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Assessment of Confocal Microscopy for the Diagnosis of Polymerase Chain Reaction–Positive *Acanthamoeba* Keratitis

A Case-Control Study

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Purpose: To determine in vivo confocal microscopy diagnostic criteria to diagnose *Acanthamoeba* keratitis (AK) using polymerase chain reaction (PCR) as the reference diagnostic technique.

Design: Retrospective case-control study. Data were recorded prospectively and analyzed retrospectively.

Participants: Fifty patients with PCR-positive AK (study group) and 50 patients with bacterial, fungal, viral, or immune keratitis featuring negative *Acanthamoeba* PCR results (control group).

Methods: In vivo confocal microscopy performed at the acute stage of keratitis.

Main Outcome Measures: Presence of in vivo confocal microscopy images suggestive of AK. Multivariate logistic regression was used to determine the relationship between types of images and presence of PCR-positive AK.

Results: The following 4 types of images were associated significantly with PCR-positive AK ($P < 0.05$): bright spots (round or ovoid hyperreflective objects with no double wall; diameter, <30 μm); target images (hyperreflective objects with hyporeflective halo; diameter, <30 μm); clusters of hyperreflective objects (diameter, <30 μm); and trophozoite-like objects (diameter, >30 μm). Specificity of both target and trophozoite images was 100%. This figure was 98.2% for clusters and 48.2% for bright spots. If the diagnosis of AK was made on presence of target images, clusters or trophozoite images (at least 1 of the 3 features), the positive predictive value of confocal microscopy was 87.5% and the negative predictive value was 58.5%.

Conclusions: *Acanthamoeba* keratitis is a serious vision-threatening disease. In vivo confocal microscopy can help in this challenging diagnosis, especially when PCR is delayed, shows negative results, or is not available. Target images and trophozoite-like images are pathognomonic of AK. Clusters of hyperreflective objects are highly specific of AK. However, the overall sensitivity of in vivo confocal microscopy features of AK is low. In addition to the clinical features, microbiological tests (direct examination and cultures of corneal scrapings), and PCR, in vivo confocal microscopy allows for more rapid diagnosis and treatment initiation, potentially leading to an improved outcome. *Ophthalmology 2017;■:1–8 © 2017 by the American Academy of Ophthalmology*

Acanthamoeba keratitis (AK) is a potentially sight-threatening corneal infection caused by a pathogenic amoeba.[1] Worldwide, *Acanthamoeba* is a free-living, opportunistic protozoan that is found in water and soil and that has a 2-stage life cycle: the active trophozoite and the quiescent cyst. The trophozoite is the dividing form and is thought to be the infective form. The cysts are dormant forms of the organism and protect the amoeba from harmful environments.[2,3]

First described in 1974 as an ocular pathogen, *Acanthamoeba* causes a chronic keratitis that is refractory to traditional antibiotic therapy. There is often a poor prognosis because of a significant delay in diagnosis and frequently a lack of effective medical treatment.[4] The initial presentation of AK is often nonspecific, leading to misdiagnosis and delay of appropriate treatment. Because early treatment guarantees a better prognosis, a rapid and accurate diagnostic method is crucial.[5,6]

Current methods of identifying the organism include corneal scraping for histopathologic analysis, tissue culture, and more recently, PCR. Cultures can take several days to weeks to reveal positive results, with positive rates ranging from 0% to 68%.[1–7] Although not available in all laboratories, our current reference technique of diagnosis is real-time PCR.[7] When the results are positive, the diagnosis is confirmed, but PCR can reveal false-negative results in cases of corneal scrapings that are too superficial or the presence of inhibitors of PCR in corneal scraping.

In vivo confocal microscopy (IVCM) is a high-resolution imaging technique that has been used in the last 2 decades as a potential diagnostic tool. Confocal microscopic features of AK such as bright spots, double-walled cyst, or signet sign have been described in a series of studies.[7–13] Its noninvasive nature makes it an important modality in early diagnosis of AK. The present study was performed to determine the relative diagnostic value

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of in vivo confocal microscopy in the diagnosis of AK in patients with keratitis clinically suggestive of AK, compared with PCR as the reference diagnostic method.

Methods

Study Design

This study was a retrospective, case-control study and was designed from a consecutive series of patients hospitalized for severe keratitis assessed with in vivo confocal microscopy and microbiologic tests including *Acanthamoeba* PCR. Inclusion criteria were the following: severe keratitis requiring hospitalization for treatment in a tertiary center (French National Eye Hospital, Paris, France) between January 2005 and December 2015, clinical presentation compatible with AK, in vivo confocal microscopy performed at the acute stage of keratitis, *Acanthamoeba* PCR performed on corneal scrapings (not in contact lens boxes) on admission, and routine microbiological workup performed on admission. Patients with AK presumed or proven by presence of trophozoites or cysts by direct examination of corneal scrapings after May-Grünwald Giemsa staining featuring negative *Acanthamoeba* PCR results were not included in the study. For patients with bilateral keratitis, only the most severely affected eye was included. Fifty eyes of 50 patients with PCR-positive AK met the inclusion criteria (study group). The control group was selected from patients with bacterial, fungal, viral, or immune keratitis; negative *Acanthamoeba* PCR results; and absence of trophozoites or cysts by direct examination of corneal scrapings after Giemsa staining. Data were recorded prospectively and then analyzed retrospectively. The study was approved by the Ethics Committee of the French Society of Ophthalmology (identifier, 00008855, Société Française d'Ophtalmologie IRB#1). Described research adhered to the tenets of the Declaration of Helsinki, and informed consent was obtained.

