American Journal of Ophthalmology Case Reports 25 (2022) 101372

Contents lists available at ScienceDirect

American Journal of Ophthalmology Case Reports

journal homepage: www.ajocasereports.com/

Risk factors, management, and outcomes of *Acanthamoeba* keratitis: A retrospective analysis of 110 cases

Brittni A. Scruggs [a,b], Tyler S. Quist [a], M. Bridget Zimmerman [c], Jorge L. Salinas [d], Mark A. Greiner [a,e,*]

[a] Department of Ophthalmology and Visual Sciences, University of Iowa, 200 Hawkins Drive, Iowa City, IA, 52242, USA
[b] Department of Ophthalmology, Mayo Clinic, 200 1st St SW, Rochester, MN, 55905, USA
[c] Department of Biostatistics, College of Public Health, University of Iowa, 145 N. Riverside Drive, Iowa City, IA, 52242, USA
[d] Departments of Infectious Diseases and Internal Medicine, University of Iowa, 200 Hawkins Drive, Iowa City, IA, 52242, USA
[e] Iowa Lions Eye Bank, 2500 Crosspark Road, W300, Coralville, IA, 52241, USA

ARTICLE INFO

Keywords:
Acanthamoeba
Contact lens
Corneal infection
Keratitis
Keratoplasty

ABSTRACT

Purpose: To evaluate the risk factors, medical and surgical management, and visual outcomes of patients affected by *Acanthamoeba* keratitis (AK) over a 16-year period.

Observations: Records were reviewed retrospectively for all AK patients treated at University of Iowa between 2002 and 2017. Main outcomes measured were risk factors, time to diagnosis, coinfection types, initial and final visual acuities, and treatment outcomes, with failure of medical therapy defined as need for therapeutic keratoplasty (TK). Effects of steroid use on these outcomes were determined. Among all AK cases occurring during the study period (N = 110), the median age of the AK cohort was 31 years (range 8–80 years), and 49.1% were men. Contact lens wear was the primary risk factor for AK (95/100, 86.4%), and the median time to diagnosis was 0.70 (0.23–1.23) months. Forty-four AK patients (40%) failed medical therapy. Vision outcomes were better for AK patients with successful medical therapy compared to those requiring TK (LogMAR 0.00 v. 0.30; $p < 0.0001$). Corticosteroid use was associated with increased time to diagnosis (1.00 v. 0.50 months; $p = 0.002$), decreased final vision (LogMAR 0.10 v. 0.00; $p < 0.05$) and increased need for TK (40/77 v. 4/33; $p < 0.001$).

Conclusions and importance: Acanthamoeba keratitis cases have increased over the past two decades at our institution. In this large retrospective study, AK was commonly misdiagnosed with delayed diagnosis and high rates of failed medical therapy. Corticosteroid use before AK diagnosis led to poorer outcomes. Our findings underscore the need for ophthalmologists to suspect *Acanthamoeba* in the setting of contact lens-associated keratitis before topical steroids are initiated.

1. Introduction

Acanthamoeba species are ubiquitous protozoans present in some public water sources such as tap water and freshwater lakes and rivers.[1–5] Due to its ability to encyst in extreme environmental conditions (*i.e.,* high temperatures, heavy chlorination), this organism becomes very difficult to kill. It is known that individuals can develop *Acanthamoeba* keratitis (AK), a blinding corneal infection, mainly from poor contact lens hygiene practices. AK commonly is misdiagnosed as herpetic, bacterial, or fungal keratitis prior to being confirmed with ancillary diagnostic testing. The combination of late diagnoses and the amoeba's resistance to killing makes these cases especially challenging for the treating ophthalmologist.[6] It is our experience at the University of Iowa Hospitals & Clinics (UIHC) that many of these patients ultimately need therapeutic keratoplasty (TK). It can be difficult to prevent the new donor cornea from becoming infected,[7–10] and intraocular infection and/or loss of the eye are late complications that can occur in these cases.[7]

The incidence of AK in the United States (US) is estimated to be 1–2 new cases per 1 million contact lens wearers annually[11]; approximately 16.7% of US adults wear contact lenses.[12] In Iowa, among the estimated

* Corresponding author. Cornea and External Diseases Department of Ophthalmology and Visual Sciences University of Iowa Carver College of Medicine Associate Medical Director, Iowa Lions Eye Bank 200 Hawkins Drive, 11290-A PFP, Iowa City, IA, 52242, USA.
E-mail addresses: Scruggs.brittni@mayo.edu (B.A. Scruggs), Tyler-quist@uiowa.edu (T.S. Quist), bridget-zimmerman@uiowa.edu (M.B. Zimmerman), jorge-salinas@uiowa.edu (J.L. Salinas), mark-greiner@uiowa.edu (M.A. Greiner).

https://doi.org/10.1016/j.ajoc.2022.101372
Received 10 July 2021; Received in revised form 25 January 2022; Accepted 25 January 2022
Available online 27 January 2022

