In Vivo Quantification of Bacterial Keratitis with Optical Coherence Tomography

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Purpose. To quantify the human corneal inflammatory response in treated bacterial keratitis with long-wavelength anterior segment optical coherence tomography (AS-OCT).

Methods. Patients with clinically suspected bacterial keratitis were recruited from the corneal service at Southampton Eye Unit, UK. Patients underwent AS-OCT and slit-lamp examination on presentation (day 0) and days 3, 7, and 14 of treatment. Corneal thickness (CT) in the infiltrated area, infiltrate thickness (IT), and infiltrate width (IW) were measured on high-resolution AS-OCT scans. Mean values for each day and rates of change for each interval were calculated and compared (one-way ANOVA, paired *t*-test).

Results. Twenty-six eyes of 26 patients were recruited. Mean CT and IT on presentation were 905 μm and 388 μm, respectively. On days 3, 7, and 14, CT and IT decreased to 753 μm and 320 μm ($P < 0.01$), 678 μm and 296 μm ($P < 0.01$), and 584 μm and 207 μm ($P < 0.01$), respectively. Mean IW, 1498 μm on presentation, did not change during treatment ($P > 0.30$). Mean daily rate of CT reduction was faster in the early (days 0–3) compared to late (days 7–14) phase (4.49% vs. 1.33%, $P = 0.006$). Mean daily rate of IT reduction was no different in early, middle, and late phases (5.41% vs. 1.19% vs. 3.38%, $P > 0.01$). In the late phase, IT decreased faster than CT (3.38% vs. 1.33%, $P = 0.003$).

Conclusions. CT and IT decreased significantly by day 3 in resolving bacterial keratitis. The rapid early phase reduction in IT and CT was followed by rapid late phase IT reduction. This study demonstrates that serial AS-OCT examination can be used to monitor in vivo the clinical course of inflammatory disease. (*Invest Ophthalmol Vis Sci.* 2011;52:1093–1097) DOI: 10.1167/iovs.10-6067

Corneal disease is a major cause of blindness, with its epidemiology encompassing a spectrum of corneal infections and inflammatory diseases.[1–4] Bacterial keratitis, an infective and inflammatory condition of the cornea, is characterized by corneal epithelial ulceration, corneal edema and stromal infiltration with inflammatory cells.[5–8] Animal studies have confirmed these morphologic changes with histopathology.[6,9,10]

In clinical practice, bacterial keratitis is routinely assessed with slit-lamp biomicroscopy, an examination and imaging modality that is primarily limited by the physical properties of light. The location of corneal infiltration can be assessed and the horizontal and vertical dimensions of epithelial ulceration and infiltration measured.[11] However, the depth of pathology and associated corneal edema cannot be measured with the slit-lamp. As a result, in vivo information regarding corneal inflammation in bacterial keratitis and the response to treatment is limited.

Objective clinical assessment of bacterial keratitis and the treatment response is difficult, especially during the early treatment phase. Subjective features, such as improvement of patient symptoms, are therefore also used as indicators of resolving infection. Recently, however, in vivo assessment of microbial keratitis has become possible with long wavelength anterior segment optical coherence tomography (AS-OCT). This modality provides cross-sectional scans of the cornea and assessment of the depth of inflammation with measurements of stromal infiltration thickness and corneal thickness in the infiltrated area.[12]

In this study we aim to quantify corneal inflammation in resolving bacterial keratitis by measuring the temporal in vivo change of corneal thickness (CT), infiltrate thickness (IT), and infiltrate width (IW) with a commercially available AS-OCT device.

Methods

Recruitment of Patients

This study was approved by the local NHS Research Ethics Committee and informed consent was obtained from all patients. The research was performed in accordance with the tenets of the Declaration of Helsinki. Subjects with a clinical diagnosis of presumed bacterial keratitis were recruited from the corneal service at Southampton Eye Unit, Southampton University Hospitals NHS Trust, UK. A clinical diagnosis of bacterial keratitis was made in the presence of a typical history and an epithelial ulceration with underlying stromal infiltration associated with signs of inflammation.[13–15] Patients with a history and clinical findings suggestive of a viral keratitis or hypersensitivity type corneal ulceration were not recruited. Bacterial keratitis was considered resolved if the epithelial defect and signs of inflammation resolved completely. Recruited patients who did not respond to antibacterial treatment were excluded.

