



The application of in vivo confocal scanning laser microscopy in the management of *Acanthamoeba* keratitis

Yukihiro Matsumoto,¹ Murat Dogru,^{2,3} Enrique Adan Sato,¹ Yasuhiro Katono,⁴ Yuichi Uchino,¹ Shigeto Shimmura,¹ Kazuo Tsubota¹

¹Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan; ²Johnson&Johnson Department of Ocular Surface and Visual Optics, Keio University School of Medicine, Tokyo, Japan; ³Department of Ophthalmology, Tokyo Dental College, Chiba, Japan; ⁴Department of Microbiology, Keio University School of Medicine, Tokyo, Japan

Purpose: To evaluate the role of in vivo laser scanning confocal microscopy (Heidelberg Retina Tomograph II, Rostock Cornea Module, HRTII-RCM) in the management of *Acanthamoeba* keratitis (AK).

Methods: Four eyes of four patients with AK seen at Keio University Hospital at the Department of Ophthalmology were studied in this single-center, prospective, interventional case series. All patients were routinely examined by slit-lamp microscopy including corneal fluorescein staining. Best corrected visual acuity (BCVA) was also measured before and after the treatment for AK. Both the scraped corneal epithelium and soft contact lens (SCL) storage solution in each patient's SCL case were cultured. Patient corneas were examined regularly using the HRTII-RCM before treatment and after commencement of medications including azoles, echinocandins, and chlorhexidine.

Results: All patients had various degrees of conjunctival injection, corneal edema, stromal opacity with radial keratoneuritis with slit-lamp examination. Cultures for AK were positive in three out of four cases by corneal scraping. Contact lens storage solutions were also positive in three of four cases. HRTII-RCM examination could detect *Acanthamoeba* cysts or trophozoites in all eyes before corneal scraping. No organisms were detectable in any of the cases in any of the corneal layers four to six weeks after treatment. The BCVA improved with treatment in three of four eyes.

Conclusions: HRTII-RCM could effectively demonstrate cysts and trophozoites and the nature of the inflammatory process in AK. In vivo laser scanning confocal microscopy employing HRTII-RCM could provide an end-point for treatment, saving the patient from additional invasive diagnostic procedures and unneeded exposure to long term topical or systemic medications.

Although *Acanthamoeba* keratitis (AK) is extremely rare, with reported US annualized incidence rates ranging between 1.65 to 2.01 cases per million contact lens wearers, it may end up to 15 times more common in the United Kingdom, Europe, and Hong Kong [1-7].

Recently, there has been a considerable increase in the number of AK cases in the US which was attributed to changes in clinical or demographic factors, organism pathogenicity, changes in lens care hygiene, and water supply disinfection standards [1-7].

Soft contact lens (SCL) wear still remains the leading risk factor for AK, although corneal trauma followed by exposure to *Acanthamoeba* alone may incite the keratitis [8].

The diagnosis and management are still challenging issues for the ophthalmologist and numerous studies have shown that a delay in diagnosis is associated with worse visual outcome and a protracted course [8]. Limitations of the diagnostic techniques may often lead to a delayed or a mistaken diagnosis. Corneal scrapings or biopsies, stained with Giemsa, periodic acid-Schiff (PAS), calcofluor white, or acridine orange stains may demonstrate cysts and trophozoites but are

invasive diagnostic tools which add an additional burden to the corneal wound healing process. Cultures using non-nutrient agar overlaid with *Escherichia coli* (*E. coli*) may show the trophozoites but not all laboratories are willing or equipped to provide this culture [8].

Confocal microscopy is a non-invasive tool that may aid in the diagnosis of AK and be useful in monitoring the treatment response. Recently, cornea-specific in vivo laser scanning confocal microscopy (Heidelberg Retina Tomograph II, Rostock Cornea Module [HRTII-RCM], Heidelberg Engineering GmbH, Dossenheim, Germany) has become available, yielding impressive, high quality images in many corneal pathologies [9]. In the literature, to the best of our knowledge, only a single case report described the confocal laser findings in a mixed corneal infection with filamentous fungi and *Acanthamoeba*. In this study, we report the application of this new technology to the diagnosis and successful new treatment of AK with a combination of miconazole and chlorhexidine described for the first time in the literature.

METHODS

Subjects and slit-lamp examinations: All four patients (three females and one male, mean age; 25.0 ± 12.2 years) were contact lens (CL) users and had severe ocular pain and decreased vision for at least a month despite medical treatment at an

Correspondence to: Dr. Yukihiro Matsumoto, Department of Ophthalmology, Keio University School of Medicine, Shinanomachi 35, Shinjuku-ku, Tokyo 160-8582, Japan; Phone: 81-3-5363-3820; FAX: 81-3-3358-5961; email: ymatsumoto8@aol.com

other clinic. Patients had to be suspected cases of AK with any of the corneal findings such as irregular punctate epithelial keratitis, pseudo-dendritic keratitis, radial keratoneuritis, ring infiltration or discoid keratitis to be included in this study. Best corrected visual acuity (BCVA) was measured at the first visit to our hospital (Keio University Hospital, Tokyo, Japan) and was routinely checked afterward. All patients were carefully examined by slit-lamp microscopy including corneal fluorescein dye staining. The slit-lamp photographs were also recorded simultaneously at each visit. The severity of AK was provided in each patient as early, late, and advanced as reported previously [10]. Briefly, early stages of AK consisted of clinical signs including pseudodendrites, epithelial haze, and epithelial defects. Late stages consisted of stromal infiltrates, nummular keratitis, and epithelial defects. Advanced stage disease had ring infiltrates, satellite lesions, and stromal abscess as major clinical signs. Confocal microscopy was carried out before any invasive procedure in this study. Informed consents were obtained from each patient.