Abbreviations

AK	*Acanthamoeba* keratitis
CHX	0.02% chlorhexidine;
CI	confidence interval
CL,	contact lens
DALK	deep anterior lamellar keratoplasty
history of present illness	HPI
HSV	herpes simplex virus
PCR	polymerase chain reaction
PHMB	0.02% polyhexamethylene biguanide;
TK	therapeutic keratoplasty
UIHC	University of Iowa Hospitals & Clinics

2.42 million adult residents in 2019, 0.4–0.8 new AK cases per year would be expected. Our team recently performed a retrospective epidemiologic investigation of all AK cases (N = 75) in Iowa residents treated at UIHC over 16 years.[13] The average number of new AK infections diagnosed per year increased from 2.9 cases during 2002–2009 to 6.5 cases during 2010–2017.[13]

In this retrospective study, we report the clinical outcomes of all AK cases treated at UIHC between 2002 and 2017 with a focus on risk factors, medical and surgical management, and visual outcomes of these patients. AK classically is misdiagnosed as HSV keratitis,[14] and initiation of topical corticosteroids prior to a diagnosis of AK is a well-documented risk factor for poor outcomes.[15] Therefore, we sought to identify AK at our institution and to study the impact of steroid use on outcomes. Given this is one of the largest AK studies to date, we anticipate that our analysis of this single-center study will help clinicians identify factors that lead to improved AK diagnosis, management, and outcomes in clinical practice.

2. Methods

Institutional Review Board (IRB) approval for this study was obtained at the University of Iowa Hospitals & Clinics, and all research adhered to the tenets of the Declaration of Helsinki.

2.1. Acanthamoeba keratitis (AK) cases

The UIHC Joint Office of Compliance provided the medical record numbers of patients associated with the following specific *Acanthamoeba* keratitis ICD-9 and ICD-10 codes: 136.21; 370.02; B60.10; B60.11; B60.12; B60.13; B60.19; B64; H16.021; H16.022; and H16.023. A total of 861 records were reviewed, and only patients with a confirmed AK diagnosed at UIHC between 2002 and 2017 were included in this study. A confirmed AK case was defined as detection of *Acanthamoeba* using confocal microscopy or corneal scraping. Cases with a history of confirmed AK diagnosis but with no treatment of active disease at UIHC were excluded. Overall, 110 AK cases (110 eyes; 109 patients) were identified for inclusion in this study, of which one patient had simultaneous, bilateral AK. Eight AK patients transferred care during active treatment, and, thus, the final visual acuities were known for 102 out of 110 AK eyes.

2.2. Retrospective analysis of medical records

Electronic medical records and/or paper charts were reviewed in all cases. Patient demographics as well as the date of diagnosis and location of residence (zip code) at the time of diagnosis were determined. Exposures and risk factors including a history of contact lens (CL) wear, poor CL hygiene practices, ocular exposure to organic material, and corneal abrasions were noted. The initial history of present illness (HPI), ophthalmic exam, and presenting symptoms and signs for each case were documented for all AK cases.

Best-corrected visual acuity (VA) documented using a Snellen chart 20 feet from the patient was noted for all cases on initial presentation and when the patient was deemed "stable" (i.e. no longer actively being treated for AK infection). The final VA was recorded as the last recorded vision at UIHC when anti-amoebic drops were no longer needed or when the decision was made to continue drops for maintenance (e.g. to minimize the chance of recurrence in a corneal graft); thus, the timing from diagnosis to final VA varied significantly given the variability of individual treatment courses and duration of UIHC care. For patients who required surgery, the final vision was recorded after optical keratoplasty was performed. For patients who transferred care, the final vision was recorded prior to transferring care to their local provider, who may or may not have performed subsequent procedures for further rehabilitation.

VA was categorized as normal vision (20/15–20/25) or mild (20/32–20/63), moderate (20/80–20/160), severe (20/200–20/400), or profound (worse than 20/500) vision loss according to the Visual Standards Aspects and Ranges of Vision Loss with Emphasis on Population Surveys by the International Council of Ophthalmology (29th International Congress of Ophthalmology; Sydney, Australia, April 2002). Excellent vision was defined as either normal vision or mild vision loss as described previously.

2.3. Diagnosis of microbial keratitis

AK was diagnosed using confocal microscopy and corneal scraping tests, and recorded results were reviewed. Confocal microscopy was performed using a tandem scanning confocal microscope (ConfoScan 4, Nidek Technologies, Fremont, CA) and evaluated by an experienced UIHC cornea specialist for trophozoites and/or bright cysts with or without double-walls consistent with *Acanthamoeba* infection. Epithelial corneal scrapings were performed in clinic and placed into Saccomanno fixative, and specimens were analyzed for amoebic cysts by an experienced UIHC ocular pathologist using direct light microscopy.

The original diagnosis, including any misdiagnoses, were noted. The "time to diagnosis" for all cases was determined as the time between first seeing an eye specialist (non-UIHC or UIHC) and the date of accurate diagnosis. If the accurate keratitis diagnosis was made on the initial visit (i.e. no misdiagnosis), the time to diagnosis was recorded as zero. This value often differed from the "time since symptom onset," which was not always recorded in the HPI. Conversely, if a patient was diagnosed at the initial UIHC visit but was previously misdiagnosed by a non-UIHC provider, the time to diagnosis was determined as the time since the non-UIHC visit when the patient first presented with symptoms related to corneal infection.