Examination and Scanning Protocol

Anterior segment imaging was carried out by OCT tomography (Visante OCT; Carl Zeiss Meditec Inc, Dublin, CA). AS-OCT imaging

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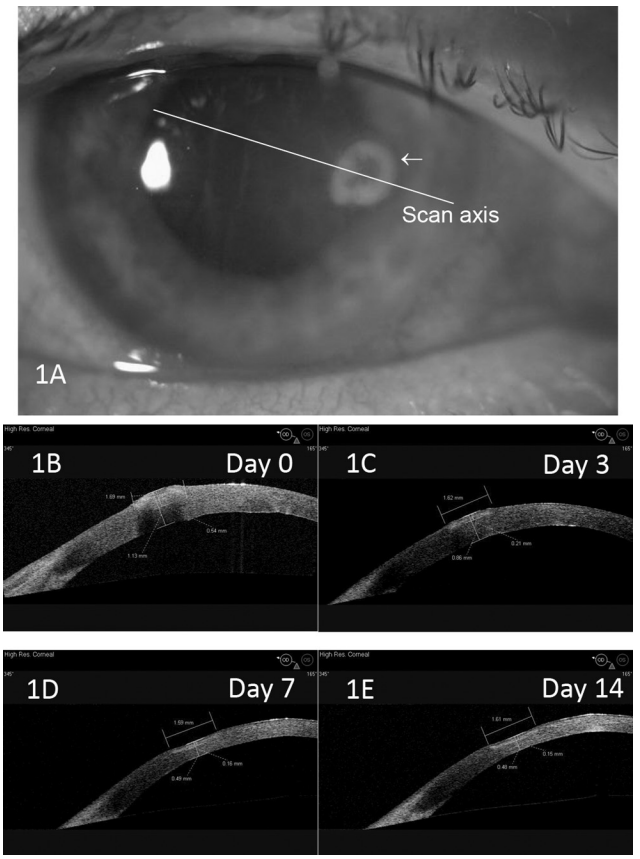

FIGURE 1. AS-OCT imaging of bacterial keratitis. (**A**) A corneal ulcer with inflammatory infiltration (←) due to *Pseudomonas aeruginosa* keratitis. (**B**) The high-resolution AS-OCT image through the center of the infiltrated area at the 165° axis on presentation. The hyper-reflective area in the anterior corneal stroma corresponds to the clinical infiltration. The epithelium and endothelium have lost the smooth profile, reflecting the increased stromal thickness due to stromal infiltration and edema. Corneal thickness, infiltrate thickness and infiltrate width measure 1130, 540, and 1690 μm respectively. (**C**), (**D**), and (**E**) illustrate the reduction of corneal and infiltrate thickness on days 3, 7, and 14 respectively. Infiltrate width does not change considerably.

and clinical slit-lamp examination were carried out on presentation (day 0) and subsequently on days 3, 7, and 14 of treatment. All patients underwent treatment based on clinical findings and requirements. Standard antibacterial treatment was intensive guttae ofloxacin 0.3% and guttae cefuroxime 5% to the affected eye. Antibiotics were instilled at an hourly frequency for 48 hours, then reduced to two hourly for a further 48 hours. The frequency was then reduced to six hourly for a total treatment period of two weeks.

A standardized scanning protocol was used. At all visits high-resolution AS-OCT scans were carried out through the same area of corneal infiltration with the scanning beam running through the center of the infiltration at a defined axis. Corneal infiltration on the AS-OCT images was defined as the hyper-reflective area that corresponded to the clinical corneal infiltration. CT, IT, and IW were measured with caliper tools of the OCT tomography software (Visante, version 1.1.2; Carl Zeiss MediTec, Inc.). CT was measured in the center of the infiltration with one caliper arm on the most anterior hyper-reflective corneal surface and the second arm on the hyper-reflective endothelium. IT, also in the center of the infiltration, was measured with one caliper arm on the most anterior hyper-reflective corneal surface and the second arm on the posterior border of the hyper-reflective area. IW was measured by placement of the caliper arms on the transverse borders of the hyper-reflective area. The scanning protocol is illustrated in Figure 1.

