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VIEWPOINT

Confocal Microscopy as an Early Relapse Marker for *Acanthamoeba* Keratitis

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Acanthameoba keratitis is a serious ophthalmological condition with a potentially vision-threatening prognosis. Early diagnosis and recognition of relapse, and the detection of persistent Acanthamoeba cysts, are essential for informing the prognosis and managing the condition. We suggest the use of in vivo confocal microscopy not only to identify the early signs of relapse after keratoplasty in patients with Acanthamoeba keratitis, but also as an additional follow-up tool after antimicrobial crosslinking. This study shows that in vivo confocal microscopy is, in experienced hands, a quick and reliable diagnostic tool. Clin. Anat. 31:60–63, 2018.

Key words: *Acanthamoeba* keratitis; keratoplasty; crosslinking; relapse marker; in vivo confocal microscopy

INTRODUCTION

The diagnosis of *Acanthamoeba* keratitis presents a real challenge, which can often lead to delayed initiation of treatment and a worse prognosis. Among patients with *Acanthamoeba* keratitis, 83–93% wear contact lenses (Bacon et al., 1993; Carvalho et al., 2009; Dart et al., 2009) It is therefore important to consider this condition as a differential diagnosis for contact lens-associated keratitis (Daas et al., 2015). At the same time, fungal keratitis should also be ruled out. Using in vivo confocal microscopy, we were able to diagnose *Acanthamoeba keratitis* on the day of admission, whilst simultaneously ruling out fungal keratitis (Daas et al., 2016). The specificity and sensitivity of in vivo confocal microscopy with respect to Acanthamoeba keratitis are both over 90%, whilst the sensitivity with respect to the diagnosis of fungal keratitis is 86% (Szentmáry et al., 2012; Nielsen et al., 2013). For a keratitis of unknown cause, in vivo confocal microscopy is recommended in combination with in vitro cultivation, polymerase chain reaction (PCR), and, if necessary, histological assessment (Seitz et al., 2015). The aim of this article, based on two case reviews, is to indicate the potential use of confocal microscopy as an early and reliable relapse marker after a keratoplasty for *Acanthamoeba* keratitis (Patient 1), and as a method for follow-up assessment in a patient with this condition after antimicrobial crosslinking (Patient 2).

CLINICAL FINDINGS

Patient 1. In January 2016, a 45-year-old female patient who wore contact lenses presented to our outpatients department with a two month history of reduced vision and pain in the left eye, and an initially keratitis without clear etiology. Assessment by slit-lamp demonstrated stromal corneal infiltrates, "pseudodendrites," and a disturbance of the epithelium, consistent with a "dirty epithelium" (Fig. 1).

Patient 2. A 27-year-old female patient, who also wore contact lenses and had a history of *Acanthamoeba*

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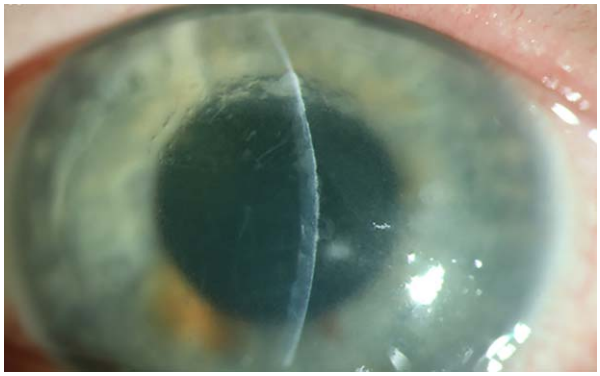


Fig. 1. Patient 1: Slit lamp: stromal corneal infiltrates "pseudodendrites" and a disturbance of the epithelium, consistent with a "dirty epithelium." [Color figure can be viewed at wileyonlinelibrary.com]

keratitis, presented to our outpatient department with a nine month history of fluctuating vision and glare sensitivity in her right eye. The best-corrected visual acuity in the affected eye was 0.1 (20/200) after nine months of conservative management and two courses of external antimicrobial crosslinking. Assessment by slit lamp demonstrated central subepithelial and stromal scar formation, consistent with "haze" (Fig. 2). The pathognomonic ring-infiltrate was absent and the anterior chamber showed no signs of inflammation.