In vivo laser confocal microscopy: In vivo confocal scanning laser microscopy was performed on all subjects with a new generation confocal microscope, the Rostock Corneal Software Version 1.2 of the HRTII-RCM. After topical anesthesia with 0.4% oxybuprocaine, the subject's chin was placed in the chin rest. The objective of the microscope was an immersion lens (Zeiss, magnification 63X) covered by a polymethylmethacrylate cap (Tomo-Cap; Heidelberg Engineering GmbH, Dossenheim, Germany). Comfort gel (Bausch&Lomb GmbH, Berlin, Germany) was used as a coupling agent between the applanating lens cap and the cornea. By adjusting the controller, the center of the Tomo-Cap was applanated onto the center of the cornea and in vivo digital images of the cornea were visualized directly on the computer screen. When the first superficial cells were seen, the digital micrometer gauge was set at zero then by pressing on the foot pedal, sequence images were recorded by a charge-coupled device (CCD) color camera (maximum 30 frames/s) while

gradually moving the focal plane forward into the corneal stroma. Corneal lesions were scanned while moving the applanating lens from the upper edge of the lesion toward the lower edge with minute vertical movements. Corneal lesions were also scanned while moving the applanating lens from the nasal edge of the lesion toward the temporal edge with minute horizontal movements. The number of cysts and trophozoites in nonoverlapping areas of each lesion were counted in each 400 $\mu\text{m} \times 400 \mu\text{m}$ frame. The depth where the organisms were found was also recorded in each case.

The laser source employed in the HRTII-RCM is a diode laser with a wavelength of 670 nm. Two-dimensional images consisted of 384x384 picture elements, covering an area of 400 $\mu\text{m} \times 400 \mu\text{m}$. Transversal field of view was captured using the "400 FOV" field lens. Digital resolution was quoted 1 $\mu\text{m}/\text{pixel}$ at transversal and 2 $\mu\text{m}/\text{pixel}$ at longitudinal by the manufacturer. Patients were regularly examined using HRTII-RCM to evaluate the efficacy of medical treatment after commencement of topical medications.

Culture for Acanthamoeba organisms: Acanthamoeba was cultured on non-nutrient agar (Agar No.1, Oxoid, Basingstoke, Hampshire, UK) overlaid with *E. coli* (*E. coli* 25922, American Type Culture Collection, Manassas, VA), which was specially prepared in the microbiology laboratory of our hospital. Corneal scrapings were done using a sterile spatula. The SCL storage solutions from each patient's SCL cases were also sent for culture. Scraped corneal epithelium was placed in sterile water. Scrape specimens and SCL storage solutions were centrifuged for 15 min at 3,000 rpm. The supernatant was carefully removed in a sterile manner and the remaining substance was seeded on the culture dish. The culture dish was observed with a light microscope everyday for at least a week. If the organism was not detected seven days after seeding, the result was regarded as negative.

Medical treatment: All patients received a combination of a topical antifungal agent (three patients: echinocandin compound; one patient: azole compound) and a surface-active agent

TABLE 1. BACKGROUND AND CLINICAL FINDINGS OF ACANTHAMOEBA KERATITIS

	BCVA before treatment	Clinical stage	Use of CL	Use of steroid	Culture of cornea scraping	Culture of CL solution	HRT II -RCM	Topical treatment	Corneal scraping	BCVA after treatment	Recur- rence	Follow-up periods
Case 1	HM	Advanced	(+)	(+)	Pos.	Neg.	(+) (-) at 6w	FCZ ChG	5 times	HM	(-)	40w
Case 2	6/200	Late	(+)	(-)	Neg.	Pos.	(+) (-) at 4w	MCFG ChG	7 times	16/20	(-)	34w
Case 3	12/20	Early	(+)	(+)	Pos.	Pos.	(+) (-) at 5w	MCFG ChG	9 times	20/20	(-)	20w
Case 4	CF	Advanced	(+)	(+)	Pos.	Pos.	(+) (-) at 4w	MCFG ChG	5 times	14/20	(-)	18w

The table demonstrates the clinical stage of acanthamoeba keratitis, risk factors for the infection, culture results, treatment strategy, visual outcome, and prognosis. Note that the combination of either fluconazole and chlorhexidine or micafungin and chlorhexidine were effective options in eliminating acanthamoeba organisms as observed by confocal microscopy. No recurrences were observed with cessation of treatment throughout the follow-up period. BCVA=best corrected visual acuity, CL=contact lens, HRTII-RCM=Heidelberg Retina Tomograph II, Rostock Cornea Module, HM=hand motion, CF=counting finger, FCZ=fluconazole, MCFG=micafungin, ChG=chlorhexidine gluconate, Pos.=positive, Neg.=negative

(chlorhexidine gluconate). Topical fluconazole (FCZ; Diflucan, Pfizer Pharmaceutical Co., Tokyo, Japan) was prepared as five bottles (10 ml/bottle) that were aliquoted from 0.2% injectable solution of FCZ (100 mg/50 ml) in a sterile manner. Topical micafungin (MCFG; Fungard, Astellas Pharmaceutical Co., Tokyo, Japan) was prepared as 0.1% eye drops by dissolving the injectable powder of the vials containing 50 mg of the antifungal agents by 50 ml of sterile saline solution. In the same manner, 0.02% topical chlorhexidine gluconate (ChG; Maskin Water, Maruishi Pharmaceutical Co., Osaka, Japan) was prepared by diluting 2.5 times the 0.05% original solution, the antiseptic, surface-active agent. At the beginning of the treatment, these eye drops were applied frequently (every 30 min) to the infected eyes, which underwent proper corneal scraping in order to reduce the quantity of the organism and to increase the permeability of these agents. Corneal scraping was stopped within a month in all cases. The patients were instructed to apply the combination of these eye drops to their infected eyes every 30 min at the beginning of treatment. The frequency of topical eye drops was tapered gradually accord-

ing to the improvement response of the corneal lesions. Topical eye drop use was terminated when successive confocal scans did not reveal any cysts or trophozoites during the follow up.