The number of patients who were prescribed oral antiviral medications (acyclovir or valacyclovir) for coinfection with clinically diagnosed herpes simplex virus (HSV) keratitis was recorded. Real-time polymerase chain reaction (PCR) assay (DiaSorin Molecular Simplexa™ HSV 1 & 2 Direct assay, Ref MOL2151) intended for the *in vitro* qualitative detection and differentiation of HSV1 and/or HSV2 viral DNA polymerase was performed for select patients. The results of HSV PCR were recorded for all cases for which testing was performed. Tear samples were obtained by swabbing the conjunctival surface, and these swabs were then submitted to the UIHC Clinical Microbiology Laboratory for HSV PCR testing.

Data pertaining to any bacterial and/or fungal cultures obtained were reviewed and recorded for all AK cases, and treatments for coinfection with bacterial or fungal species were noted.

2.4. Treatment of microbial keratitis

The number and types of medications (both topical and oral) were documented for all patients. The initiation and duration of use were

recorded specifically for the following medications: chlorhexidine (CHX) 0.02%, polyhexamethylene biguanide (PHMB) 0.02%, brolene, fluconazole, itraconazole, ketoconazole, voriconazole, pentamidine, moxifloxacin 0.5%, tobramycin 1.4%, vancomycin 25 mg/mL, prednisolone acetate 1%, and prednisone. AK cases were stratified into those without prescribed topical or oral steroids ("none"), those with steroids used *prior* to AK diagnosis ("before"), and those receiving steroids *only after* receiving the AK diagnosis ("after"). The use of steroids after surgical intervention was not included in this analysis. The "before" group included patients who may have also received steroids after AK diagnosis. For analysis of these steroid groups, topical and oral steroid agents included difluprednate, fluorometholone (FML), loteprednol, prednisolone, prednisone, and/or tobramycin/dexamethasone. In all cases, the success of medical therapy was determined by the ability of the medical regimen to prevent surgery, with failure of medical therapy defined as the need for TK. TK included all partial (e.g. DALK) or full-thickness penetrating keratoplasties performed for failure of medical therapy, *excluding* transplants performed to address visually significant corneal scars.

All surgical procedure notes were reviewed to determine the type, number, and timing of surgical interventions required for each patient. The number of patients requiring repeat corneal transplants was recorded.

2.5. Statistical analyses

Statistical analyses were conducted using a two sample two-tailed Mann-Whitney *U* test, or Wilcoxon rank-sum test, where appropriate, to compare ages, time to diagnosis, and visual acuities between two groups. Data regarding total percentages were analyzed by Pearson Chi-square test to determine differences among two groups. Significance for comparisons was defined as $p < 0.05$ for all analyses. All statistical tests were performed using GraphPad Prism 4.0b for Macintosh (GraphPad Software, San Diego, CA). All values of age, time, and visual acuity were reported as medians (25th − 75th percentiles). GraphPad Prism 4.0b for Macintosh were used to generate figures. Box plots represented 25th to 75th percentiles with vertical bars providing range and horizontal bars representing median values.

3. Findings

3.1. Epidemiology and demographics

The average number of new AK cases per year at UIHC increased from 4.0 cases during 2002–2009 to 9.8 cases during 2010–2017. The number of new UIHC AK cases tripled during 2011–2015 (Fig. 1). Patient demographics for AK patients are shown in Table 1. The AK median age was 31 [21.0–44.3] with 16 (14.5%) AK patients aged <18 years old. AK cases were most prevalent in the summer (Table 1).

3.2. Risk factors

Reported exposures and risk factors for AK patients are noted in Table 2. Most patients were soft contact lens (SCL) wearers with reported poor CL hygiene practices (Table 2). Of the 89 SCL wearers, 15 reported wearing extended wear contacts (e.g. monthlies) and 9 reported wearing daily CLs. All other SCL wearers did not specify the type or intended duration of their SCL. There were 15 (13.6%) non-CL wearers, and seven of these patients developed AK without documented risks or exposures. Some of the AK patients who did not wear contact lenses reported a prior history of various exposures, including swimming daily in a stock tank, rubbing eyes after handling frogs, topical anesthetic use, and/or corneal abrasions with organic material. Thirty-three CL wearers (34.7%) reported sleeping in CL, which was the most reported risk factor for AK development.