CT, IT and IW were measured on days 0 (presentation), 3, 7, and 14 of treatment. Mean values were calculated for each day and compared. For parameters that showed statistically significant change, the daily rate of change was calculated for the early (days 0 to 3), middle (days 3 to 7), and late (days 7 to 14) phases of treatment. Daily rate of change of each parameter was calculated for each eye according to the formula: [*X* day B − *X* day A] /*X* day A /time interval in days, where *X* is the parameter value and A and B are the first and last day of the treatment phase, respectively. Mean values were calculated and compared.

Statistical Analysis

Power of the study to detect rate of change in IT and CT, at 5% significance level, using one-way analysis of variance (ANOVA) was 93% and 92%, respectively. Mean values of CT, IT, IW and their daily rate of change were compared for statistical difference using paired *t*-test and one-way ANOVA. P values < 0.01 were considered statistically significant. Statistical Analysis was performed with analysis software (The Statistical Package for Social Science, version 15; SPSS Inc, Chicago, IL).

RESULTS

Twenty seven patients (27 eyes) with a clinical diagnosis of bacterial keratitis were recruited. One patient who did not respond to standard antibacterial treatment had fungal keratitis and was excluded. In the remaining 26 patients with bacterial keratitis the infection resolved. Patient and keratitis characteristics are summarized in Table 1. Serial AS-OCT imaging and the change in CT, IT and IW with treatment are illustrated in Figures 1 and 2.

CT in the infiltrated area was largest on presentation (day 0) with a mean [SD] value of 905 [206] μm. On days 3, 7, and 14 mean [SD] CT had decreased significantly to 753 [161] μm

TABLE 1. Characteristics of Patients and Bacterial Keratitis

Patients, *n*	26
Sex, *n*	
Male	13
Female	13
Age, y	
Mean	48.4
Range	21–80
Eyes, *n*	26
Laterality	
Left	8
Right	18
Risk factor	
Contact lens wear	20
Recurrent corneal erosion syndrome	2
Lagophthalmos	2
Atopy with dry eye	1
Corneal surgery—limbal relaxing incisions	1
Presentation OCT measurements	
Corneal thickness	
Mean, μm	905
Range, μm	540–1490
Infiltrate thickness	
Mean, μm	388
Range, μm	130–840
Microbiology, *n*	
Pseudomonas aeruginosa	9
Diphtheroids	1
Coagulase-negative staphylococcus	1
Staphylococcus aureus	1
Negative	8
Not performed	6

OCT, optical coherence tomography.

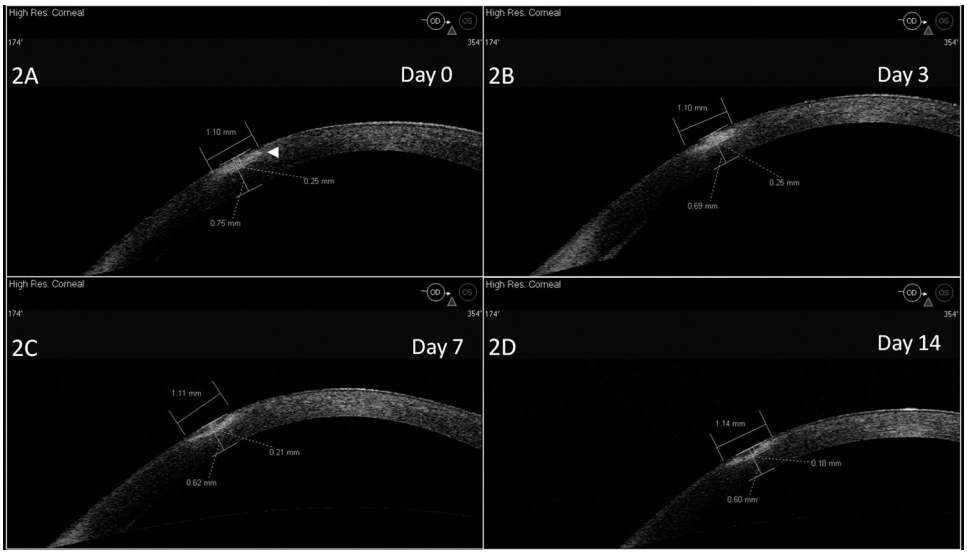

FIGURE 2. Culture-negative keratitis imaged serially with AS-OCT showing the change in corneal parameters in a contact lens wearer with presumed bacterial keratitis. Serial high-resolution AS-OCT scans have been carried out through the center of the corneal infiltration at the 174° axis. (**A**) The stromal infiltration ◀ at presentation associated with posterior bowing of the endothelium due to infiltration and edema. Corneal thickness, infiltrate thickness and infiltrate width measure 750, 250, and 1100 μm respectively. (**B**), (**C**), and (**D**) illustrate the reduction of corneal and infiltrate thickness on days 3, 7, and 14 of treatment. Infiltrate width does not decrease.