RESULTS

The characteristics and clinical outcome of our cases are summarized in Table 1.

Slit-lamp examination: None of the patients received a proper diagnosis of AK at their previous institutions and were treated by topical antibiotics, ointment of acyclovir, or topical steroids for bacterial, herpetic, or unknown keratitis for at least one month. At the first visit to our hospital, we observed corneal epithelial irregularity and disarray with sub-epithelial infiltration and radial keratoneuritis in each case by slit-lamp microscopy. Specifically, case 1 (Figure 1) showed advanced AK with remarkable stromal ring infiltration, satellite lesions, and epithelial breakdown. Case 2 (Figure 2) had late AK with stromal infiltrates. Case 3 (Figure 3) had early AK with epithelial haze and pseudodendrites. Case 4 (Figure 4) had ad-

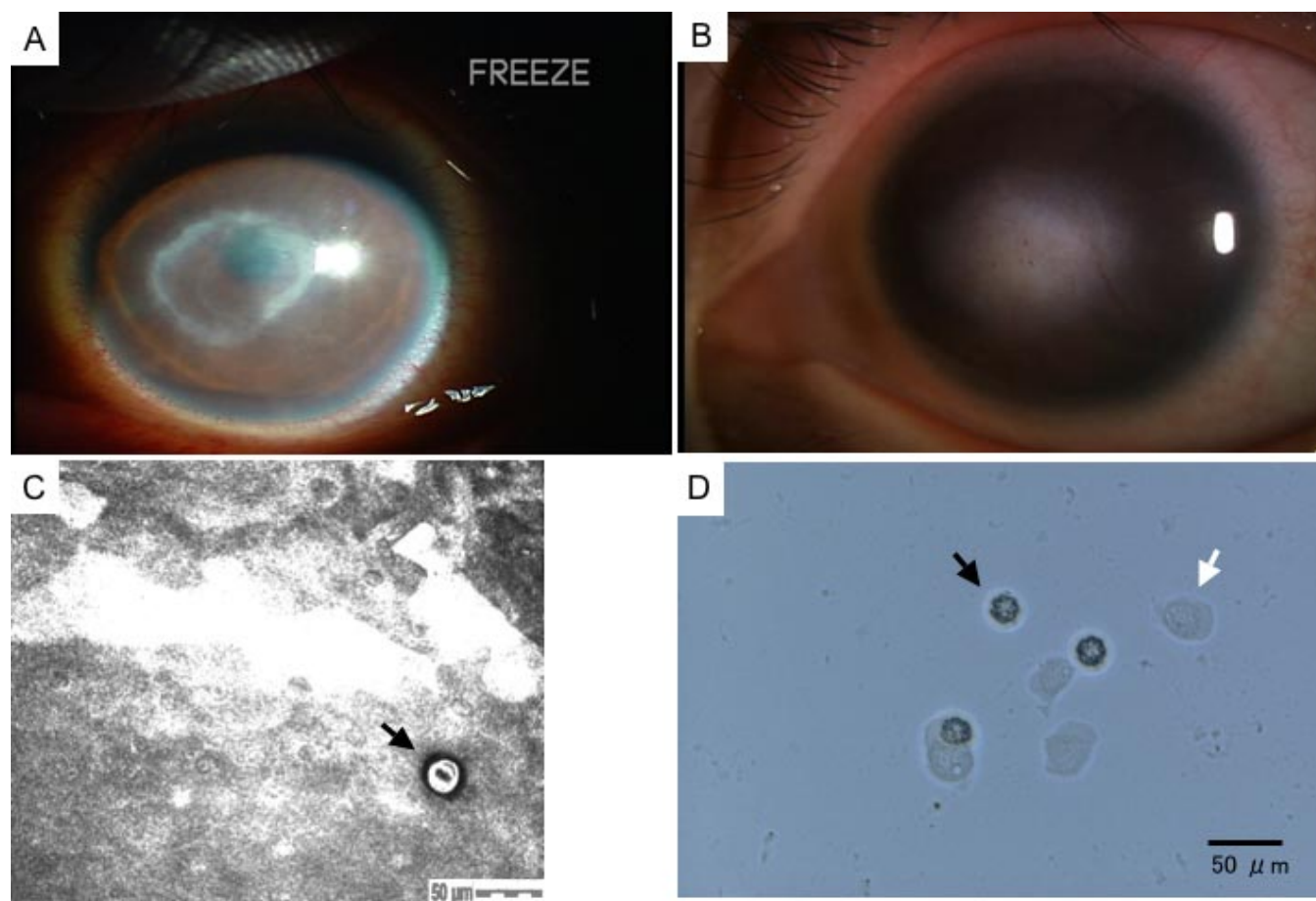


Figure 1. Clinical outcome, confocal scan, and culture photographs of case 1. **A:** The pre-treatment slit-lamp photograph of the cornea revealed conjunctival injection and advanced stage *Acanthamoeba* keratitis with ring infiltration in the central cornea. **B:** The post-treatment slit-lamp photograph of the cornea revealed round corneal opacity with deep new vessels in the central cornea. **C:** The HRTII-RCM showed a double-walled and round *Acanthamoeba* cyst (indicated by the black arrow; size: 25 μ m; depth: 1 μ m). **D:** Note the confirmation of the presence of the *Acanthamoeba* cysts (indicated by the black arrow) and trophozoites (indicated by the white arrow) in the culture of corneal scrapings.

vanced AK with ring infiltrate and stromal opacity. The infiltrations started to decrease one week after commencement of topical eye drop application and could not be detected after four to six weeks in all cases. Subsequently, the frequency of each eye drop was reduced to five times per day and stopped altogether when the confocal scans revealed no organisms. No recurrences were observed in our cases throughout their follow up.