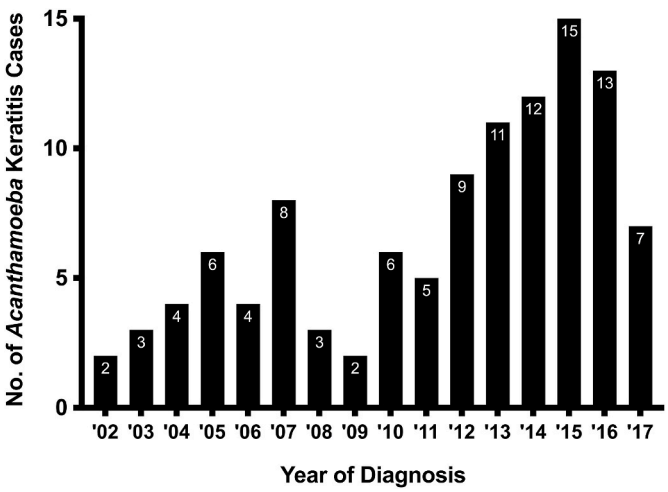

Fig. 1. Confirmed cases of *Acanthamoeba* keratitis diagnosed at UIHC between 2002–2017. AK was confirmed by confocal microscopy and/or corneal scraping in 110 cases with microbial keratitis treated at UIHC. There was an increase in incidence over time with two new cases confirmed in 2002 and 15 new cases confirmed in 2015. Abbreviations: AK, *Acanthamoeba* keratitis; UIHC, University of Iowa Hospitals & Clinics.

Table 1
Characteristics of patients with *Acanthamoeba* keratitis.

	All AK Cases N = 110 [%]
Age at diagnosis (years)	
Median (25th-75th percentiles)	31 (21.0–44.3)
Range	8–80
Gender	
Male	54 [49.1]
Female	56 [50.9]
Time to diagnosis (mo)	
Median (25th-75th percentiles)	0.70 (0.23–1.23)
Initial acuity (LogMAR)	
Median (25th-75th percentiles)	0.55 (0.20–2.0)
20/200 (LogMAR1.0 or worse)	45 [40.9]
Season of initial presentation	
Winter	25 [22.7]
Spring	28 [25.5]
Summer	35 [31.8]
Fall	22 [20.0]

Abbreviations: AK, *Acanthamoeba* keratitis.

Table 2
Exposures and/or risk factors of patients diagnosed with *Acanthamoeba* keratitis.

	All AK Cases N = 110, [%]
Contact lens wearers	95 [86.4]
Type of contact lens[a]	
Soft contacts	89 [93.7]
Rigid contacts	6 [6.3]
Contact lens-related risks[a]	
Poor contact lens hygiene	67 [69.8]
Sleeping in contacts	33 [34.7]
Wear longer than intended	26 [28.4]
Swimming in contacts	24 [25.3]
Showering in contacts	21 [22.1]
Tap water use for cleaning	11 [11.6]
Other exposures	
Organic material (*e.g.* wood)	14 [12.7]
Corneal abrasion	14 [12.7]

Abbreviations: AK, *Acanthamoeba* keratitis.
[a] Percentages calculated based on total number of contact lens wearers.

3.3. Presenting symptoms and signs

Reported clinical symptoms and signs of AK patients were noted at the time of initial presentation to UIHC (Table 3). The most common symptoms were pain (70/110; 63.6%) and photophobia (53/110; 48.2%). Ninety percent of AK patients reported at least one symptom other than decreased vision, and the number of symptoms reported by these patients is included in Table 3. The most common corneal findings, reported in Table 3, were stromal infiltrate (67/110; 60.9%), epithelial defect (50/110; 45.5%) and ring infiltrate (40/110; 36.4%).

3.4. Diagnosis

The sensitivities of confocal microscopy and corneal scraping for the AK cohort were 97.2% and 87.8%, respectively (Table 3). The three AK cases with negative confocal results had positive scrapings and clinical findings consistent with AK. Conversely, the 10 cases with negative scrapings were found to have positive confocal microscopy results and clinical findings consistent with AK.

Table 3
Symptoms, signs, and diagnostic testing of patients treated for *Acanthamoeba* keratitis at UIHC between 2002 and 2017.

	AK N = 110, [%]
Clinical symptoms	
Reported at presentation	
Significant pain	70 [63.6]
Photophobia	53 [48.2]
Irritation/Foreign body sensation	43 [39.1]
Redness	37 [33.6]
Tearing	21 [19.1]
No. of symptoms reported[a]	
Zero, decreased vision only	11 [10.0]
One	22 [20.0]
Two	38 [34.5]
Three	31 [28.2]
Four or more	8 [7.3]
Clinical findings	
Stromal infiltrate/opacity	67 [60.9]
Epithelial defect	50 [45.5]
Ring infiltrate	40 [36.4]
Corneal edema	37 [33.6]
Perineuritis	20 [18.2]
Keratic precipitates	16 [14.5]
Hypopyon	11 [10.0]
Corneal neovascularization	9 [8.2]
Corneal ulcer with stromal thinning	8 [7.3]
Diagnostic testing	
Confocal performed[b]	108 [98.2]
Positive confocal testing	105 [95.5]
Confocal sensitivity	97.2%
Scraping performed[c]	82 [74.5]
Positive scraping	72 [65.5]
Scraping sensitivity	87.8%
Bacterial/fungal cultures performed	66 [60]
No growth	34 [30.9]
Confirmed growth[d]	32 [29.1]
Bacterial	26 [23.6]
Fungal	8 [7.3]
Bacterial and fungal growth	2 [1.8]

Abbreviations: AK, *Acanthamoeba* keratitis; UIHC, University of Iowa Hospitals & Clinics.