COURSE, DIAGNOSIS, AND MANAGEMENT

In the case of Patient 1, we were able to use in vivo confocal microscopy on the day of admission to identify the *Acanthamoeba* cysts non-invasively (Fig. 3). The diagnosis was later confirmed using PCR and also through histological examination after an (8.0 mm/8.1 mm) penetrating excimer laser keratoplasty with corneal cryocoagulation (Fig. 4). One month after keratoplasty, in vivo confocal microscopy demonstrated *Acanthamoeba* cysts both in the host and donor corneas (Fig. 5).

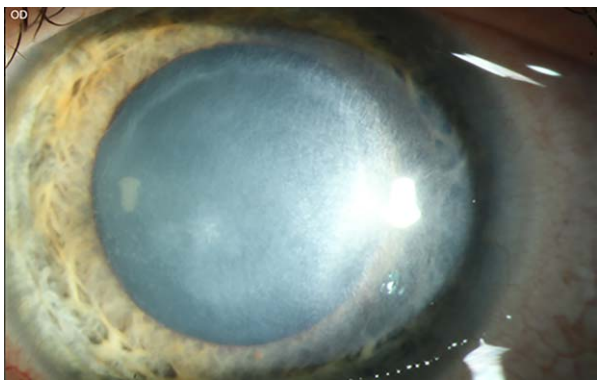


Fig. 2. Patient 2: Slit lamp: central subepithelial and stromal scar formation, consistent with "haze." [Color figure can be viewed at wileyonlinelibrary.com]

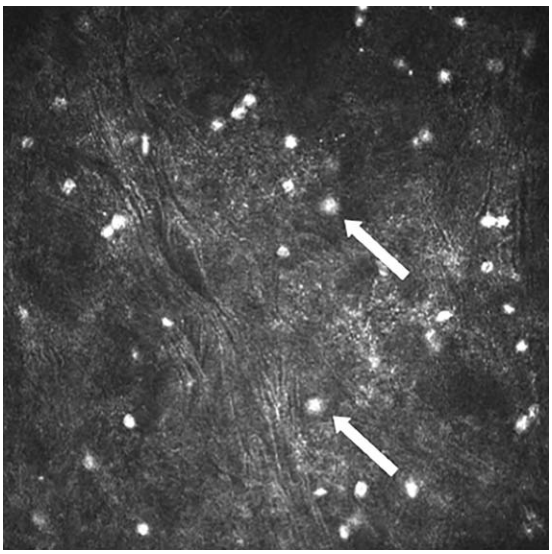


Fig. 3. Patient 1: In vivo confocal microscopy on the day of admission demonstrated *Acanthamoeba* cysts (arrows).

A repeat keratoplasty (10 mm) using hand-held trephine and multiple single sutures (10–0 Nylon) was conducted with additional corneal cryocoagulation (Fig. 6).

In the case of Patient 2, the preliminary clinical findings did not allow us to differentiate between a persisting *Acanthamoeba* keratitis and scars from a previous infection. Through the use of in vivo confocal microscopy we were able to identify double-walled *Acanthamoeba* cysts at a depth of 250 to 300 μm, i.e., below the crosslinking demarcation line (Figs. 7a and 7b). As the cystic form could not be managed by medical therapy, we recommended a penetrating excimer laser keratoplasty (8.0 mm/8.1 mm) with additional corneal cryotherapy, which would also help to rehabilitate vision.