The BCVA improved in cases 2-4 (Figure 2, Figure 3, Figure 4). Although the inflammatory infiltration decreased, severe corneal opacity ensued after treatment and the BCVA did not change in case 1 (Figure 1) who remained undiagnosed without proper medication and developed advanced AK until her referral.

In vivo laser confocal microscopy: The HRTII-RCM showed double-walled and round *Acanthamoeba* cysts and/or homogeneous intense and multiform trophozoites in all cases. All images of *Acanthamoeba* cysts captured by this device basically revealed the double-walled cyst structures; however, the cysts had various sizes and shapes. The diameter of cysts ranged between 20-100 μm . The space between the outer and

the inner wall was not fixed and the tracery structure inside the inner wall also varied. The size of the trophozoites was more than 100 μm . Confocal scans also revealed inflammatory cells consisting of neutrophils or dendritic cells in the lesions. Many *Acanthamoeba* cysts and trophozoites together with inflammatory cells were detected by the HRTII-RCM examination in all eyes before corneal scraping. The cysts were superficially located in the corneal epithelium in cases 1 (Figure 1) and 3 (Figure 3), were somewhat deeper within the epithelium at 30 μm in case 2 (Figure 2) and were intrastromal in case 4 (Figure 4) at a depth of 62 μm . Confocal scan frames revealed between zero and six cysts in case 1 (advanced AK; Figure 1). Confocal scan frames in the other case with advanced AK (case 4; Figure 4) showed between zero and three cysts. Confocal scan frames from case 2 (Figure 2) with late AK had zero to one cyst. Case 3 (Figure 3) with early AK had zero to one cyst and zero to one trophozoite in the confocal scan frames. The inflammatory reaction was mixed with neutrophils and more of dendritic cells in case 1 (Figure 1), mixed again with dendritic cell predominance in case 2 (Figure 2), mainly neutrophilic with a few dendritic cells in case 3 (Fig-