[a] Symptoms reported other than decreased or blurry vision.

[b] The three cases with negative confocal results had positive scrapings and clinical findings consistent with AK.

[c] The 10 cases with negative scrapings were found to have positive confocal microscopy results and clinical findings consistent with AK.

[d] The following pathogens were isolated in culture: *Alternaria* (N = 1), *Aspergillus* (N = 4), Coagulase negative *Staphylococcus* (N = 10), fungus not otherwise specified (N = 4), Gram negative rods (N = 4), Gram positive cocci (N = 3), *Propionibacterium acnes* (N = 7), *Pseudomonas* spp. (N = 2), *Serratia marcescens* (N = 1), *Staphylococcus epidermidis* (N = 4), *Streptococcus mitis* (N = 2), and *Streptococcus oralis* (N = 1).

The initial diagnoses made by an eye specialist were determined. AK commonly was misdiagnosed at initial presentation. Of the 102 AK cases with a known initial diagnosis, only 22 cases (21.6%) were diagnosed accurately as AK. The most common misdiagnoses were bacterial keratitis (36; 35.3%), HSV keratitis (17; 16.7%), and combined bacterial and HSV keratitis (7; 6.9%). Other initial diagnoses included conjunctivitis (4; 3.9%), corneal abrasion (4; 3.9%), combined bacterial and fungal keratitis (4; 3.9%), fungal keratitis (3; 2.9%), contact lens overwear (3; 2.9%), recurrent erosions (1; 1.0%), and scleritis (1; 1.0%). The median time to diagnosis was 0.70 (0.23–1.23) months for AK.

3.5. Visual outcomes

The visual acuities at presentation were compared to the final (i.e., stable) acuities once the corneal infection was inactive (Fig. 2). Although 40.9% of all AK patients presented with LogMAR 1.0 or worse vision, most cases at UIHC had normal or mild vision loss once stable (Fig. 2). All AK patients with a presenting vision of LogMAR 0.4 or better had excellent final acuities (Fig. 2).

There were four endophthalmitis complications (3.6%, Table 4); these patients all presented with a visual acuity of HM or worse. One patient with endophthalmitis developed choroidal infiltration and tractional retinal detachment, which required extensive retinectomy. Two patients with endophthalmitis required enucleation due to 1) panophthalmitis with severe glaucoma, and 2) corneal melt in setting of reinfected graft and severe medicamentosa. All AK patients requiring enucleation (N = 4, 3.6%) presented with visual acuities of LogMAR 1.17 or worse. Final vision outcomes were statistically inferior for patients who failed medical therapy (LogMAR 0.30 [0.00–1.10] vs. LogMAR 0.00 [0.00–0.10], $p < 0.0001$; Fig. 3).

3.6. Treatment

All prescribed medications were documented for each AK patient (Supplemental Fig. 1). The median number of medications used during active treatment was 2 [1–3]; however, 21 (19.1%) AK patients required four or more anti-amoebic topical agents. Regardless of initial vision or keratoplasty requirement, CHX was the medication of choice with

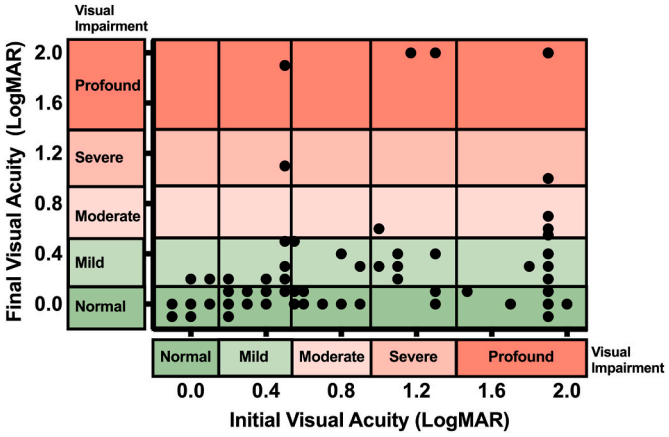

Fig. 2. Initial and final visual acuities of *Acanthamoeba* keratitis cases diagnosed at UIHC between 2002–2017. Visual acuities of AK cases (Black circles, N = 102) were determined in clinic using Snellen chart testing; these acuities have been converted to LogMAR. Severity levels of visual impairment are labeled according to the Visual Standards Aspects and Ranges of Vision Loss with Emphasis on Population Surveys by the International Council of Ophthalmology (29th International Congress of Ophthalmology; Sydney, Australia, April 2002). Abbreviations: AK, *Acanthamoeba* keratitis; UIHC, University of Iowa Hospitals & Clinics.

Table 4
Clinical outcomes of patients with *Acanthamoeba* keratitis.

