PERSPECTIVE

Acanthamoeba Keratitis: Diagnosis and Treatment Update 2009

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• PURPOSE: **To describe the current management of Acanthamoeba keratitis (AK).**

• DESIGN: **A perspective based on the literature and author experience.**

• RESULTS: **Early diagnosis and appropriate therapy are key to a good prognosis. A provisional diagnosis of AK can be made using the clinical features and confocal microscopy, although a definitive diagnosis requires culture, histology, or identification of *Acanthamoeba* deoxyribonucleic acid by polymerase chain reaction. Routine use of tissue diagnosis is recommended, particularly for patients unresponsive to treatment for AK. Topical biguanides are the only effective therapy for the resistant encysted form of the organism in vitro, if not always in vivo. None of the other drugs that have been used meet the requirements of consistent cysticidal activity and may have no therapeutic role. The use of topical steroids is controversial, but probably beneficial, for the management of severe corneal inflammatory complications that have not responded to topical biguanides alone. The scleritis associated with AK is rarely associated with extracorneal invasion and usually responds to systemic anti-inflammatory treatment combined with topical biguanides. Therapeutic keratoplasty retains a role for therapy of some severe complications of AK but not for initial treatment. With modern management, 90% of patients can expect to retain visual acuity of 6/12 or better and fewer than 2% become blind, although treatment may take 6 months or more.**

• CONCLUSIONS: **Better understanding of the pathogenesis of the extracorneal complications, the availability of polymerase chain reaction for tissue diagnosis, and effective licensed topical anti-amoebics would substantially benefit patients with AK. (Am J Ophthalmol 2009;148: 487–499. © 2009 by Elsevier Inc. All rights reserved.)**

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MOST OPHTHALMOLOGISTS WILL KNOW THAT Acanthamoeba keratitis (AK) is a recently recognized infectious disease entity that is difficult to treat. There are good recent reviews of this subject.[1,2] This perspective focuses on the diagnosis and treatment of this complex corneal infectious disease. In particular, we consider the management of diagnostic dilemmas, evidence for choice of initial therapy, and treatment of the challenging clinical problems of persistent ulceration, severe inflammation, and persistent infection, and provide guidelines for surgery.

A knowledge of some aspects of the biology of *Acanthamoebae* and of the epidemiology and pathogenesis of AK is required to understand diagnostic and treatment strategies.

BIOLOGY

ACANTHAMOEBA SPP. ARE A FAMILY OF FREE-LIVING CYST-forming protozoans that are ubiquitous in air, soil, dust, and water and can be isolated from the upper respiratory tracts of humans, 50% to 100% of whom have antibodies to Acanthamoeba.[3,4] *Acanthamoeba* spp. have been classified by their cyst morphology and by using isoenzyme or mitochondrial deoxyribonucleic acid (DNA) analysis. *Acanthamoeba castellanii and A. polyphaga* are the most common of the 8 species reported to cause keratitis. Other genera of amoebae, *Hartmannella, Naegleria*, and *Vahlkampfia*, are reportedly isolated from keratitis, although the exact role of these organisms in AK remains uncertain. Their life cycle consists of trophozoite and cyst stages (Figure, Top left and right). The trophozoite has an amoeboid shape with pseudopodia and feeds on small algae, bacteria, and other protozoans. In the cornea, they are thought to feed on keratocytes. Reproduction is asexual by binary fission. The cyst is the dormant form of the organism. Trophozoites and cysts vary in size from 25 to 50 μm and 15 to 30 μm respectively, which is well within the 2-μm resolution of modern in vivo confocal microscopes. Trophozoite encystment allows the organism to survive an adverse environment, including the nutrient deficiency and noxious chemicals that it is exposed to in keratitis, and is the form of the organism responsible for persistent

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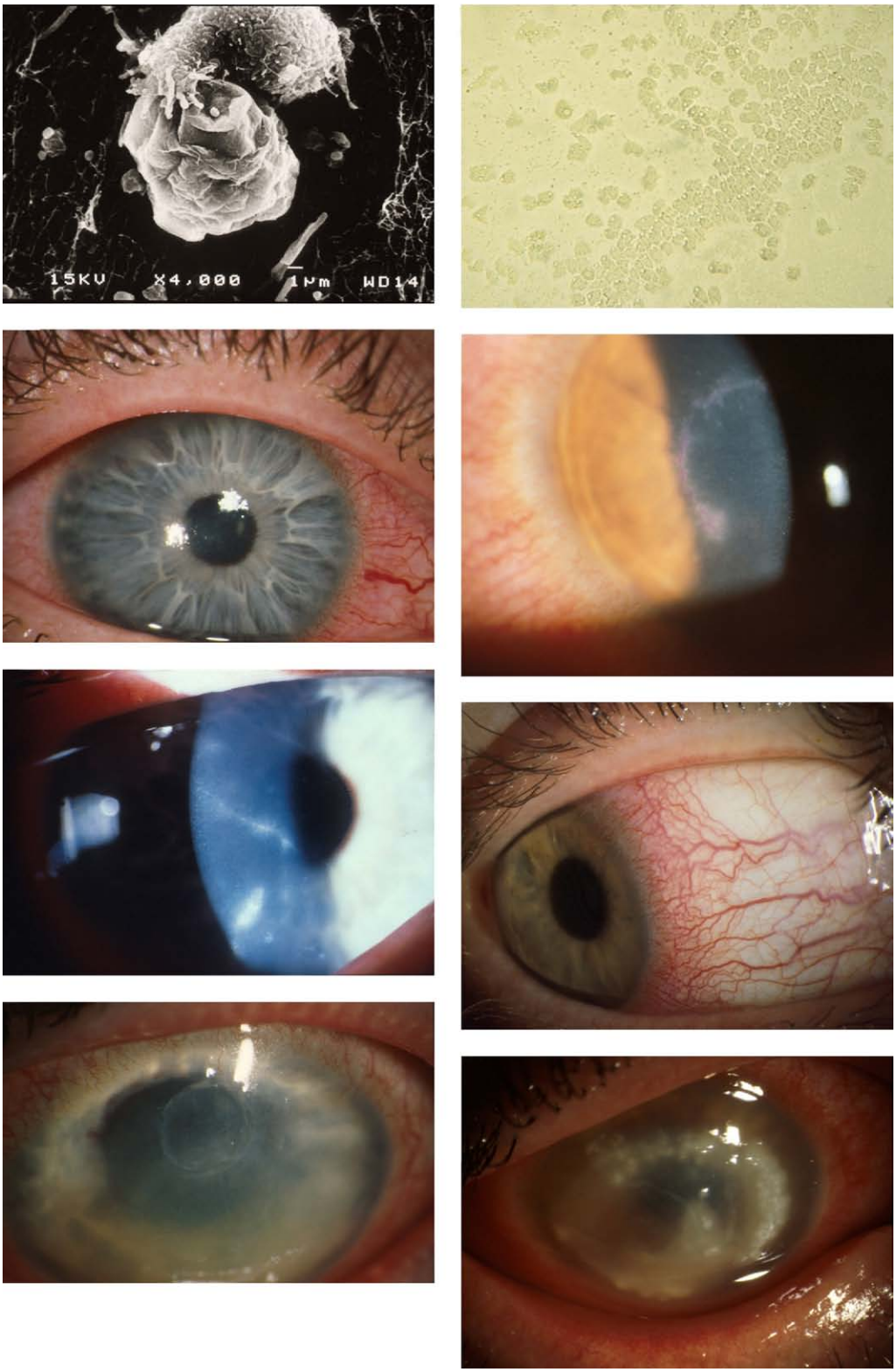