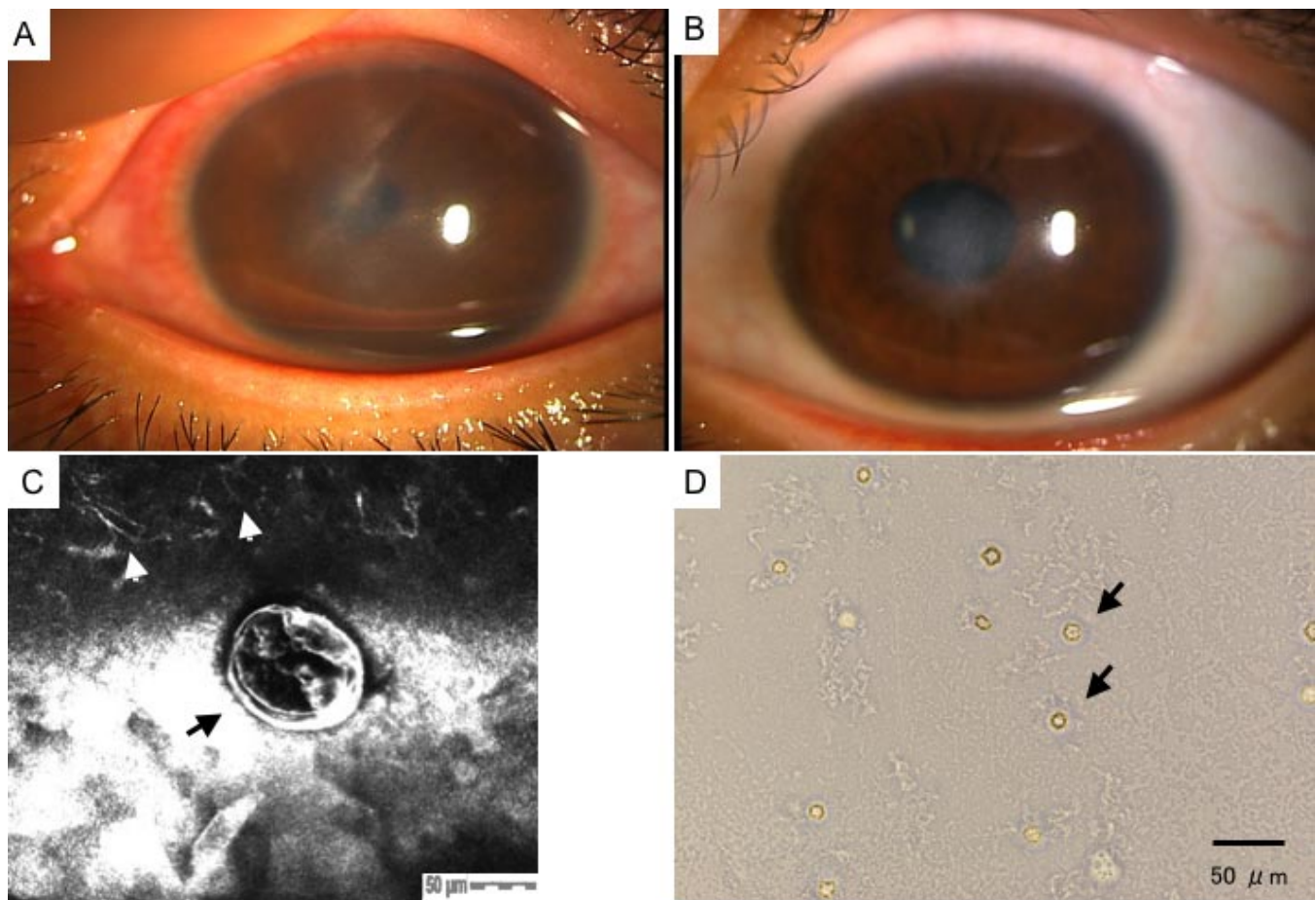


Figure 2. Clinical outcome, confocal scan and culture photographs of case 2. **A:** The pre-treatment slit-lamp photograph of cornea revealed subepithelial infiltration with radial keratoneuritis in the central cornea with conjunctival injection. **B:** The post-treatment slit-lamp photograph of the cornea revealed mild remaining corneal opacity. **C:** The HRTII-RCM showed a double-walled, round *Acanthamoeba* cyst (indicated by the black arrow; size: 100 μm ; depth: 30 μm). Please note the Langerhans cells (indicated by the white arrows) in the close vicinity of the cyst. **D:** Note the *Acanthamoeba* cysts (indicated by the black arrows) confirmed by culture of soft contact lens (SCL) storage solution.

ure 3) and mainly dendritic in case 4 (data not shown). No organisms or extensive inflammation were detected with four to six weeks after commencement of treatment for AK.

Cultures for Acanthamoeba: We performed a culture test for *Acanthamoeba* in all patients. Three out of four cases had positive cultures for *Acanthamoeba* from the scraped corneal epithelium and SCL storage solutions. Either cysts or trophozoites were shown on the culture dish with a light microscope in these cases. The cysts characteristically showed double-walled structures and the endocysts showed round, star-shaped, or irregular polygons. The trophozoites had acanthopodia and karyosome in the vesicular nucleus. The shape and size of trophozoites showed variation (25-50 μm in diameter).

DISCUSSION

A previous official report on ophthalmic technology assessment by the American Academy of Ophthalmology stated that confocal white light scanning holds the potential to instantaneously provide a diagnosis of AK and a measure of the effec-

tiveness of its treatment [11]. In this report, we provided high resolution images of the *Acanthamoeba* cysts and trophozoites within the corneal epithelium or the stroma using the in vivo laser scanning confocal microscope (HRTII-RCM). Images of the cysts in all cases were characteristic with double-walled, round, or ovoid shaped structure, from 20 to 100 μm in diameter. The HRTII-RCM provided high resolution sharp image details of the cysts such as the space and lack of fixation between the outer and inner walls and the presence of variation of the tracery structure inside the inner walls. Although Tandem Scanning Confocal Microscopy (Tandem Scanning Corporation, Reston, VA) and ConfoScan (Nidek Technologies Srl, Vigonza, Italy) images from patients with AK have been reported in the literature, only a single recent case report demonstrated *Acanthamoeba* cyst image together with fungus filaments with the HRT II-RCM [9,12-15]. We revealed the images of trophozoites employing the HRTII-RCM for the first time in the literature in this report, which we believe will provide an easier diagnosis of trophozoites