($P < 0.001$), 678 [178] μm ($P < 0.001$) and 584 [146] μm ($P < 0.001$), respectively. CT measurements were possible in all 26 (100%) eyes. The temporal change of CT is illustrated in Figure 3.

IT was also largest on presentation with a mean [SD] value of 388 [184] μm. On days 3, 7, and 14, mean [SD] IT had decreased to 320 [163] μm ($P = 0.001$), 296 [135] μm ($P = 0.004$), and 207 [87] μm ($P < 0.001$), respectively. IT measurements could be carried out in 21 (80.8%) of cases. The temporal change of IT is illustrated in Figure 4.

Mean [SD] IW on presentation was 1530 [1048] μm. IW did not change significantly during treatment with mean [SD] values of 1498 [855] μm ($P = 0.591$), 1547 [956] μm ($P = 0.878$), and 1680 [1014] μm ($P = 0.308$), on days 3, 7, and 14 respectively. IW measurements were possible in 13 (50%) eyes (Fig. 5).

CT decreased most rapidly in the early phase of treatment, with a mean [SD] daily rate of 4.49 [3.78]%. The mean [SD] daily rate of CT reduction in the middle phase was 2.69 [3.62]% (4.49 vs. 2.69, $P = 0.21$) and decreased in the late phase to 1.33 [2.29]% per day (4.49 vs. 1.33, $P = 0.006$).

IT also decreased most rapidly in the early phase of treatment, with a mean [SD] daily rate of 5.41 [5.13]%. In the middle phase, the mean [SD] daily rate of IT reduction was slower at 1.19 [6.80]% (5.41 vs. 1.19, $P = 0.042$), but accelerated again in the late phase to 3.38 [3.08]% per day (5.41 vs. 3.38, $P = 0.70$).

The mean daily rates of CT and IT reduction were not significantly different in the early (4.49% vs. 5.41%, $P = 0.49$) and middle phases of treatment (2.69 vs. 1.19, $P = 0.40$). In the late phase, however, IT decreased at a significantly faster rate than CT (3.38% vs. 1.33%, $P = 0.003$; Table 2).

Pseudomonas aeruginosa was cultured in 9 cases. A subgroup analysis was therefore carried out to compare pseudomonas to non-pseudomonas keratitis. CT on presentation was larger in the pseudomonas group (mean CT [SD]: 1072 [196] vs. 807 [142] μm, $P = 0.001$), as was IT (mean IT [SD]: 501 [117] vs. 344 [189] μm, $P = 0.057$), and IW (mean IW [SD]: 2628 [1057] vs. 1035 [565] μm, $P = 0.003$). CT and IT differences between the two pathogen groups did not persist on days 3, 7, and 14. The IW difference persisted on day 3 (mean IW [SD]: 2460 [894] vs. 1138 [566] μm, $P = 0.009$), day 7 (mean IW [SD]: 2660 [927] vs. 1052 [391] μm, $P = 0.001$), and day 14 (mean IW [SD]: 2655 [873] vs. 1167 [649] μm, $P = 0.005$) of treatment.

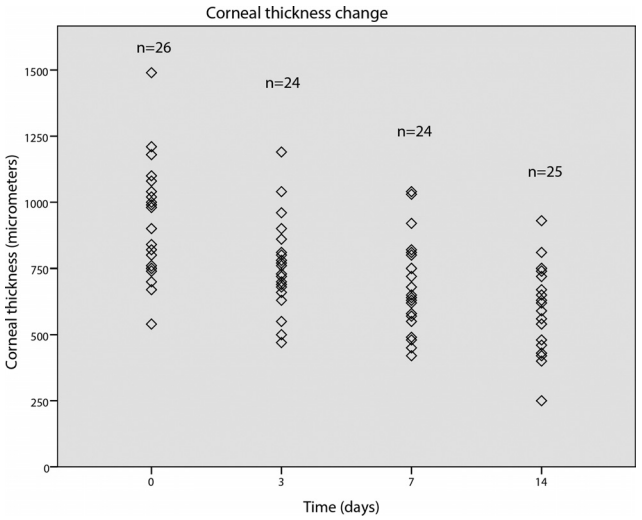

FIGURE 3. Corneal thickness reduction during treatment of bacterial keratitis.