FIGURE. Acanthamoeba keratitis (AK). (Top row, left) Electron micrograph of an *Acanthamoeba* trophozoite excysting. The cyst wall is below the trophozoite which is extending its acanthapodia. (Top row, right) *Acanthamoeba* trophozoites migrating on non-nutrient agar overlain with *E. coli*. The pale areas in some trophozoites are contractile vacuoles. (Second row, left) Early AK showing an epitheliopathy and epithelial opacities. (Second row, right) Pseudodendritic epitheliopathy in early AK. (Third row, left) Perineural infiltrates in the stroma in early AK. (Third row, right) Limbitis in early AK. (Fourth row, left) Three-mm corneal biopsy site in AK, also showing a ring infiltrate. (Third row, right) Ring infiltrate and severe scleritis in advanced AK.

disease. It is widely accepted that because of this behavior, effective treatment of AK demands cysticidal drugs.[1–3]

EPIDEMIOLOGY

CLINICIAN AWARENESS OF AK IS AN IMPORTANT STEP IN establishing or excluding the diagnosis. There are ten-fold variations in the reported incidence of the disease in the national populations, from as few as 0.15 per million in the United States to as high as 1.4 per million in the United Kingdom, with other countries for which figures are available, such as Sweden and New Zealand, having intermediate incidences. These differences have been shown, at least in large part, to relate to the prevalence of contact lens (CL) use, the contamination of domestic water and swimming pools by *Acanthamoeba*, the amoebicidal efficacy of CL care systems, regional variations in the availability of different CL care systems, the use of reusable soft CLs, and the prevalence of the use of diagnostic techniques for AK.[5,6] Although still an uncommon cause of keratitis in India, where CL use is much less widespread, AK has been reported in 1% of culture-positive cases of microbial keratitis[7,8] as opposed to 4% to 8% for countries where CL use is common.[9,10]

• **ACANTHAMOEBA KERATITIS IN NON–LENS USERS:** Acanthamoeba keratitis in non–CLs wearers is often overlooked as a cause of keratitis and diagnosed late despite comprising 3% to 15% of AK cases in the United Kingdom and United States.[11–13] In non–lens users AK is usually associated with trauma and exposure to contaminated water or soil, often in agricultural workers.[7,12,14] Additional potential risk factors are the use of contaminated tank-fed water in the home,[9,15] warmer weather,[16,17] and poor socioeconomic conditions.[14] AK has also been reported after surgical trauma including penetrating keratoplasty (PK) and radial keratotomy.[3]

• **ACANTHAMOEBA KERATITIS IN CONTACT LENS USERS:** In countries with a high prevalence of CL wear, 85% to 88% of AK cases occur in CL users. National incidence estimates for CL users vary widely, from 1.65 to 2.01 per million in the United States to 17.53 to 19.50 per million in the United Kingdom.[13] In most series, AK comprises less than 5% of CL related microbial keratitis cases[9–11] and is usually unilateral, although bilateral disease occurred in 8 of 106 cases (7.5%) in a United Kingdom national survey.[13] There is an increased incidence of AK during the summer/early autumn that is probably related to the increased presence of *Acanthamoeba* in the environment during warmer weather[5,9,17] or increased recreational activity in environmental waters.

Contact Lens Types. None of the epidemiologic studies have been either designed or powered to identify differences in the risks for AK associated with different lens types. However, soft lenses probably carry a higher risk for AK than daily-wear rigid lenses,[17] and planned-replacement soft CLs, which require daily cleaning and overnight storage, probably carry a higher risk than daily disposable lenses, possibly for hygiene-related reasons.[13] More recently orthokeratology lenses, which are rigid and worn overnight, have been associated with AK.[18]

The development of AK in CL users is much more strongly related to poor lens hygiene and contaminated water than is bacterial keratitis,[13,15,19–21] for which the overnight wear of lenses remains the dominant risk factor.[22,23]

PATHOGENESIS

• **CORNEAL INVASION:** The mechanisms of corneal invasion by Acanthamoeba and the immune response to it have been established for animal models and have been recently reviewed.[24,25] Findings that may be relevant to the management of human infection are that corneal stromal degradation results from the release of multiple organisms and host-derived proteases. However, despite the ability of *Acanthamoeba* trophozoites to penetrate the Descemet membrane and the corneal endothelium, intraocular infection does not occur in animal experiments because of the elimination of trophozoites by the intense neutrophil response in the anterior chamber.[26] This mirrors the findings in human infection, although in humans the situation is more complex and extracorneal infection with *Acanthamoeba* has been reported occasionally (see the section on "The Extracorneal Complications of Acanthamoeba Keratitis").

• **THE IMMUNE RESPONSE:** In animals the innate immune response mediated by macrophages and neutrophils is pivotal to the resolution of experimental AK.[27,28] Understanding this cellular response is important to our management of the disease, which involves control of inflammation without exacerbating infection. The use of topical dexamethasone (Decadron; Merck & Co, Whitehouse Station, New Jersy, USA), in the hamster model, accelerates both trophozoite excystment and proliferation. In addition, dexamethasone-treated trophozoites or cysts induced a significant cytopathic effect on corneal epithelial cells compared with untreated organisms. Topical steroids suppress the activity of neutrophils and macrophages and intramuscular injection of dexamethasone had a profound effect on the incidence, severity, and chronicity of keratitis in the Chinese hamster model of AK. The use of steroids in the management of human disease must be considered in the light of these findings and it is probably important that effective anti-amoebics be used when steroids are used in the management of AK.[29] The promotion of excystment using steroids, if this happens in humans, could

TABLE 1. Commoner Causes of Persistent Microbial Keratitis

Causative Organisms	Most Common Isolates
Aerobic bacteria	Most commonly nontuberculous *mycobacteria* and *Nocardia* spp.
Anaerobic bacteria	Most commonly *Capnocytophaga* spp., *Propionibacterium acnes*, and *Clostridium* spp.
Fungi	Yeasts and filamentous fungi
Herpes simplex virus	
Protozoa	Most commonly *Acanthamoeba* spp. and *Microsporidia* spp.

be potentially beneficial by increasing susceptiblity to anti-amoebic therapy.