Clinical Assessment

The following features were recorded: age, gender, ophthalmologic history, date of initial diagnosis, time elapsed between symptom onset and diagnosis, clinical presentation, laboratory results, season at the time of diagnosis, medical and surgical treatments, hospitalization, and initial and final best-corrected visual acuity. *Acanthamoeba* keratitis was classified as follows: stage 1, epitheliitis; stage 2, epitheliitis with radial neuritis; stage 3, anterior stromal disease; stage 4, deep stromal keratitis; and stage 5, ring infiltrate or scleritis.

Microbiological Investigations

Routine microbiological workup was performed as previously described by Bouheraoua et al.[14] Briefly, corneal ulcers were scraped and 2 slides were prepared for direct microscopic examination. May-Grünwald Giemsa staining was carried out systematically for the first slide and, according to images, Gram, periodic acid−Schiff, or acridine orange staining was used for the second slide.

Supplementary samplings with cotton swabs were carried out for bacterial and fungal infections. Chocolate Polyvitex agar, Schaedler broth with globular extract, Portagerm-Amies agar swabs (Biomérieux, Craponne, France), and Sabouraud-chloramphenicol-gentamicin medium were inoculated. The chocolate Polyvitex agar was incubated at 99°F (37.2°C) in an atmosphere containing 3% carbon dioxide and Sabouraud was incubated the first day at 99°F (37.2°C) and then at 86°F (30°C). Real-time PCR analysis was used for viral diagnosis. Polymerase chain reaction analysis for *Acanthamoeba* was carried out systematically as previously reported by Goldschmidt et al.[15,16]

In Vivo Confocal Microscopy

The Heidelberg Retina Tomograph III in vivo confocal microscope (Heidelberg Engineering, Dossenheim, Germany) was used to assess keratitis during the first days of hospitalization. After topical anesthesia and instillation of eye high-viscosity gel, patients were asked to fixate using an external fixation target. The instrument objective then was brought into optical contact with the corneal tissue by a disposable sterile polymethyl methacrylate cup and a high-viscosity gel. Images of all corneal layers were obtained from the superficial epithelial layer down to the corneal endothelium in the diseased corneal zone by trained orthoptists (C.G.). The acquired images consisted of 384×384 pixels over a 400×400-μm field of view with a transversal resolution of approximately 1 μm.

At least 800 images were obtained for each patient. They were assessed by 2 trained physicians (S.D.C., J.K.) in a masked fashion. For each image, the following data were recorded: layer (epithelium, anterior stroma, mid stroma, posterior stroma, or endothelium), presence of images suggestive of AK (round or ovoid hyperreflective objects either with or without double wall, round or ovoid hyperreflective objects with a hyporeflective halo [target sign], signet sign images, polygonal or stellate hyperreflective objects, coffee bean hyperreflective objects, rod hyperreflective objects with a hyperreflective halo, single file of hyperreflective objects, cluster of hyperreflective objects, trophozoite-like hyperreflective objects, keratoneuritis images, and spindle-shaped hyperreflective objects), and size of hyperreflective objects (larger or smaller than 30 μm).[17] We considered hyperreflective objects to be trophozoite-like objects when they had spiny surface structures suggestive of acanthopodia. Figure 1 shows the classification of the various images considered to be compatible with AK diagnosis.

Statistical Analysis

Statistical analysis was performed with the software program Statistica version 6.1 (StatSoft France, Maisons-Alfort, France). The chi-square test, *t* test, and logistic regression were used to compare the study group with the control group. Variables were selected first in univariate analysis and then were analyzed in multivariate logistic regression.

Results

Table 1 shows patient characteristics. Compared with the control group, the study group featured younger age of patients, higher prevalence of contact lens wear, higher frequency of topical steroid treatment before the onset of keratitis, longer first symptom-to-diagnosis time, higher frequency of radial neuritis, absence of hypopyon, and longer duration of medical treatment. Conversely, there were no significant differences between both groups for gender ratio, initial and final visual acuity, keratitis presentation (other than radial neuritis and hypopyon), and percentage of cases requiring surgical intervention.

Diameter of round or ovoid hyperreflective objects either with double wall, round or ovoid hyperreflective objects with hyporeflective halo (target sign), signet sign images, polygonal or stellate hyperreflective objects, coffee been hyperreflective objects, and rod hyperreflective objects was always less than 30 μm. Conversely, diameter of trophozoite-like objects was always more than 30 μm. Round or ovoid hyperreflective objects with no double wall were observed in the epithelium and in the anterior, mid, and posterior stroma, but not in the endothelium. Round or ovoid hyperreflective objects with double wall, signet sign images, polygonal or stellate hyperreflective objects, and coffee bean hyperreflective objects