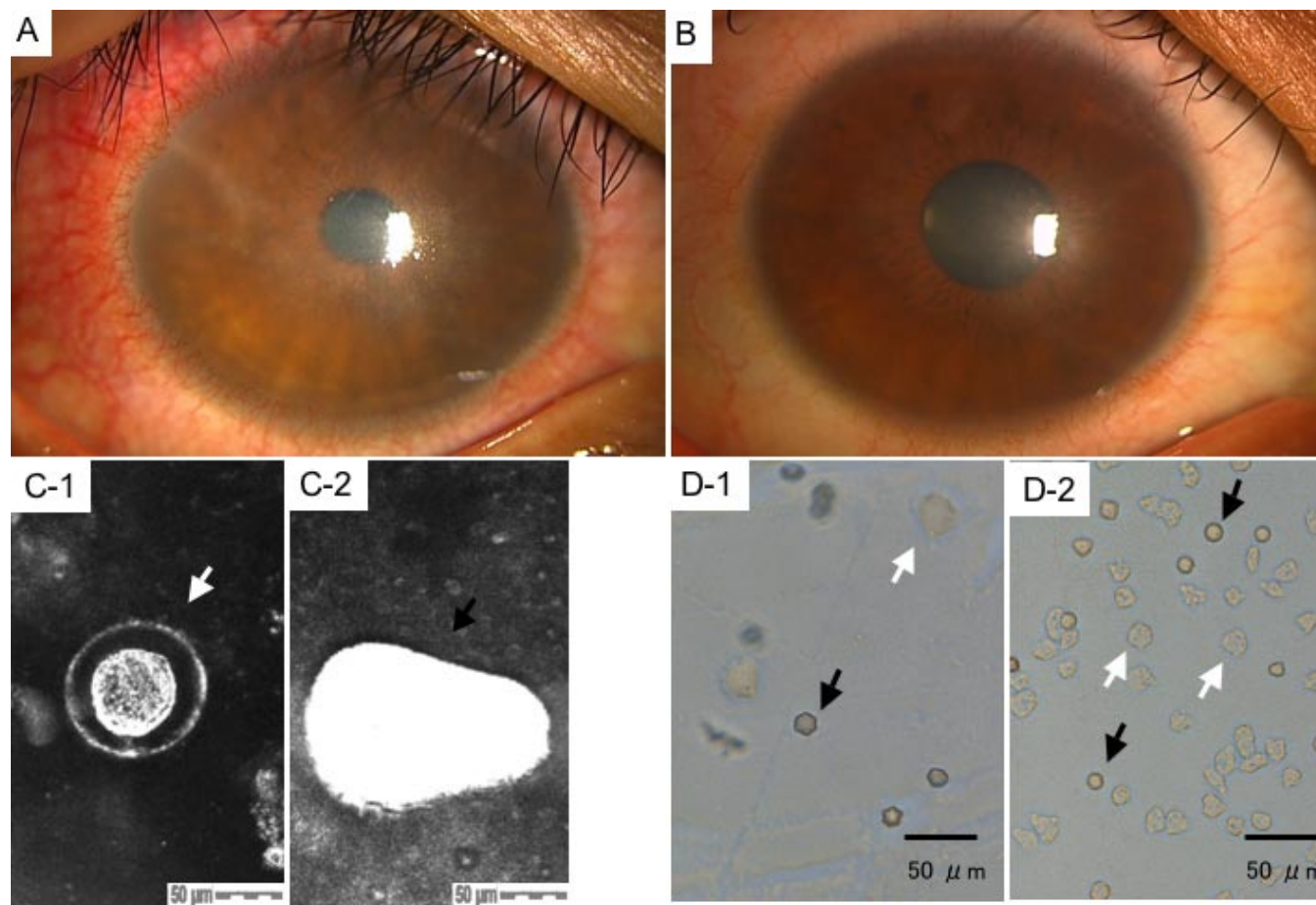


Figure 3. Clinical outcome, confocal scan and culture photographs of case 3. **A:** A slit-lamp photograph of the pre-treatment cornea revealed central subepithelial infiltration with radial keratoneuritis, corneal edema with conjunctival injection. **B:** A slit-lamp photograph of the post-treatment cornea revealed centrally clear cornea with mild nasal corneal opacity. **C:** The HRTII-RCM scan of the lesion showed double-walled and round *Acanthamoeba* cyst (C-1: indicated by the white arrow, size: 100 μm ; depth: 2 μm) and highly reflective and irregular-shaped trophozoite (C-2: indicated by the black arrow, size: 175 μm ; depth: 2 μm). **D:** Note that the *Acanthamoeba* cysts (indicated by the black arrow) and trophozoites (indicated by the white arrow) were reconfirmed by cultures of corneal scrapings (D-1). Cysts (indicated by the black arrows) and trophozoites (indicated by the white arrows) were also confirmed in the culture of the SCL storage solution (D-2).

for users of this new technology. The trophozoite forms have been reported to be more difficult to discern with previous confocal device technologies as they appear similar to normal corneal keratocyte nuclei with ovoid, S-shaped structures within the corneal stroma [11]. With HRTII-RCM, the trophozoites had homogeneous intense, high-reflective, and multifiform structures that were generally over 100 μm in diameter. We also detected many inflammatory cells including dendritic cells, neutrophils, and others surrounding the *Acanthamoeba* organisms. In our experience, the HRTII-RCM permitted more details with sharper and higher image quality compared to white-light confocal microscopy images (Tandem Scanning Confocal Microscopy or ConfoScan) of AK published in the literature due to a better axial resolution than that obtained with the conventional white-light confocal microscope (1 μm versus 10 μm by ConfoScan 2).

The cyst and trophozoite sizes differed from the published sizes in the literature in this study. The variation in the size of the intrastromal cysts observed in the corneal stroma in our

study may be explained by the differences of the biological behavior of the amoeba organisms in living tissues compared to the in vitro environment in cultures. Different cyst sizes may also reflect the presence of different pathogenetic strains in the tissues. Future studies employing HRTII-RCM and species identification concomitantly may very well increase our diagnostic ability of pathogenetic strains through their size and morphological features.

Acanthamoebic cultures and smears are still the standard diagnostic methods used to detect the infectious organisms [16,17]. In our study, microbiological evidence of *Acanthamoeba* in the corneal epithelial scrapings and CL solutions was found in three out of four cases. Cultures, however, are usually useful before treatment and require several days to obtain growth. On the other hand, confocal microscopy allowed both a qualitative and quantitative analysis of the entire cornea to be performed rapidly, delivering immediate diagnosis in vivo, and the examination could be done repeatedly and noninvasively. Repetitive scans performed by