The role of the cell-mediated immune response has been less studied in the animal models of disease. Cell-mediated immunity is of relatively little importance in the response to infection in the Chinese hamster model and does not develop[30] unless a population of resident antigen-presenting cells is induced, although, when these are present, AK is prevented.[31] Chronic or recurrent corneal and scleral inflammation in affected humans is common.[32] Necrotic organisms and amoebic cyst walls have been shown to elicit an adaptive immune response in the animal model of keratitis[33] and, in humans, can remain for years in corneal tissue, where they may cause persistent corneal and scleral inflammation even when not apparently viable.[32] Two severe inflammatory complications of AK in human disease, scleritis[34] and severe ischemic posterior segment inflammation,[35] have not been described in animal models. The role of corneal antigen-presenting cells may be much more important in human than in animal disease; it has been proposed that corneal antigen-presenting cells in human AK may result in a T-cell response that results in an inflammatory response in vascularized ocular tissues or that AK might induce autoimmunity through molecular mimicry.[35] A better understanding of the pathogenesis of these conditions is needed.

DIAGNOSIS

EARLY DIAGNOSIS AND PROMPT DELIVERY OF APPROPRIATE medical therapy is essential to secure a good prognosis. If effective therapy is delayed for 3 weeks or more the prognosis deteriorates.[36–38] AK should be considered in any case of corneal trauma complicated by exposure to soil or contaminated water, and in all CLs wearers. This is particularly the case when the onset is slow, and the features are atypical for bacterial or fungal keratitis. In addition, the disease must be considered when there is a failure to respond to first-line therapy for bacterial or herpes simplex virus (HSV) keratitis, even when there has been a positive culture for another organism, because 10% to 23% of cases of AK may be polymicrobial[14,39,40] or co-infected with HSV.[41] In the United Kingdom, *Acanthamoeba* is more common than fungal keratitis and, in CLs wearers, is probably more common than HSV keratitis; failure to consider *Acanthamoeba* as a cause of progressive keratitis is negligent. Table 1 lists the commoner causes of progressive keratitis that will fail to respond to first-line therapy for bacterial keratitis. We take corneal cultures and biopsies for this panel of organisms when investigating such cases of progressive keratitis.

TABLE 2. Comparison of Clinical Features in Patients With Acanthamoeba Keratitis at Presentation With Early vs Late Diagnosis[39]

Clinical Feature	Early Disease[a] (Percentage)	Late Disease[b] (Percentage)
Punctate keratitis	46	21
Dendritiform ulcer	14	4
Epithelial loss	38	75
Perineural infiltrate	57	29
Limbitis	95	96
Ring infiltrate	19	83
Uveitis	5	79

[a]Less than 1 month of symptoms.
[b]Greater than 2 months of symptoms.

• CLINICAL FEATURES: Familiarity with the clinical features of AK is a first step in diagnosis, although this may be complicated by co-infection with bacteria or HSV. However, in uncomplicated AK there is a common pattern of progression from epithelial to stromal disease. Most patients complain of photophobia, pain, and tearing, usually in one eye, although disease may be bilateral in CLs users. The pain in early AK may be severe, and disproportionate to the clinical signs, although some patients are pain free. Absence of pain does not preclude the diagnosis.[25,40] Typically the clinical features of AK vary according to the duration of symptoms before presentation (Table 2).[1,39] Early disease, within the first month, is characterized by an epitheliopathy (Figure, Second row, left) including a punctate keratopathy, pseudodendrites (Figure, Second row, right), epithelial or subepithelial infiltrates, and perineural infiltrates (Figure, Third row, left), with ring infiltrates in less than 20% of patients. Anterior uveitis is uncommon at this stage. Perineural infiltrates are virtually pathognomonic for AK, being present in up to 63% of cases diagnosed within 6 weeks in some centers. They are believed to occur because trophozoites cluster around nerves. A careful slit-lamp examination may be necessary to identify them, as only 1 or 2 nerves may be affected. Limbitis (Figure, Third row, right) is a common finding in both early and late disease. Later disease, after 1 month, is characterized by ring infiltrates (Figure, Fourth row), frank

ulceration, and a secondary sterile anterior uveitis, sometimes with hypopyon. In some patients endothelial plaques or a disciform reaction may cause corneal edema. Perineural infiltrates are less common in late disease. Corneal sensation is usually reduced. Further evolution to severe forms of late disease, including abscess formation, hypopyon, scleritis (Figure, Fourth row, right), glaucoma, cataract, corneal melt, corneal perforation, and posterior segment inflammation, is more common in late-presenting disease. The extracorneal manifestations of AK are discussed in the next section. Bacterial superinfection of amoebic ulcers, including infectious crystalline keratopathy, is not uncommon during prolonged treatment and should be suspected if the clinical picture worsens during the course of treatment.[3,5,5,39]

The Extracorneal Complications of Acanthamoeba Keratitis. Cataract, iris atrophy, glaucoma, and peripheral ulcerative keratitis have all been reported as complications of severe and prolonged AK[42,43] and have been attributed to toxicity from the use of topical biguanides and/or diamidines. Cataract may develop in about 20% of cases.[3,43] However, other potential causes of these complications include chronic inflammation, corticosteroid effects, and the effects of vascular thrombosis or possibly intraocular infection.[35,43] Vascular thrombosis was reported in 4 of 5 enucleated eyes with chronic chorioretinal inflammation, in the absence of any histopathologic evidence of dissemination of *Acanthamoeba* outside the cornea. Acanthamoeba scleritis (Figure, Fourth row, right) is believed to be unrelated to direct invasion of *Acanthamoeba* and attributable to an inflammatory response, of uncertain etiology, triggered by the corneal infection. The potential pathogenic mechanisms for these inflammatory responses have been discussed in the summary of the pathogenesis previously. Spread of *Acanthamoeba* outside the cornea has been reported in only 4 cases: 1 of keratitis, uveitis, and meningoencephalitis; another of endophthalmitis in a patient with acquired immunodeficiency syndrome; a further case of retinitis following 4 corneal transplants; and 1 case of scleral invasion. Histology on at least a further 11 eyes enucleated as a result of AK have shown no extracorneal spread.[35] However, accurate histologic diagnosis can often be difficult, and polymerase chain reaction (PCR) would aid definitive confirmation of the presence or absence of extracorneal spread.

