated a high accuracy for the detection of Acanthamoeba cysts and fungal filaments [5, 12, 15]. A recent prospective study conducted in India by Chidambaram et al. also reported high sensitivity and specificity for the detection of fungal and Acanthamoeba infection in cases of moderate to severe microbial keratitis [13]. To our best knowledge, our study is the first prospective study in the United States which reports the high diagnostic accuracy of HRT3/RCM confocal microscope in the diagnosis of BK and VK.

In our study, the intra-observer agreement was found to be excellent (Kappa = 0.94). This is consistent with what was previously reported in the literature [12, 13, 15]. The inter-observer agreement was also good or substantial with a Kappa of 0.68.

Our study detected a high level of pooled sensitivity and specificity of IVCM in identifying atypical infectious keratitis (85% and 100% respectively). This is consistent with what Vaddavalli et al. had reported using the ConfoScan 3.0 with 2 IVCM graders in which sensitivity of 88.3% and specificity of 91.1% were detected for the identification of FK and AK [15]. Specifically for AK, the sensitivity (91.7%) and specificity (100%) detected in our study were also extremely high. This is in agreement with what was recently published by Chidambaram et al., where they found a sensitivity of 88.2% and specificity of 98.2% in detecting AK in an Indian population using the same model of IVCM as in our study [13]. Our study thus confirms the utility of HRT3/RCM in diagnosis of AK at all levels of severity.

In the AK subgroup, there were two false negative diagnoses where confocal failed to make the correct diagnosis. In one case, IVCM diagnosed BK instead of AK, secondary to the lack of any atypical elements such as cysts or trophozoites. This likely is due to the large amount of inflammation precluding a clear image quality. In the other case, AK was misdiagnosed as FK. Hyphae-like linear structures from possible degradation products of neutrophils, collagen fibers, were identified as fungal hyphae [26, 27].

In the FK subgroup, the sensitivity was moderate (66.7%) but the specificity was high (100%). The lower sensitivity detected in our study when compared to what was previously reported might be attributed to the small sample size of FK in our study population [13]. In this particular subgroup, we had 4 cases of false negative confocal diagnosis, where 3 cases of FK were diagnosed as BK, and one diagnosed as VK. A central dense white infiltrate was presented in all four cases, which likely precluded a clear iamge by IVCM of the deeper corneal structure. This, in combination with the significant amount of inflammatory cells at the area of the corneal ulcer, likely hindered the identification of any fungal elements.

To our knowledge, our study is the first to report the sensitivity and specificity of HRT3/RCM in detecting BK and VK. In the BK subgroup, though limited by the small sample size, we found similar sensitivity and specificity of detecting BK as that of detecting FK. Furthermore, in all 10 cases of pure BK, no atypical elements such as Acanthamoeba cysts or fungal elements were identified. This again underscores the specificity of the IVCM in detecting these atypical organisms. No existing consensus on IVCM diagnostic criteria for BK exists. Characteristic confocal

presentations include clusters of neutrophils and lymphocytes, in addition to the lack of atypical elements [21]. Future studies of larger sample size, and imaging system with high resolution with the possibility of visualizing bacterial organisms, is necessary.

In the VK subgroup, the sensitivity (100%) and specificity (91%) of IVCM in making the diagnosis were both very high. This is very likely biased due to our very small sample size. There are 3 cases of IVCM false positives for VK. Two were misdiagnosed BK cases, which could be secondary to a lack of characteristic features of atypical infectious keratitis. Additionally, this could be secondary to previous episodes of HSV keratitis which resulted in decreased corneal nerve endings with no active infective processes. The other case was a misdiagnosed FK case, which is likely due to the deep location of the fungal elements not visualized by IVCM. To date, there is no published consensus on confocal diagnostic criteria for VK, and the resolution of commercially available confocal imaging system precludes visualization of any potential viral particles [28]. Future studies designed specifically to look at the characteristics of VK with a large population is needed to further elucidate the utility of IVCM in diagnosing VK.