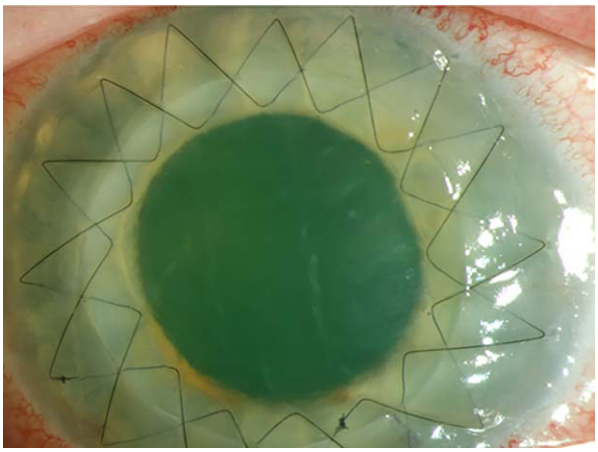


Fig. 4. Patient 1: Slit lamp: three days after an (8.0 mm/8.1 mm) penetrating excimer laser keratoplasty with additional corneal cryocoagulation. [Color figure can be viewed at wileyonlinelibrary.com]

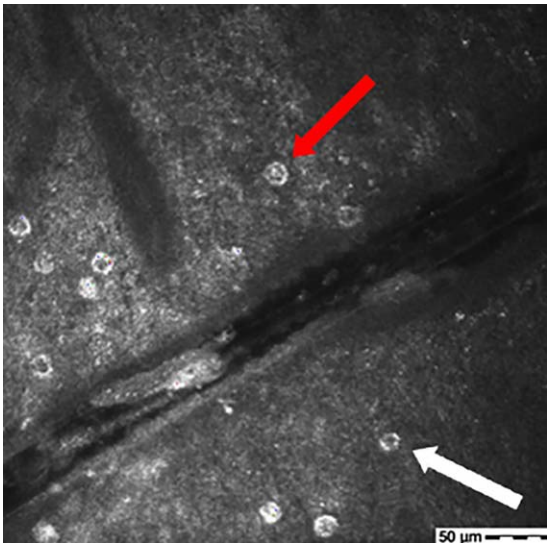


Fig. 5. Patient 1: In vivo confocal microscopy: one month after keratoplasty, *Acanthamoeba* cysts in the host (white arrows) and donor (red arrow) corneas. [Color figure can be viewed at wileyonlinelibrary.com]

DISCUSSION

Acanthamoeba keratitis is a rare and often late-diagnosed form of keratitis with a frequently prolonged clinical course. In its early stages it often presents in a similar manner to herpetic keratitis. Therefore, differential diagnosis is a particular challenge for ophthalmologists (Meltendorf and Dunker, 2011). In the German *Acanthamoeba* keratitis register ($n = 172$), 47.6% of cases initially had incorrect diagnoses of herpes-simplex viral keratitis, 25.2% of bacterial keratitis, and 3.9% of mycotic keratitis (Daas et al., 2015).

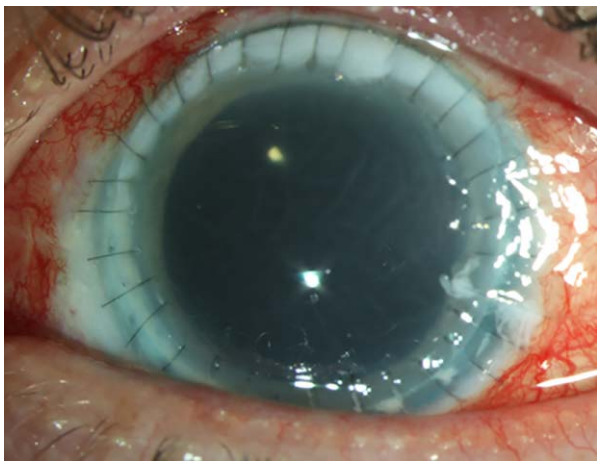
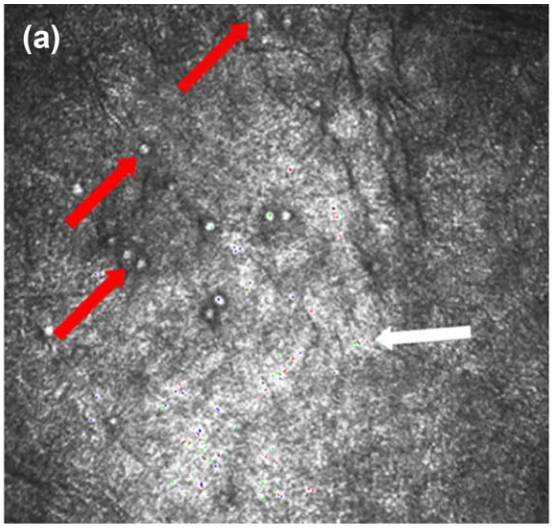