Although a possibility, it is currently uncertain that the anterior segment ocular complications are drug induced. There are several studies reporting use of topical biguanides without any apparent ocular complications. It is more likely that they are immune phenomena unrelated to drug toxicity, particularly given the other observed immune phenomena of scleritis and posterior segment inflammatory complications. Further clarification of the cause of these complications is needed.

• **CONFOCAL MICROSCOPY:** Confocal microscopy is the preferred diagnostic technique in some centers,[44–46] with sensitivity and specificity exceeding 90% for individual unmasked observers. However, there are no well-designed masked studies published of its sensitivity and specificity compared with culture- or histology-positive cases. The diagnosis of AK by PCR has been shown to have a sensitivity of over 80% and specificity of 100% in one study,[47] whereas only 24 of 31 cases of AK diagnosed by confocal microscopy were confirmed by PCR in another study.[48] We are concerned that the high sensitivity and specificity values for confocal diagnosis of AK may be center related and not representative of what can be expected elsewhere, leading to either misdiagnosis as AK or a false sense of security when there are no features of AK on confocal microscopy. Although we use the technique in the initial evaluation of cases, we do not use it for making a definitive diagnosis if there has been a poor response to therapy in cases with a negative tissue diagnosis (culture, histology of biopsies/smears, or PCR).

• **TISSUE DIAGNOSIS:** A definitive diagnosis of AK can only be made on the basis of culture or histology, or by the identification of the presence of amoebic DNA with PCR. A positive culture is invaluable for organism identification, drug sensitivity testing, and typing for epidemiologic information. The protocols we use for specimen collection and handling, histopathology, and culture techniques are described in the Supplemental Text available at AJO.com because these techniques are often cited as being difficult and unproductive,[45] which is not the case in the United Kingdom where they are widely used, or in some centers in the United States.[46] A tissue diagnosis was obtained in 177 of 349 cases (50%) reported in two United Kingdom national surveys.[13,17] In our own center, we have reported positive cultures in up to 58 of 101 cases (57%) and histology in 17 of 55 (31%)[49] and 13 of 20 (65%) cases respectively.[39] Identification of *Acanthamoeba* by PCR, with two different pairs of primers, showed a sensitivity of 84% and a specificity of 100%; it was positive in 16 of 19 epithelial samples (84%) compared to 10 of 19 (53%) culture positive,[47] similar to results reported elsewhere.[46,48]

As PCR is not routinely available, our protocol for tissue diagnosis includes corneal cultures and a corneal epithelial and stromal biopsy (Figure, Fourth row, left) in some cases. All diagnoses of AK are regarded as provisional unless there is a positive tissue diagnosis, and repeated efforts are made to achieve this in cases that respond poorly to treatment.

TREATMENT

WE INSTITUTE TREATMENT FOR AK, AFTER TAKING TISSUE for diagnosis as described, on the basis of clinical signs consistent with the diagnosis, particularly when perineural

TABLE 3. Case Series Describing Medical Therapies for Acanthamoeba Keratitis

Author	Year	Numbers	Treatment	Outcome	PK	Relapsing Disease
Bacon[39]	1993	72 patients	25/72 (35%) PHMB 55/72 (76%) propamidine 22/72 (31%) other	≥6/9 in 58/73 (79%) ≤6/36 in 9/73 (12%)	23/72 (32%) Acute PK 21/72 (29%)	8/72 (11%)
Larkin[59]	1992	6 patients	PHMB 0.02%	5/6 resolved		
Ishibashi[75]	1990	3 patients	Miconazole 0.1% + oral itraconazole	≥6/12 in all 3 patients		
Duguid[49]	1997	105 patients	PHMB 0.02% + propamidine	≥6/12 in 88/105 (79%) ≤6/36 in 18/105 (16%) 2/105 (2%) loss of eye	10/105 (9%)	19/105 (17%)
Mathers[55]	2006	58 patients [letter to editor]	0.02% or 0.04% chlorhexidine alone 8 patients: 0.06% chlorhexidine + oral ketaconazole + topical steroids	Apparently all successes 1/8 failure		
Seal[76]	1996	12 patients	Chlorhexidine + propamidine	"all successfully treated"		
Azuara-Blanco[77]	1997	10 patients	PHMB 0.02% + propamidine	≥6/12 in 10/10 (100%)	2/10 (20%)	
Park[67]	1997	36 patients	19 patients topical steroid + anti-amoebics 17 patients anti-amoebics alone	14/19 (73%) steroid patients "cured" 13/17 (76.5%) nonsteroid patients "cured"	5/19 (26%) steroid patients 4/17 (23%) nonsteroid patients	
Kosrirukvongs[64]	1999	6 eyes	Chlorhexidine 0.006%	≥6/18 in 5/6 eyes (83%)		
Hargrave[78]	1999	60 eyes	Propamidine + neomycin-polymyxin-gramicidin	50/60 (83%) "success" 7/60 (12%) lost/enucleated	17/60 (28%)	
Perez-Santonja[61]	2003	—	PHMB or chlorhexidine ± hexamidine or brolene ± topical steroids			11/180 (6%)
Butler[10]	2005	20 patients	PHMB + propamidine ± other 17/20 (85%) "other," ie, chlorhexidine 0.02% or Neosporin	≥6/12 in 15/20 (75%) ≤6/36 in 1/20 (5%)	6/20 (30%) Acute PK 5/20 (25%)	5/20 (25%)
Sun[40]	2006	20 patients (all unilateral)	Chlorhexidine 0.02% + neomycin+ metronidazole 0.4% ± oral itraconazole	≥20/100 in 7/20 (35%) ≤count fingers in 10/20 (50%)		
Thebpatiphat[79]	2007	20 eyes	18/20 PHMB + propamidine + Neosporin 2/20 PHMB + Neosporin 11/20 oral itraconazole 11/20 topical steroids	≥20/30 in 10/20 (50%) ≤count fingers in 5/20 (25%)		
Lim[63]	2008	51 eyes	Randomized controlled trial 28 eyes chlorhexidine 0.02% 23 eyes PHMB 0.02%	24/28 (86%) "success" with chlorhexidine 18/23 (78%) "success" with PHMB	2/28 (7%) chlorhexidine patients 3/23 (13%) PHMB patients	

PHMB = polyhexamethylene biguanide; PK = penetrating keratoplasty.

infiltrates are present, supported by confocal microscopy findings when these are available. The diagnosis is regarded as provisional until we have a positive tissue diagnosis, but we do not continue to culture a patient, or take biopsies, if the response to treatment for AK is good.