We found two cases of concurrent infection involving both fungal and Acanthamoeba organisms. In the first case, microbiologic analysis was negative for bacteria and fungi. Upon obtaining IVCM imaging, both Acanthamoeba cysts and fungal hyphae were identified in the cornea. In the second case, all cultures including bacterial, fungal and Acanthamoeba returned negative. Confocal analysis revealed both fungal hyphae and Acanthamoeba cysts, yielding the diagnosis of acanthamoeba and fungal co-infection. Both of these cases support the use of IVCM in making the initial clinical diagnosis of IK, not only in cases with atypical clinical presentations, or inconclusive culture results, or complicated ocular history; but also in cases with classic presentations suggestive of a certain type of infection.

We also look at the agreement between initial clinical diagnosis, confocal diagnosis, and the final diagnosis. At initial presentation DEC corneal specialists correctly identified only 17 of 24 cases of final diagnosis-defined AK and 6 of 10 cases of final diagnosis-defined FK. For these initially mis-diagnosed caes, IVCM was able to arrive at the correct diagnosis (Figs. 4, 5) in 72.7% of the cases and change the management for those patients.

Furthermore, we look at the subgroup of patients who underwent microbial study.

In the final diagnosis-defined AK group (including co-infectious cases), 18 patients had undergone corneal scraping for Acanthamaeba culture, where 6 turned out positive. In this particular group, IVCM outperformed both microbiology analysis and the corneal specialists' clinical judgement in making the correct diagnosis, highlighting its utility in making the diagnosis of AK. This is particularly true in situations of co-infections with an atypical organism such as AK or FK and a bacterial agent.

In the final diagnosis-defined FK group, 4 out of the 7 non-culture proven FK cases had no fungal elements identified on IVCM. Similarly, initial clinical assessment also missed 4 out of the 7 cases. Interestingly, both initial clinical assessment and IVCM missed the same 3

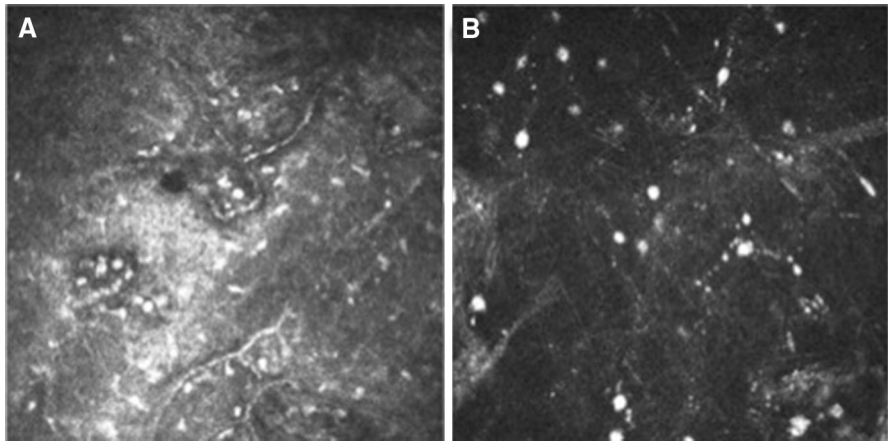

Fig. 5 **a** Representative image at 61 micron depth, showing groups of hyper-reflective linear structures with branching, typical of fungal hyphae. **b** Representative image at 134 micron depth, showing round and oval shaped hyper-reflective structure, typical of Acanthamoeba cysts

cases of FK, diagnosing them as either HSV keratitis or BK. IVCM also misdiagnosed the fourth case of FK as BK, due to lack of fungal elements identified; but clinically this case was correctly classified as FK. In contrast, in a case of non-culture proven FK, while clinically misclassified as AK, IVCM was able to identifiy fungal hyphae and hence correctly make the diagnosis of FK. This suggests that neither IVCM nor clinical assessment should be used alone as the sole diagnostic tool when evaluating fungal keratitis.