	All AK Cases N = 110 [%]
Final acuity (LogMAR)[a]	
Median (25th-75th percentiles)	0.10 (0.00–0.30)
20/200 (LogMAR 1.00 or worse)	12 [11.8]
Surgical intervention required	51 [46.4]
TK[b]	44 [40.0]
Repeat TK	19 [17.3]
Enucleation	4 [3.6]
Glaucoma surgery	6 [5.5]
Endophthalmitis	4 [3.6]
HSV coinfection	
PCR positive	5 [4.5]
Treated for HSV[c]	54 [49.1]

Abbreviations: AK, *Acanthamoeba* keratitis; DALK, deep anterior lamellar keratoplasty; HSV, herpes simplex virus; PCR, polymerase chain reaction; TK, therapeutic keratoplasty.

[a] Data based on total number of patients with final visual acuities noted without transfer of care (N = 102).

[b] Includes therapeutic partial (e.g. DALK) or full-thickness penetrating keratoplasties.

[c] Patients with a clinical diagnosis of active HSV corneal infection with or without PCR confirmation who received HSV treatment concurrently with AK treatment.

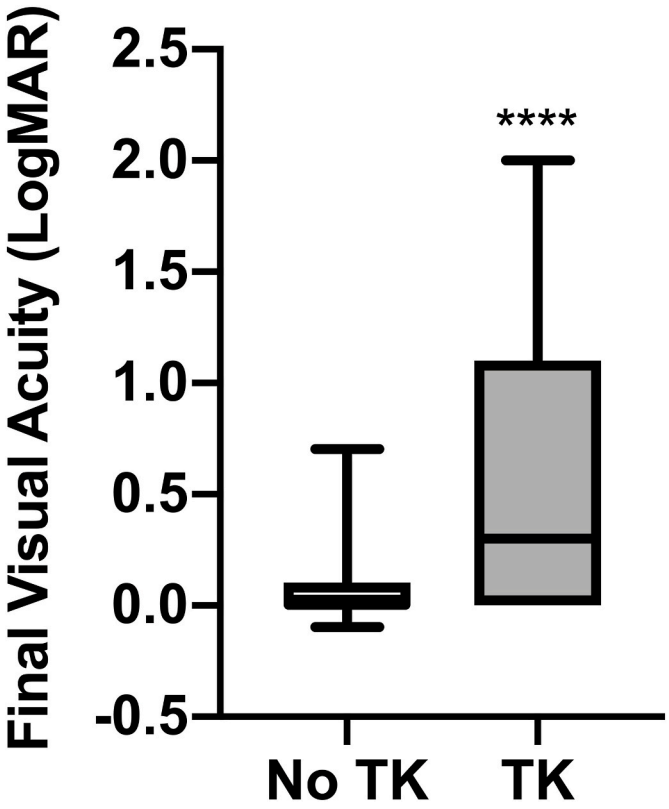

Fig. 3. Comparison of final visual acuities in *Acanthamoeba* keratitis cases with or without need for therapeutic keratoplasty. The median final visual acuities for all AK patients treated without TK were compared to those who required TK. Box plots represent 25th to 75th percentiles with vertical bars providing range. Using Mann-Whitney *U* test, significance was defined as ****, $p < 0.0001$ compared to patients with no TK. Abbreviations: AK, *Acanthamoeba* keratitis; TK, therapeutic keratoplasty (partial or full-thickness).

approximately 95% patients (N = 104) using this agent (Supplemental Fig. 1). PHMB (N = 61) and brolene (N = 31) were used more frequently in patients requiring keratoplasty. Pentamidine (N = 23) was used almost exclusively in the perioperative period. Oral prednisone (N = 11) and topical steroids (N = 64) were used more frequently in patients who ultimately required keratoplasty. Oral voriconazole was used as adjunct therapy in 28 patients (25.5%; Supplemental Fig. 1).

Fifty-one (46.4%) AK cases required surgery, and Table 4 documents the various surgical interventions needed. Forty-four AK patients (40%) required TK. A limited number of AK patients also required superficial keratectomy (8), cross-linking (2), pars plana vitrectomy for retinal detachment (2), Gundersen flap (2), tarsorrhaphy (2), and synechiolysis (2).

3.7. HSV and other coinfections

Ninety-four (85.5%) of the AK cases were tested for HSV coinfection via PCR testing. An unexpectedly high number of AK patients were clinically diagnosed and treated for HSV (54/110 [49.1%]). Of the AK cases with clinically diagnosed and treated HSV (N = 54), only five patients (9.3%) had a positive PCR test, and seven patients (13.0%) had no HSV testing. Despite negative PCR testing, antiviral therapy was continued for any patient with high suspicion of viral infection using confocal microscopy (e.g. Langerhans cells) or clinical examination (e.g. concurrent lip lesions, dendritic corneal lesions). Of the AK cases without clinically diagnosed HSV (N = 56), forty-five (80.3%) had a negative PCR test and 11 (19.7%) were not tested for HSV.

Sixty-six (60%) AK cases had corneal cultures performed to detect the presence of secondary bacterial or fungal infections; of these cases, 32 (48.5%) tested positive for culture growth, and most of these coinfections were bacterial (Table 3). Seven AK cases had more than one secondary infection with two cases having both bacterial and fungal growth (Table 3). Patients with bacterial coinfections were treated with fortified antibiotic topical drops, such as vancomycin or tobramycin. Fungal coinfections were treated with amphotericin B topical drops. AK patients who required TK tended to have coinfection more frequently than AK patients treated successfully with medical therapy (17/44 [38.6%] v. 15/66 [22.7%]; p = 0.09).