• **AMOEBICIDAL AGENTS:** There are currently no drugs that are licensed for use in AK in any country, and information about safety and efficacy has to be derived from published case series. Acanthamoeba trophozoites are sensitive to most available chemotherapeutic agents (antibiotics, antiseptics, antifungals, antiprotozoals including metronidazole, antivirals, and antineoplastic agents). However, persistent infection is related to the presence of *Acanthamoeba* cysts, against which very few of these agents have any effect,[50,51] and only agents that are cysticidal in vitro against cysts can be expected to be effective as therapy. Although neomycin has been widely used, it is ineffective against cysts in vitro.[50] In addition, like all aminoglycosides it is toxic to the corneal epithelium and can often result in indolent corneal ulceration that may be incorrectly attributed to disease activity. We do not think that neomycin has any role in modern therapy. Cysts have also shown a high in vitro resistance against the imidazoles,[50] the newer antifungals, caspofungin[52] and voriconazole,[53] and the antineoplastic agent miltefosine,[53] even though these agents have been used successfully in cases of systemic *Acanthamoeba* infection (in which the role of the cyst may be less important). The diamidines and biguanides are currently the most effective cysticidal antiamoebics in vitro and their use is supported by substantial case series. Table 3 summarizes treatments delivered in case series of AK. Metronidazole (Flagyl; Pfizer Inc, New York, New York, USA) has been used for several cases in one series[40] but has no effect in vitro.[54] For these reasons topical therapy with biguanides with or without the addition of diamidines is currently the mainstay of treatment for AK.

Biguanides. The two biguanides that are in use are polyhexamethylene biguanide (PHMB) 0.02% to 0.06% (200 to 600 μg/ml) and chlorhexidine 0.02% to 0.2% (200 to 2000 μg/ml) (available from Moorfields Pharmaceuticals, London, United Kingdom and other manufacturing pharmacies elsewhere). Concentrations of the biguanides over 0.02% have been used by us and others for resistant cases;[55] the 0.2% preparation of chlorhexidine has been shown to be effective in a randomized clinical trial of fungal keratitis in which development of cataract or other toxicity was not identified as a clinical problem.[56] Other biguanides such as alexidine and the amidoamine myristamidopropyl dimethylamine have also shown to have good anti-acanthamoebal efficacy in vitro and merit further investigation, but they have not yet been used clinically.[57,58]

The biguanides interact with the cytoplasmic membrane, resulting in loss of cellular components and inhibition of respiratory enzymes.[59] In vitro studies have shown the cationic agents chlorhexidine and PHMB to have the best and most constant amoebicidal and cysticidal activity of drug classes studied to date.[50,60] Both drugs have been effective clinically as primary therapy, as well as in cases in which medical failures have occurred with other agents[50,59,60] (Table 3). PHMB and chlorhexidine each have minimal cysticidal concentrations (MCC) approximating 2 μg/ml, 100 times lower than the concentration of the topical solutions in widespread use, although MCC as high as 3 μg/ml for PHMB and 12.5 μg/ml for chlorhexidine have been reported for a series of clinically resistant cases.[58,61] Clinically, corneal epithelial toxicity has been minimal for both chlorhexidine 0.02% and PHMB 0.02%. However, chlorhexidine 0.05% has corneal toxicity in the rabbit model, whereas PHMB has been shown to be nontoxic to mammalian epithelia at a concentration of 20%.[62] There has been no evidence of chlorhexidine toxicity in several large case series using a range of chlorhexidine concentrations.[56,63,64] These findings support the use of biguanides as first-line treatment for AK either alone or in combination with diamidines, with which there may be a synergistic or additive effect.[60]

Diamidines. Available diamidines include propamidine isethionate 0.1% (1000 μg/ml) (Brolene; May and Baker, Dagenham, United Kingdom) and hexamidine 0.1% (1000 μg/ml) (Desomedine; Chauvin, Montpellier, France); these are licensed as antibacterials in some European countries.

The antimicrobial effects of the diamidines result from the cationic surface-active properties inducing structural membrane changes affecting cell permeability. When these molecules penetrate into the amoebic cytoplasm, denaturation of cytoplasmic proteins and enzymes occurs. Propamidine and hexamidine have been effective clinically against both the trophozoite and cyst forms of *Acanthamoeba*. The mean MCC reported for propamidine is 17 to 46 μg/ml, although clinical isolates vary widely with a range of up to 500 μg/ml.[50] The mean MCC for hexamidine has been reported as 41 μg/ml.[60] However, clinically resistant isolates have been reported with MCC of 125 to 500 μg/ml for both propamidine and hexamidine.[58,61] For these reasons, we do not believe diamidines should be used as monotherapy for AK. Clinically, the diamidines are well tolerated by ocular tissues, although prolonged treatment with propamidine may lead to toxic keratopathy.[39] Hexamidine has shown a faster amoebicidal effect than propamidine against both trophozoites and cysts in vitro.[65]

Toxicity of Biguanides and Diamidines. Cataract, iris atrophy, and peripheral ulcerative keratitis are all complications of AK that have been attributed to the use of topical biguanides and/or diamidines, but inflammatory complica-

tions of the disease are most likely to be involved (see section on "The Extracorneal Complications of Acanthamoeba Keratitis").

• **MEDICAL TREATMENT PROTOCOLS:** *Anti-amoebic Therapy*. The goals of medical therapy in AK include the eradication of viable cysts and trophozoites and rapid resolution of the associated inflammatory response. We use cysticidal drugs for treating AK because the encysted form is more resistant than the trophozoite to treatment, and failure to eradicate all viable cysts results in recurrence of the disease on reducing treatment intensity.