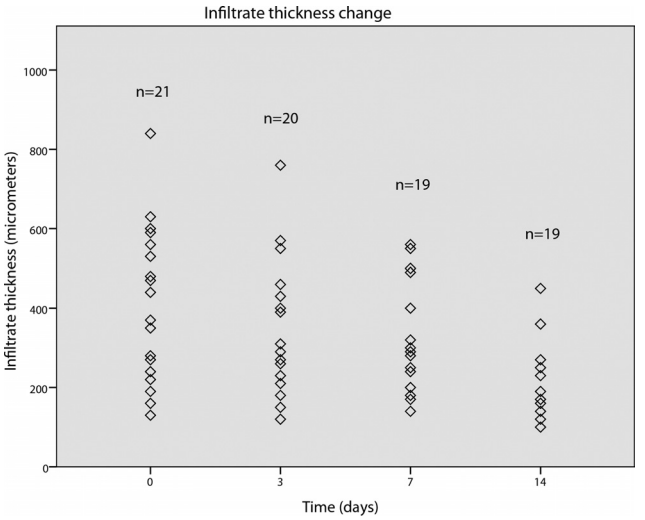

FIGURE 4. Infiltrate thickness reduction during treatment of bacterial keratitis.

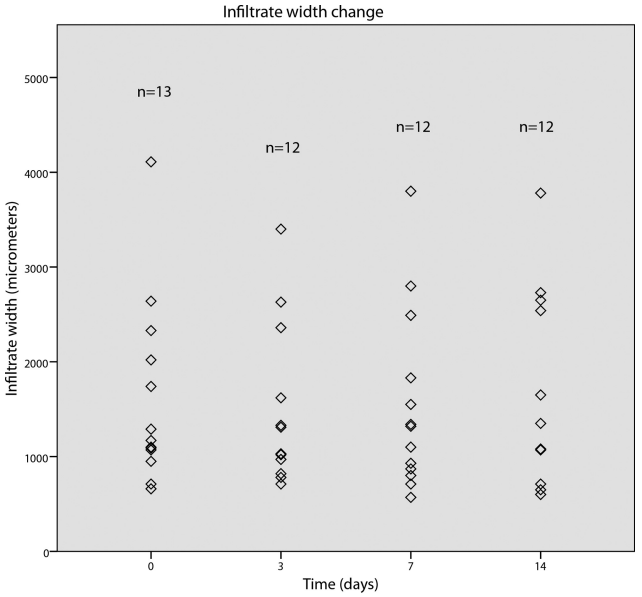

FIGURE 5. Infiltrate width in resolving bacterial keratitis.

The rate of decrease of CT was statistically larger in the pseudomonas group during the early treatment phase (mean [SD] daily rate: 7.58 [4.57] vs. 3.22 [2.61]%, $P = 0.007$), but of borderline statistical difference in the middle (mean [SD] daily rate: 4.48 [3.66] vs. 1.74 [3.32]%, $P = 0.09$) and late (mean [SD] daily rate: 2.18 [1.61] vs. 0.78 [2.54] %, $P = 0.16$) phases. The rate of decrease of IT was not statistically different in the two groups.

DISCUSSION

In this quantification study of human bacterial keratitis both CT and IT decreased significantly within three days of starting treatment, providing objective evidence that successful treatment results in an early reduction in corneal edema and inflammation. In contrast, IW did not change. This study has also shown that AS-OCT can be applied to the in vivo quantification of the course of an inflammatory condition, providing serial measurements of CT, IT, and IW in bacterial keratitis.