3.8. Effects of corticosteroids on AK outcomes

The majority of patients (77/110; 70%) used steroids before AK diagnosis and/or during treatment for active AK (Fig. 4A). AK cases were stratified by those never prescribed steroids (N = 33), those prescribed steroids *prior* to AK diagnosis (N = 48), and those prescribed steroids *only after* AK diagnosis (N = 29) (Fig. 4). Most AK patients (42/77; 54.5%) receiving steroids were also treated with oral antiviral therapy (Fig. 4B). The steroid groups collectively had more secondary bacterial infections compared to the group without steroids (27/77 [35.3%] v. 4/33 [12.1%], $p < 0.05$; Fig. 4B); fourteen of these patients received steroids *prior* to bacterial keratitis diagnosis. The time to diagnosis was longer for patients using steroids prior to AK diagnosis compared to those without steroids (1.00 [0.48–1.88] v. 0.50 [0.23–1.00] months; p = 0.002; Fig. 4C). The final visual acuities were significantly decreased in AK patients with steroid use (LogMAR 0.10 [0.0–0.50] v. 0.00 [0.0–0.20], $p < 0.05$; Fig. 4D). Steroids increased the need for TK surgeries (40/77 [51.9%] v. 4/33 [12.1%], $p < 0.0001$; Fig. 4E). Patients without steroid use did not require enucleation, yet four eyes in the steroid groups were enucleated (data not shown).

4. Discussion

Our group previously reported a recent epidemic of *Acanthamoeba* keratitis in Iowa.[13] This alarming increase in severe corneal infections prompted us to develop this retrospective study to reevaluate all AK cases starting in 2002, when confocal microscopy was implemented at UIHC. The AK patients in this study exhibited typical clinical features of this disease. AK infections were slightly more prevalent during the summer months. Consistent with prior AK studies,[14,16] more than 85%

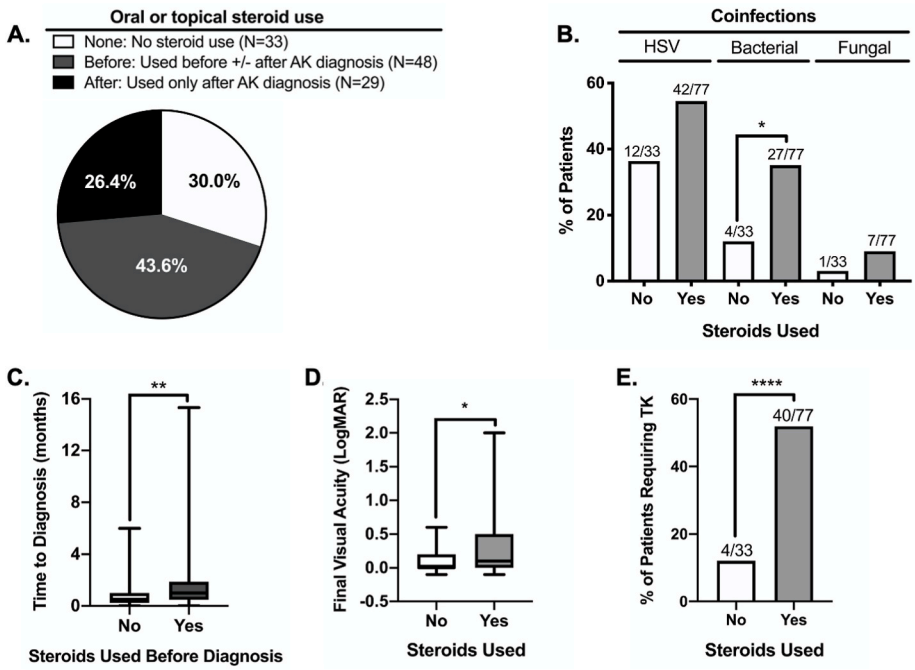

Fig. 4. Comparison of *Acanthamoeba* keratitis outcomes with and without steroid use. A, All AK cases (N = 110) treated at UIHC between 2002 and 2017 were stratified into three groups: (1) those who used no oral or topical steroids (None; White); (2) those who used steroids prior to the diagnosis of AK with or without steroid use after AK diagnosis (Before; Dark grey); or (3) those who used steroids during *active* keratitis treatment only after the diagnosis of AK (After; Black). **B,** The percentages of AK patients *treated* for HSV and confirmed to have bacterial and/or fungal coinfections were stratified based on steroid use. Note that most patients treated for HSV had negative HSV PCR testing. **C,** The median time to diagnosis (horizontal bar) was determined for patients who did not use steroids prior to AK diagnosis compared to patients who did receive steroids prior to diagnosis. **D,** The median final visual acuities (horizontal bar) were stratified by steroid use. **E,** The percentages of AK patients requiring TK were stratified by steroid use. Box plots in C & D represent 25th to 75th percentiles with vertical bars providing range. Significance was defined as *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$ based on Mann-Whitney *U* test (C & D) or Pearson chi-square test (B & E). Abbreviations: AK, *Acanthamoeba* keratitis; HSV, herpes simplex virus; PCR, polymerase chain reaction; TK, therapeutic keratoplasty (partial or full-thickness); UIHC, University of Iowa Hospitals & Clinics.

wore contact lenses; as expected, inadequate contact lens practices increased AK susceptibility.[17] In an important point of departure from the expected clinical behavior, AK patients reported pain at their initial visit less frequently than documented by Hargrave et al. (63.6% v. 95%).[18] This may be due to the high coincidence of corneal coinfections, well known to be associated with corneal neuropathy,[19] among AK patients in this study.