The treatment protocol used at Moorfields Eye Hospital, London, is empirical but based on both the clinical and laboratory data discussed previously and is outlined. Therapy is usually started with a biguanide (PHMB 0.02% or chlorhexidine 0.02%) and a diamidine (propamidine 0.1% or hexamidine 0.1%), although there is no clinical evidence to suggest that this is more effective than monotherapy with PHMB alone. PHMB 0.02% and hexamidine drops are administered every hour, day, and night, for 48 hours initially, followed by hourly drops by day only for a further 72 hours. Intensive early treatment is given because organisms may be more susceptible before cysts have fully matured. Epithelial toxicity is common if the dosage is maintained at this intensity. For this reason the frequency of therapy is reduced after 5 days to 2-hourly by day for 3 to 4 weeks, and then tailored to the individual case. These anti-amoebics are tapered with the goal of maintaining topical therapy 4 times daily for several weeks. In a United Kingdom multicenter study of 218 patients, the average duration of medical therapy was 6 months (range, 0.5 to 29 months).[17] If drug toxicity is suspected then the anti-amoebics can be stopped for 3 to 7 days to assess the response, and if toxicity is confirmed by an improvement in clinical signs and comfort, they may be discontinued for longer before switching to or restarting a low-toxicity agent, usually PHMB, without addition of the more clinically toxic diamidines.

Although the current anti-amoebics show low or very low minimal cysticidal concentrations, the clinicopathologic correlation between the in vitro sensitivities and the clinical outcome has been less than satisfactory,[50,61] resulting in treatment failure despite in vitro sensitivity to the anti-amoebic agent and the use of topical preparations at 66 times the minimal cysticidal concentration for PHMB and 16 times for chlorhexidine. Although the reasons for this are not well understood, it is possible that therapeutic intrastromal concentrations are not being achieved, that drugs might bind to tissue components or that they might be inactivated in vivo, or that organisms in vivo are intrinsically more resistant than in vitro. Strategies for managing this situation are outlined in the section on "Managing Persistently Culture-Positive Keratitis". The possibility of bacterial superinfection, and possibly concurrent herpes simplex infection, should also be considered in cases that do not respond to the anti-amoebic regimen outlined.[39]

Topical Corticosteroids. The role of steroids is controversial, but we believe that topical corticosteroids can have an important and beneficial role in the management of some cases of AK. Steroid treatment is unnecessary in most cases diagnosed early, which usually respond rapidly to anti-amoebic drugs. However, persisting inflammation (anterior scleritis, severe pain, indolent ulcers, corneal inflammation, and anterior chamber inflammation) may respond dramatically to the addition of even low-potency topical steroid therapy, eg, prednisolone 0.5%, 4 times daily. This is not commenced at our center until a minimum of 2 weeks of biguanide treatment has been completed, when most organisms should have been killed. Severe inflammation may require higher doses of steroids at any time during the course of the disease and dexamethasone 0.1% (or equivalent) at varying frequency may be needed to control corneal and extracorneal inflammation. Steroid therapy is compatible with a medical cure, providing anti-amoebic therapy is maintained throughout and continued after the steroids have been withdrawn.[3,66,67] This is necessary to control the recrudescence of active infection from viable cysts and we have seen recurrences in patients treated with steroids alone. We do not discontinue topical steroids until there is good control of inflammatory activity but we continue anti-amoebic therapy with a biguanide, 4 times daily for 4 weeks after steroids have been withdrawn. When the eye has been free of inflammation for 4 weeks, while using a biguanide alone, we regard the condition as cured and have successfully discontinued all therapy.

Treatment for Limbitis and Scleritis. The presence of limbitis is common in both early and late disease.[39] The presence and importance of this clinical sign is often overlooked and it may account for significant pain. In most cases, it can usually be successfully managed with oral nonsteroidal anti-inflammatory treatment, such as flurbiprofen 50 to 100 mg, 2 or 3 times a day. Scleritis is less common, occurring in approximately 10% of our cases,[34] but it can be severe and result in scleral necrosis and uncontrollable pain (see section on "Extracorneal Complication of Acanthamoeba Keratitis"). If it does not respond to flurbiprofen, then high-dose systemic steroid therapy (prednisolone 1 mg/kg/day), with systemic cyclosporine (3 to 7.5 mg/kg/day), can be used for successful control.[34] Systemic immunosuppression is used for an average of 7 months.[34] Systemic anti-amoebic therapy with oral itraconazole 100 mg daily[51] is probably a useful adjunct in these cases to prevent the potential spread of trophozoites into adjacent tissues, although this complication has not been documented. Without this anti-inflammatory treatment, these eyes may need retrobulbar alcohol or enucleation for symptomatic relief, and in our experience severe inflammatory complications are the most common reason for a poor outcome. This approach to

managing the inflammatory complications of AK might also be useful in the management of severe ischemic posterior segment inflammation[35] if its onset can be recognized early enough.

It is important to appreciate that persistent scleritis and limbitis may not be associated with the presence of viable *Acanthamoeba* in the cornea, and that clinical signs are unhelpful in making the distinction. Repetition of corneal cultures or biopsy may be helpful, but false negatives are common. In our experience, immunosuppression has had a beneficial effect on outcome even when viable organisms have been shown to be present in the cornea,[34] providing that topical biguanides have been used at the same time. No systemic dissemination of *Acanthamoeba* has been observed in any patient receiving systemic immunosuppression to date. The use of intensive topical anti-amoebic therapy, with or without topical steroids, may be inadequate to control scleritis. Until the introduction of oral immunosuppressive therapy, the pain of uncontrolled scleritis was a leading cause for enucleation in affected eyes at our center.[34]

Treatment for Persistent Epithelial Defect. This is a common problem in severe AK. Treatment involves excluding bacterial superinfection and persistent *Acanthamoeba* infection, as far as possible, with repeat cultures. Reducing the toxicity of treatment by discontinuing all topical therapy, except for prophylactic use of a low-frequency nonpreserved broad-spectrum antibiotic to prevent bacterial superinfection, for several days may result in a rapid improvement in clinical signs, after which anti-amoebic therapy can be reintroduced if necessary. In very inflamed eyes treatment of inflammation, as described previously, will often encourage epithelialization. If these measures have failed then a lamellar keratectomy of necrotic tissue, within the ulcer, is both therapeutic and diagnostic when the specimen is divided for culture and histology. An amniotic membrane may also, but not invariably, help re-epithelialization when combined with a keratectomy.[2]

• **SURGICAL MANAGEMENT:** *Epithelial Debridement.* Extensive debridement of the affected area of corneal epithelium may in itself be therapeutic if it is performed early when the disease is intraepithelial. This is usually done in our center as part of the initial investigation of the disease when the epithelium is removed for cultures and histology as well as to promote penetration of topical therapy. Repeated debridement is used in some centers to improve drug penetration.[40]

Cryotherapy. In vitro studies of AK have shown that cryotherapy kills trophozoites but not cysts, unless combined with medical treatment.[68,69] We have used cryotherapy rarely for persistently culture-positive AK unresponsive to the less destructive measures described in the upcoming section on this problem. It is critical that persistent infection be distinguished from persistent inflammation before using this treatment, because endothelial failure is a side effect. The technique uses a double freeze-thaw using a retinal cryoprobe, with maintenance of each freeze until an ice ball has formed in the stroma around the applicator. Numerous contiguous applications are needed to treat the whole cornea.