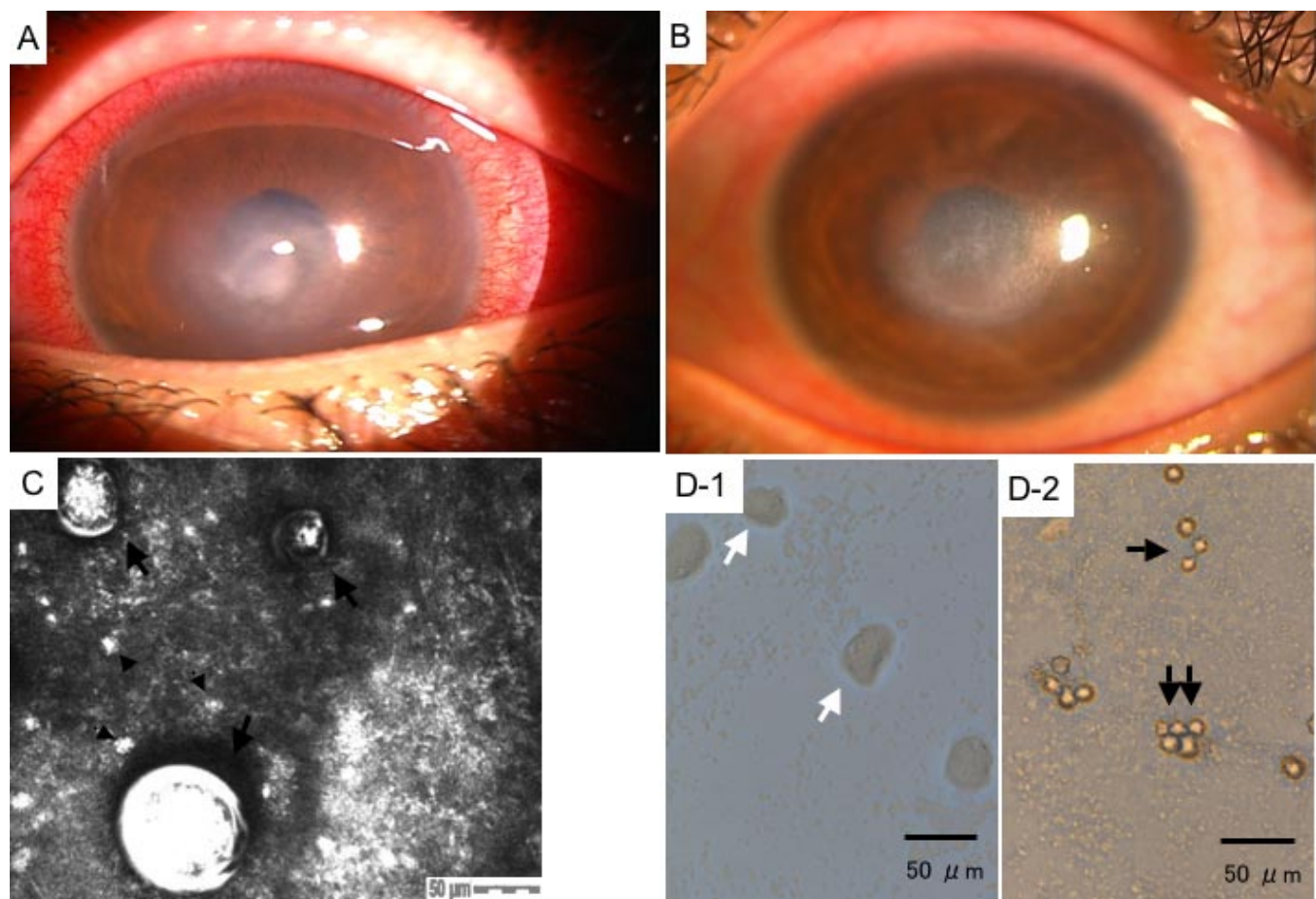


Figure 4. Clinical outcome, confocal scan and culture photographs of case 4. **A:** The pre-treatment slit-lamp photograph of the cornea revealed extensive subepithelial infiltration in the central cornea with conjunctival injection. **B:** The post-treatment slit-lamp photograph of the cornea revealed moderate remaining stromal opacity. **C:** The HRTII-RCM scan of the corneal lesion showed double-walled and round *Acanthamoeba* cysts (indicated by the black large arrows; upper left cyst size: 50 μm , upper right cyst: 40 μm , lower left cyst: 90 μm , depth: 62 μm) and small and round inflammatory cells (indicated by the black small arrow heads). **D:** Note that the presence of the *Acanthamoeba* trophozoites (indicated by the white arrows) was also confirmed by culture of corneal scrapings (D-1). Cysts (indicated by the black arrows) were also confirmed in the SCL storage solution (D-2).

HRTII-RCM showed eventual clearing and disappearance of either the trophozoites or the cysts and subsidence of the inflammatory reaction within four to six weeks after initiation of *Acanthamoeba* treatment in our cases, suggesting the efficacy of the device in evaluating the treatment responses. Application of this device as an end point for the cessation of the topical treatments resulted in a shorter duration of treatment compared to other reports in the literature. We believe that the confocal laser scanning microscopy may provide the best means to diagnose the disease but may not be the most efficient means to evaluate the effectiveness of treatment, which typically requires months to resolve.

Another important observation in this study was the efficacy of the combination of MCFG and ChG in the treatment of AK achieving a rapid resolution with a reduction in corneal infiltration and injection within a month. No recurrences were observed after cessation of treatment. A PubMed and Medline review revealed that this treatment modality has not been reported previously. The treatment of AK is challenging because the cystic form is highly resistant and may persist for years. Along with propamidine and neomycin, chlorhexidine and polyhexamethylene biguanide have been shown to be just as effective [8,18-20]. Imidazoles were reported to be effective against the trophozoites but not the cysts thus recommended to be prescribed together with an antiseptic agent [8,21]. A recent study reported that caspofungin, a new group of echinocandin anti-fungal, had in vitro activity against trophozoites and cysts of *Acanthamoeba* [22]. MCFG, a new addition to the armamentarium of echinocandin compounds, has been reported to be effective and safe in rabbit models of keratomycosis [23]. We previously reported the efficacy and safety of MCFG in patients with fungal keratitis [24]. MCFG has also been recently reported to be amoebacidal (Tei et al, unpublished data, presented at the 28th Japan Cornea Conference, December 2004, Yonago, Japan). We treated AK by frequent topical application of antifungal and antiseptic agents after diagnosis in this series. Either 0.2% FCZ or 0.1% MCFG as antifungal agents and 0.02% ChG as an antiseptic agent were applied for AK. Both MCFG and caspofungin of the same family are available in the United States, which we believe will be stimulating for the physicians to challenge AK with this new and promising therapeutic approach.

In our series of patients, it was our clinical impression that cases with advanced stages of AK with deeper location of *Acanthamoeba* organisms in the corneal epithelium or stroma as well as cases with mixed inflammatory infiltrates in abundance of dendritic cells ended up with considerable stromal opacity and relatively lower visual acuity values. The depth in which the organisms were located rather than the numbers seemed to have an influence on the final clinical outcome. Although the cysts were superficially located in case 1 at 1 μm , this case had the worst final acuity and significant central corneal opacity. This outcome may be due to late diagnosis and improper medication including topical steroids for a longer duration of time. In future trials, it would be interesting to study if confocal parameters such as the depth and number of the parasites in the corneal tissue, the nature of the inflamma-

tory infiltrates, and clinical parameters such as the duration of improper treatment until institution of *Acanthamoeba* treatments have prognostic implications on final visual acuities and remaining opacities in patients with AK.

ACKNOWLEDGEMENTS

The authors have no proprietary interest in any of the products mentioned in this paper nor received any funding from any company for the conduct of the current study. This work was presented at the 31st Japan Cornea Conference, February 9-11, 2007, Miyazaki, Japan.