In addition to this early phase reduction in IT and CT, resolving bacterial keratitis showed a late phase reduction in IT. During the early and middle phases of treatment, IT and CT decreased at similar rates, indicating that a similar process may be taking place in the infiltrated and deeper non-infiltrated cornea. Reduction in corneal edema is the most likely explanation for these early changes. In the late phase, however, the reduction of CT decelerated significantly compared to the early phase and IT decreased at a significantly faster rate than CT. This suggests a different biological process to the early phase. We hypothesize that resolution of inflammatory cells and other components of the stromal infiltrate takes place in this late phase.

Pseudomonas keratitis had a larger corneal inflammatory response than non-pseudomonas keratitis. All parameters, CT, IT, and IW, were larger in corneal infection due to pseudomonas. In addition, early CT reduction was more rapid in this group. Histopathology studies have shown that inflammation in bacterial keratitis is characterized by stromal infiltration and corneal edema.[6,9,10] Experimental animal models of pseudomonas keratitis have shown intense PMN infiltration and edema on light microscopy.[10,16] The in vivo quantitative and morphologic information provided by AS-OCT is consistent with these microscopy examinations. AS-OCT technology, however, does not have the capability to distinguish inflammatory infiltration from scarring, as both appear hyper-reflective on AS-OCT.

In animal studies of keratitis, in vivo assessment of the infection severity and course is carried out with slit-lamp grading. Ocular parameters, such as conjunctival injection, chemosis, corneal infiltration, corneal edema, and iritis are quantified on a grading scale of 0 to 4 to produce a total severity score.[6] Enucleation of the eye and microscopy examination are often required to objectively assess the extent and depth of corneal infection.[9,10] The development of a portable AS-OCT device may overcome these limitations and provide objective in vivo quantification information without the requirement for enucleation.

We found that IW, as defined in this study, did not decrease with resolution of the infection. This may be explained by the proximity of the superficial margins of the infiltrated area to the immune laden tear film and limbus. In the early phase of treatment, any reduction in edema is offset by a waning immune response that is more likely to infiltrate the superficial margins rather than the compact deeper stroma. In the later phases, as the inflammatory components are gradually replaced with collagen and scar tissue, the measured width of the infiltrate would not change, as co-existing scar tissue and inflammatory infiltration both appear hyper-reflective on AS-OCT.

We could measure CT in all cases and IT in 80.8% of cases. However, IW could only be measured in 50% of cases, as the transverse margins of the infiltrate could not be identified in the remaining cases. This is most likely due to two factors. First, infiltrations have soft, ill-defined transverse margins from ongoing inflammatory activity, and second, the AS-OCT transverse imaging resolution (60 μm) is lower than the axial resolution (18 μm).[17]

This study has also demonstrated the application of AS-OCT to monitoring bacterial keratitis in clinical practice. Reiterative scans and measurements of CT, IT, and IW can be used for objective and quantitative assessment of disease severity and treatment response. Although slit-lamp biomicroscopy is the gold standard in clinical practice, evaluation of corneal inflammation is subjective and assessment of the treatment response is difficult in the early stages. AS-OCT showed a mean IT reduction of 68 μm in the early phase, a change that cannot be

TABLE 2. Rate of Change of Corneal Thickness and Infiltrate Thickness in Resolving Bacterial Keratits

Rate of Change (% per day)	Days 0–3	Days 3–7	Days 7–14
Mean daily CT decrease [SD]	4.49 [3.78]	2.69 [3.62]	1.33 [2.29]
Mean daily IT decrease [SD]	5.41 [5.13]	1.19 [6.80]	3.38 [3.08]
*P**	0.49	0.40	0.003

CT, corneal thickness; IT, infiltrate thickness.
* *P*: 2-tailed *t*-test.

detected or measured with slit-lamp biomicroscopy. The use of long wavelength light (1310 nm) in AS-OCT achieved good penetration through structures that highly scatter light, such as corneal infiltration and opacification, by non-contact examination.[12,18,19] Alternative imaging modalities, such as ultrasound biomicroscopy and Scheimpflug imaging (Pentacam), have a limited role in corneal inflammation.

In summary, AS-OCT can directly visualize, measure and monitor in vivo parameters of corneal inflammation in bacterial keratitis. Resolving corneal infection is characterized by an early reduction in corneal edema, followed by a later reduction in infiltration. Serial standardized AS-OCT scanning can be used in clinical practice to quantify and objectively assess bacterial keratitis.

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