The main limitation of our study is the small sample size, especially in the FK, BK and VK subgroups which results from a bias of atypical cases, particularly suspected amoebic keratitis, being referred to our institution specifically for IVCM. Furthermore, our study included a larger number of Acanthamoeba keratitis when compared to other forms of keratitis, most likely due to the increased number of referrals at a tertiary eye care center, and the relative unavailability of confocal imaging system in the community. This higher proportion of AK can inflate the sensitivity of the test. The study images for each patient were obtained by different technicians at different visits. The fact that all three microscopists participated in imaging acquisition introduced a component of bias in the form of image quality (but the data in this study shows they had high agreement with masked grading, and we think this is because our imagers are highly experienced). The definition of BK and VK cases in our study are not as specific as those for AK/FK cases. Since no organisms could be identified with currently available imaging modality, our diagnosis rely greatly on clues from the sub-epithelial nerve plexus, dendritic cells and other cell types in the infected cornea. Although we were able to use some of the characteristics reported previously for IK [29], we understand that some of the features might be present in other corneal conditions or even normal eyes [27, 29, 30]. The fact that we also scanned uninvolved areas of the cornea surrounding the pathologic area, helped to make comparisons between normal and abnormal tissues in our patients, which helped to mitigate the issue. Next, the gold standard set in our study is clinical diagnosis and improvement or resolution of disease after initiation of the appropriate treatment. Not all patients included in the study underwent microbiological evaluation. The accessibility of culture media and microbiology laboratory is the major limitation that prevents community ophthalmologists to obtain cultures for atypical organisms. The required incubation time for fungal and acanthamoeba culturing also may lead to delay of treatment. When these patients are referred to our tertiary center for further evaluation and management, clinicians are more likely to initiate treatment for atypical organisms given failure of previous treatment, which likely were antibiotics or antiviral agents. In the cases of VK, when patients present with typical exam findings such as dentritic epithelial defects, our very experienced corneal specialists feel confident in initiating topical and/or oral antiviral agents without obtaining culture or PCR analysis. IVCM images in these cases are not used to augment clinical diagnosis and treatment plan. In cases of BK, microbial analysis also had a high culture negative rate, likely due to initiation of antibiotic treatment by referring physician prior to arriving to our center. These patients recovered remarkably well with antibiotics treatment, without any antiviral, antifungal or anti-acanthamoeba medications. Nonetheless, the lack of confirmative microbiology data in our study, may create uncertainty as to the offensive microbial agent and subsequently confound our sensitivity and specificity analysis.

In conclusion, IVCM is a non-invasive imaging modality that can not only rapidly but also accurately diagnose various atypical infectious keratitis, especially in cases of atypical infectious keratitis. In complex cases of infectious keratitis with simultaneous infection by multiple types of organisms, IVCM may influence the differential diagnosis and initiation of the appropriate treatment, altering the clinical course of the disease. The ophthalmologist should consider IVCM a powerful tool in the armamentarium for the evaluation of IK cases that can change the clinical course of a patient's care.

Compliance with ethical standards

Conflict of interest All authors except for Dr. Olivia Lee certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Financial disclosure None of the authors have relevant financial disclosures.

Appendix

See Table 1.

Table 1 Sensitivity and specificity calculation 2 × 2 table by etiology

	Clinical positive	Clinical negative	Total
Acanthamoeba keratitis			
Confocal positive	22	0	22
Confocal negative	2	25	27
Total	24	25	49
Fungal keratitis			
Confocal positive	8	0	8
Confocal negative	4	37	41
Total	12	37	49
Bacterial keratitis			
Confocal positive	8	4	12
Confocal negative	4	33	37
Total	12	37	49
Viral keratitis			
Confocal positive	5	3	8
Confocal negative	0	41	41
Total	5	44	49

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