REFERENCES

- Joslin CE, Tu EY, McMahon TT, Passaro DJ, Stayner LT, Sugar J. Epidemiological characteristics of a Chicago-area *Acanthamoeba* keratitis outbreak. *Am J Ophthalmol* 2006; 142:212-7.
- Radford CF, Minassian DC, Dart JK. *Acanthamoeba* keratitis in England and Wales: incidence, outcome, and risk factors. *Br J Ophthalmol* 2002; 86:536-42.
- Seal DV, Kirkness CM, Bennett HG, Peterson M, Keratitis Study Group. Population-based cohort study of microbial keratitis in Scotland: incidence and features. *Cont Lens Anterior Eye* 1999; 22:49-57.
- Lam DS, Houang E, Fan DS, Lyon D, Seal D, Wong E, Hong Kong Microbial Keratitis Study Group. Incidence and risk factors for microbial keratitis in Hong Kong: comparison with Europe and North America. *Eye* 2002; 16:608-18.
- Radford CF, Lehmann OJ, Dart JK. *Acanthamoeba* keratitis: multicentre survey in England 1992-6. National *Acanthamoeba* Keratitis Study Group. *Br J Ophthalmol* 1998; 82:1387-92.
- Schaumberg DA, Snow KK, Dana MR. The epidemic of *Acanthamoeba* keratitis: where do we stand? *Cornea* 1998; 17:3-10.
- Seal DV. *Acanthamoeba* keratitis update-incidence, molecular epidemiology and new drugs for treatment. *Eye* 2003; 17:893-905.
- Hammersmith KM. Diagnosis and management of *Acanthamoeba* keratitis. *Curr Opin Ophthalmol* 2006; 17:327-31.
- Babu K, Murthy KR. Combined fungal and *acanthamoeba* keratitis: diagnosis by in vivo confocal microscopy. *Eye* 2007; 21:271-2.
- Alizadeh H, Niederhorn JY, McCulley JP. *Acanthamoeba* keratitis. In: Krachmer JH, Mannis MJ, Holland EJ, editors. *Cornea*. Vol 1. Philadelphia: Elsevier Mosby; 2005. p. 1115-22.
- Kaufman SC, Musch DC, Belin MW, Cohen EJ, Meisler DM, Reinhart WJ, Udell IJ, Van Meter WS. Confocal microscopy: a report by the American Academy of Ophthalmology. *Ophthalmology* 2004; 111:396-406. Erratum in: *Ophthalmology*. 2004; 111:1306.
- Cho BJ, Holland EJ. In vivo tandem scanning confocal microscopy in *acanthamoeba* keratitis. *Korean J Ophthalmol* 1998; 12:112-7.
- Mathers WD, Nelson SE, Lane JL, Wilson ME, Allen RC, Folberg R. Confirmation of confocal microscopy diagnosis of *Acanthamoeba* keratitis using polymerase chain reaction analysis. *Arch Ophthalmol* 2000; 118:178-83.
- Parmar DN, Awwad ST, Petroll WM, Bowman RW, McCulley JP, Cavanagh HD. Tandem scanning confocal corneal microscopy in the diagnosis of suspected *acanthamoeba* keratitis. *Ophthalmology* 2006; 113:538-47.
- Winchester K, Mathers WD, Sutphin JE, Daley TE. Diagnosis of *Acanthamoeba* keratitis in vivo with confocal microscopy. *Cor-*

- nea 1995; 14:10-7.
16. Bharathi MJ, Ramakrishnan R, Meenakshi R, Mittal S, Shivakumar C, Srinivasan M. Microbiological diagnosis of infective keratitis: comparative evaluation of direct microscopy and culture results. *Br J Ophthalmol* 2006; 90:1271-6.
 17. Yera H, Zamfir O, Bourcier T, Ancelle T, Batellier L, Dupouy-Camet J, Chaumeil C. Comparison of PCR, microscopic examination and culture for the early diagnosis and characterization of *Acanthamoeba* isolates from ocular infections. *Eur J Clin Microbiol Infect Dis* 2007; 26:221-4.
 18. Seal D, Hay J, Kirkness C, Morrell A, Booth A, Ridgway A, Armstrong M. Successful medical therapy of *Acanthamoeba* keratitis with topical chlorhexidine and propamidine. *Eye* 1996; 10:413-21.
 19. Perez-Santonja JJ, Kilvington S, Hughes R, Tufail A, Matheson M, Dart JK. Persistently culture positive *acanthamoeba* keratitis: in vivo resistance and in vitro sensitivity. *Ophthalmology* 2003; 110:1593-600.
 20. Sun X, Zhang Y, Li R, Wang Z, Luo S, Gao M, Deng S, Chen W, Jin X. *Acanthamoeba* keratitis: clinical characteristics and management. *Ophthalmology* 2006; 113:412-6.
 21. van der Bijl P, van Eyk AD, Seifart HI, Meyer D. In vitro transcorneal penetration of metronidazole and its potential use as adjunct therapy in *Acanthamoeba* keratitis. *Cornea* 2004; 23:386-9.
 22. Bouyer S, Imbert C, Daniault G, Cateau E, Rodier MH. Effect of caspofungin on trophozoites and cysts of three species of *Acanthamoeba*. *J Antimicrob Chemother* 2007; 59:122-4.
 23. Kaji Y, Hiraoka T, Oshika T. Potential use of (1,3)-beta-D-glucan as target of diagnosis and treatment of keratomycosis. *Cornea* 2004; 23:S36-41.
 24. Matsumoto Y, Dogru M, Goto E, Fujishima H, Tsubota K. Successful topical application of a new antifungal agent, micafungin, in the treatment of refractory fungal corneal ulcers: report of three cases and literature review. *Cornea* 2005; 24:748-53.