Corneal Graft Surgery. Therapeutic keratoplasty for management of the acute complications of AK has, with few exceptions in small numbers of patients,[3,70] generally been reported to have poor results.[71–73] Since the introduction of the biguanides as medical therapy, PK has not been recommended as a treatment for the elimination of organisms;[1–3] therapeutic keratoplasty was not required in a consecutive series of 111 patients in our center treated with PHMB and propamidine.[49] Therapeutic keratoplasty should therefore be reserved as a treatment for: 1) corneal perforation that does not respond to corneal gluing, 2) intumescent cataract, or 3) fulminant corneal abscess. Many of these eyes will be severely inflamed with uncontrolled scleritis and limbitis, which we treat before surgery with systemic immunosuppression using prednisolone (0.5 to 1 mg/kg/day) and cyclosporine (3 to 7.5 mg/kg/day), which is tapered as inflammation is controlled in the postgraft period. The extent of the infected corneal tissue cannot be identified and should be assumed to include the entire cornea so that, unlike grafts for other corneal infections, which should be large enough to remove all contaminated tissue, the graft for AK should be kept to the minimum size required to excise all ulcerated and necrotic tissue, retaining clinically healthy (but usually subclinically infected) tissue. This is because of the risk of rejection with large grafts and because repeat grafting may be needed as a result of recurrence; a further graft represents a new food source for the organism and can be used to attract residual amoebae.[71] Recurrence of disease in a graft was frequent in the first 2 weeks after surgery when keratoplasty was performed in an inflamed eye before the introduction of biguanides;[59] it typically involved the donor periphery, usually without clinical involvement of the host.[71] Late recurrences, several months after surgery, may also occur. Anti-amoebic therapy should be used before surgery and be continued postoperatively using drugs and doses that will minimize or avoid signs of toxicity. PHMB 0.02% has low clinical toxicity in most patients and is clinically less than that with either of the diamidines. We use PHMB 0.02%, 6 to 8 times daily immediately after surgery, with an adequate level of topical steroid to control inflammation. This should be continued for at least 3 weeks while results of culture of the host keratectomy specimen are awaited. If viable organisms are cultured it is prudent to continue anti-amoebic therapy 4 times daily while high-doses of steroids are needed, usually 6 months after surgery, as recurrent AK has occurred up to 3 months after an initially successful transplant. If culture

of the excised host cornea is negative after 3 weeks we assume that most viable amoebae have been treated, and the topical anti-amoebic therapy is reduced to 4 times daily and stopped after 1 month.

In our center therapeutic keratoplasty is rarely used but lamellar or PK to improve vision is carried out in patients with scarred corneas and/or irregular astigmatism. The outcome of corneal transplantation is good in this group of patients.[3,74,71] Exacerbations of scleritis and limbitis may occur following graft surgery in these eyes and may need to be treated with systemic anti-inflammatory treatment.

• **MANAGING PERSISTENTLY CULTURE-POSITIVE KERATITIS:** In our center 11 of 180 culture-positive cases of AK developed disease that was attributable to persistence of viable organisms in the cornea (shown by one or more positive cultures) following therapy with a biguanide, usually combined with a diamidine. Of these cases, 1 of 11 was enucleated (for pain relief) and 3 of 11 required therapeutic keratoplasty. In vitro sensitivity testing showed that all these cases were attributable to organisms resistant to diamidines but fully sensitive to biguanides. All cases, with one exception, were misdiagnosed for 6 or more weeks and in 40% exacerbations of disease followed a reduction in topical steroid therapy. It is important to try to differentiate persistently culture-positive cases from those with persistent inflammation, which may be caused by the severe inflammatory response in the cornea or in extracorneal tissues, and is unrelated to infection.[61] Neither confocal microscopy nor PCR can be expected to distinguish between viable and nonviable *Acanthamoeba* cysts; this leaves culture of corneal scrapes or biopsies as the only certain way of identifying persistent culture-positive disease. Unfortunately, a negative culture result does not confirm that all the viable organisms have been treated unless a substantial corneal excision biopsy has been taken.

Our recommendations for treatment of these cases of persistently culture-positive AK are: first, exclude bacterial, fungal, or herpes superinfection; then, providing the patient is on a biguanide, control the inflammation adequately with topical steroids and systemic immunosuppression as described previously. Next, we review the anti-amoebic therapy given and document the duration and combinations of therapy already used to identify which alternative therapies have been untried. We then combine a biguanide with a diamidine to gain the potentially enhanced effect of dual therapy, if this has not been done. We next switch between PHMB 0.02% and chlorhexidine 0.02% as well as switching between the diamidines (propamidine and hexamidine). Once these combinations have been used we increase the concentration of PHMB to 0.06% or chlorhexidine to 0.2% for a trial period. We have not yet used other agents topically or systemically as the in vitro data do not suggest any that are likely to be effective against *Acanthamoeba* cysts. If these measures fail then cryotherapy, as described previously, and therapeutic keratoplasty are the last options available for treatment. Repeated corneal cultures must be carried out to identify the continuing persistence of viable *Acanthamoeba* and possible development of resistance.

PROGNOSIS

THE MOST IMPORTANT FACTORS AFFECTING PROGNOSIS are severity of disease at presentation[38] and the interval between the onset of symptoms and the start of effective therapy; more than 3 weeks is associated with a worse prognosis.[36,37] Very few patients do badly who are diagnosed within this period. The best data on current outcomes, in a large number of patients, come from the two United Kingdom national surveys reporting on 349 cases. One limitation of these studies is that the disease process is so prolonged that 86 patients were still under review or lost to follow-up at the end of the studies. However, 40 of 349 (11%) had a keratoplasty, 2 were enucleated, 21 of 349 (6%) had cataract surgery. The mean duration of therapy was between 4 and 5 months with a range of 0.5 to 50 months. In the 229 eyes of patients who had completed treatment episodes, the visual acuity outcomes were 6/6 or better in 150 (65.5%), 6/9 to 6/12 in 70 (30.5%), 6/18 to 6/60 in 5 (2.0%), perception of light in 2 (2.0%), and enucleated in 2 (2.0%). These data give an overview of current therapeutic outcomes in the United Kingdom.

WHERE ARE WE NOW?