Fig. 6. Patient 1: Slit lamp: three months after a (10 mm) hand-held trephine repeat keratoplasty with additional corneal cryocoagulation and multiple interrupted 10–0 nylon sutures. [Color figure can be viewed at wileyonlinelibrary.com]



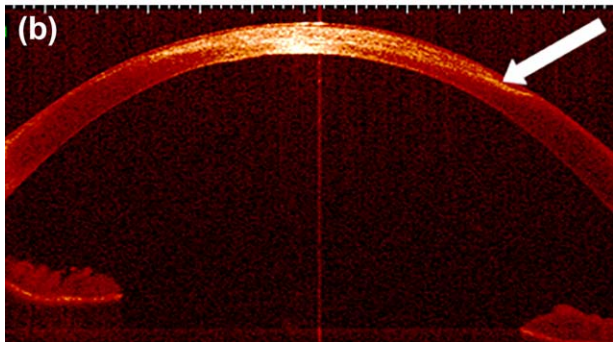


Fig. 7. (a,b) Patient 2: In vivo confocal microscopy: double-walled *Acanthamoeba* cysts at a depth of 250–300 µm (red arrow), i.e., below the crosslinking demarcation line (white arrow) (**a**), CASIA 2 OCT (Tomey Croup, Nagoya, Japan): demarcation line (white arrow) (**b**). [Color figure can be viewed at wileyonlinelibrary.com]

The sensitivity and specificity of in vivo confocal microscopy for diagnosis of *Acanthamoeba* keratitis are reported to be 100% and 84%, respectively, and 94% and 78% respectively for the identification of fungi (Kanavi et al., 2007; Messmer, 2012).

Both early detection of a recurrence of acanthamoeba keratitis following keratoplasty and detection of persistent acanthamoeba cysts in follow-up assessments are crucial for prognosis. Clinical findings alone cannot always demonstrate a relapse after successful keratoplasty, nor can they always differentiate between persistent *Acanthamoeba* keratitis and stromal scars from a previous infection.

In vivo confocal microscopy can also be extremely helpful in diagnosing fungal keratitis, or in identifying the early stages of a relapse of the aforementioned condition (Daas et al., 2017). This enables us to rule out the differential diagnosis of fungal keratitis quickly and reliably by a non-invasive investigation.

The case of the first patient allows us to demonstrate that in vivo confocal microscopy is a fast and,

in experienced hands, reliable relapse marker after a keratoplasty for *Acanthamoeba* keratitis. We were able to identify the *Acanthamoeba* cyst infestation quickly in the host and donor corneas. This was essential for deciding on further surgical therapy (repeat keratoplasty with larger diameter). The diagnosis of acanthamoeba keratitis with in vivo confocal microscopy depends on the expertise of the user. It is therefore difficult to agree a definitive sensitivity and specificity for this diagnostic tool (Hau et al., 2010).

The case of the second patient allows us to demonstrate that in vivo confocal microscopy is a quick diagnostic tool for the follow-up assessment of *Acanthamoeba* keratitis. The effectiveness of antimicrobial crosslinking of the cornea is limited to either the superficial corneal stroma or the trophozoites. Complete elimination of *Acanthamoeba* cysts through this treatment option was reportedly unsuccessful (Hager et al., 2015).

CONCLUSIONS

In a case of deteriorating clinical image after corneal transplantation, and during the follow-up of *Acanthamoeba* keratitis, we were able to identify the recurrence or persistence of *Acanthamoeba* cysts using in vivo confocal microscopy. In our opinion, in vivo confocal microscopy belongs to the spectrum of diagnostic modalities available for infectious keratitis. However, it is not to be used as stand-alone diagnostic tool for this condition (Behrens-Baumann et al., 2015).

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