A PRINCIPAL CHALLENGE FOR PATIENTS WITH AK, AND their physicians, is to obtain a diagnosis and appropriate therapy with biguanides, probably combined with diamidines, within 3 weeks of the onset of symptoms; the prognosis is excellent for patients treated within this period. Early recognition of disease is less frequent in non–CLs users; this can be remedied by prompt specialist investigation of patients with keratitis that is persistent after an appropriate treatment trial for more common causes of infection.

In CL users the epidemiologic studies suggest that the disease is largely preventable by adherence to good hygiene practice, or the use of disposable lenses, in addition to avoiding contamination of the CLs and storage cases with domestic tap or swimming pool water. Although much is understood about the pathogenesis of corneal invasion, we have no understanding of the causes of the severe noninfectious extracorneal inflammatory disorders of scleritis and ischemic posterior segment inflammation that lead to blindness in a few patients with severe disease. Further study into the causes and treatment of these conditions is needed.

Diagnosis is based on clinical signs, which may be complicated by the effect of co-infection with other

organisms, compounded by the poor sensitivity of culture or histology that may be negative in 40% to 50% of cases at the first attempt. Confocal microscopy has been recommended as an alternative to culture, but we believe the sensitivity and specificity of this technique is too low to rely on it for a definitive diagnosis. Detection of *Acanthamoeba* DNA by PCR has a sensitivity of 80% or more and is very specific but is not routinely available; a commercial kit for this would be a major step forward in the management of the disease. Currently, unless culture, histology, or PCR are positive, cases that are deteriorating should be treated as though the diagnosis is provisional. Culture and sampling techniques are within the remit of any microbiology laboratory.

Therapy is dependent on the elimination of viable cysts. The only available and consistently cysticidal agents are the two biguanides, chorhexidine and PHMB, which can be used in various concentrations. The diamidines (propamidine and hexamidine) have also been widely used, but some organisms are fully resistant to these in vitro and this has been associated with poor clinical outcomes. There is concern about the potential toxicity of the biguanides, neither of which is licensed for use in keratitis; another major step forward will be the licensing of one of these as an orphan medication, such that associated data on toxicity, antimicrobial efficacy, and pharmacokinetics would result. The use of both topical steroids and systemic anti-inflammatory therapy remains controversial, but we believe that both are essential in the treatment of the few cases with severe corneal and extracorneal inflammatory complications of the disease and that they are safe to use, providing biguanide therapy is used concurrently.

The role of keratoplasty is now largely restricted to the visual rehabilitation of eyes in which a medical cure has been achieved. Despite the in vitro efficacy of the biguanides, there is in vivo resistance in about 5% of cases, for uncertain reasons, and the measures for managing this situation are complex. Clearly, there is a need for more effective drugs in the treatment of AK.

Acanthamoeba keratitis, like herpes keratitis (with which it is often confused), remains a challenging disease to treat because of the resistance of the pathogen to conventional antimicrobial therapy and the ill-understood role of the host inflammatory response that so often complicates the therapy of severe disease.

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LABORATORY DIAGNOSIS

• **SPECIMEN COLLECTION AND HANDLING:** Progression is slow in Acanthamoeba keratitis (AK) and it is likely that the probability of recovering organisms will be optimized if antimicrobial therapy is stopped 1 to 2 days before taking cultures or biopsies in patients already on treatment. Intensive treatment may be initiated after obtaining appropriate specimens for culture and staining.

Our protocol for diagnosis includes culture and histology of affected corneal epithelium and stroma. Epithelial specimens are taken with a blade and divided in half; one specimen is fixed in formaldehyde and sent for histopathologic analysis and the other is plated directly onto non-nutrient agar or placed in normal saline (0.9%) for culture in the laboratory. Epithelial specimens are also smeared onto a glass slide for microbiological stains. Corneal scrapes of the underlying stroma are taken, when Bowman membrane has been breached, and plated directly onto non-nutrient agar and glass slides for histology. Bacterial and fungal investigations are also performed routinely (glass slide smear, blood agar, brain-heart-infusion broth, Robertson's cooked meat broth, and Sabouraud agar) and a specimen taken for herpes polymerase chain reaction. A corneal biopsy is performed when the initial cultures have been negative and if there is a poor clinical response to treatment after 1 to 2 months. A corneal biopsy will often provide either histologic or microbiological confirmation if processed appropriately, because of the long time the organisms remain within the cornea.[32] When there is no extensive area of ulceration, a 3-mm skin trephine is used to mark an area on the edge of the pupillary zone, including clinically involved and uninvolved tissue, and a lamellar corneal biopsy is taken with a 30-degree or crescent blade; the specimen is then divided for culture and histology.[80] If an extensive area of necrotic tissue is present, it is excised with some healthy tissue at the margin. Excision biopsy of necrotic tissue facilitates epithelialization and provides a large specimen, allowing the status of the infection to be established. Amniotic membrane grafts can be placed after removing necrotic tissue and sometimes aid epithelialization.[2]

• **HISTOPATHOLOGY TECHNIQUES:** It is rarely possible to identify *Acanthamoeba* on Gram stain. Smears may be stained with periodic acid schiff, hematoxylin-eosin, or calcafluor white. The latter requires a fluorescent microscope and is probably not superior to the alternatives. All of these stains are also useful for fungi. Immunostaining with immunoperoxidase using a polyclonal antibody for *Acanthamoeba* is our method of choice but the antibody is not commercially available.[32] Epithelial specimens and corneal biopsies (Figure, Fourth row, left) are prepared for histology in the same way.

• **CULTURE TECHNIQUES:** Non-nutrient agar plates are directly inoculated over an area of approximately 0.5 cm × 0.5 cm. On receipt in the laboratory, the inoculated area of agar is excised from the plate and inverted onto an *E. coli*–seeded non-nutrient agar plate. This procedure avoids exposing the patient to the risk of *E. coli* contamination. Epithelial and stromal biopsies that have been placed in normal saline are partially homogenized and then plated directly onto *E. coli*–seeded non-nutrient agar. All culture plates are incubated at 37 C for 72 hours. If there is no growth at this stage, the plates are incubated at 30 C for a further 72 hours. If there has been no growth by 6 days the specimen is cultured at room temperature because some isolates grow better at lower temperatures. Cultures may occasionally take 3 weeks to grow using this protocol and specimens are not discarded in our laboratory until the end of this period. On *E. coli*–seeded non-nutrient agar plates, *Acanthamoeba* will feed on the bacteria and leave identifiable tracks. Trophozoites in these tracks can be seen with the binocular microscope, particularly when their vacuoles contract. In vitro sensitivity testing of isolates is available in specialized centers only, and may be indicated in persistent or recurrent cases with a poor clinical response to treatment.

Biosketch

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