There have been fewer than 10 cases of AK and HSV coinfection reported.[14,20,21] Analysis of our 110 AK cases demonstrated a high percentage of clinical HSV diagnoses (54/110, 49%). HSV culture testing was not available and not performed at UIHC over the study period, but only five AK cases (5/110, 4.5%) had positive HSV PCR testing. A study by Randag et al. similarly found only two cases of laboratory confirmed HSV coinfection in their 224 AK patients despite 58% of AK cases being initially diagnosed as HSV.[14] Our results prompted our team to further investigate the effects of corticosteroids, which are commonly used in HSV treatment regimens, on AK outcomes of cases analyzed in this study.

All AK cases in this study had either positive confocal microscopy[22] or corneal scraping results. This demonstrates the utility of confocal microscopy, corneal scraping with direct light microscopy, or other diagnostic tests (e.g. PCR testing, *Acanthamoeba* culture) in making the appropriate diagnosis, but also indicates the need to perform specific AK testing early in a microbial keratitis work-up to make this difficult diagnosis quickly. Muiño et al. showed that corneal biopsy provided the highest likelihood ratio for a positive result when performing *Acanthamoeba* culture, but corneal scrapings were still superior to corneal swabbing for AK diagnosis.[23]

Similar to previous studies,[14,24] most AK patients in this study were misdiagnosed initially. We found that the presence of coinfection and/or steroid use delayed AK diagnosis significantly. These findings underscores the need for ophthalmologists to suspect *Acanthamoeba* infection particularly when HSV is suspected in the setting of contact lens associated keratitis. However, for cases of coinfection, it remains unknown if clinicians truly miss AK in the setting of microbial keratitis or if HSV and/or bacterial infections increase the susceptibility to AK due to epitheliopathy.

Our findings indicate that clinicians should also have a low threshold for culturing all infiltrates associated with AK infection, as nearly one-third of all AK patients had confirmed growth of bacteria and/or fungi. Coinfections can exacerbate AK by prolonging healing and providing an additional food source for the protozoa.[25,26] While our findings highlight the importance of detecting AK symbionts, our study was underpowered for determining differences in vision outcomes attributable to coinfection particularly. Diagnostic testing for HSV should be considered for cases of suspected AK that also have high suspicion of viral infection by history, confocal microscopy, or clinical examination. More importantly, clinicians should avoid diagnosing HSV and treating presumed viral disciform keratitis with corticosteroids without fully considering AK as an alternate or concurrent diagnosis.

Despite a significant delay to obtain an accurate diagnosis, the AK patients achieved excellent visual outcomes. We believe this is due to excellent presenting visual acuities for some patients, and the high rate of surgical intervention required for infection treatment and control for others. The proportion of AK cases requiring TK was significantly higher in the current study compared to a recent study by Höllhumer et al. (44/110 vs. 3/52),[24] yet the median final visual acuities were superior in our study (LogMAR 0.10 vs. LogMAR 0.57). Randag et al. published the largest AK study to date demonstrating 49 of 224 patients required acute keratoplasty.[14]

Our data do provide insight into important treatment considerations in AK, particularly because of the frequent use of topical corticosteroids. In this study, most patients (77.8%) with AK and clinically diagnosed HSV–most of whom had negative HSV PCR testing–used steroids before or after AK diagnosis. Corticosteroids have been shown to limit the clearance of protozoa, increase *Acanthamoeba* pathogenicity, and lead to worsened visual outcomes.[15,27] Our study also demonstrates that patients receiving steroids prior to AK diagnosis had significant delay in diagnosis and required more therapeutic and repeat keratoplasty procedures to control infection. Corticosteroid use in AK patients was associated with increased bacterial coinfections, possibly due to local immunosuppression. There was no difference in initial vision between the steroid groups to suggest an alternate reason for outcomes other than the impact of corticosteroid use. Our data underscore the importance of withholding topical corticosteroids until AK is ruled out.

4.1. Limitations

This is one of the largest studies evaluating AK in the United States, but important limitations exist for this single-center retrospective investigation. Our analysis was underpowered with respect to ascertaining a truer impact of secondary coinfections on outcomes. Because of the relatively low number of AK patients studied, we could only prove indirectly that delay in diagnosis led to vision loss. The corneal specialists interpreting the confocal images were not blinded to clinical information; thus, there is potential for examiner bias in the interpretation of the results and sensitivity for AK diagnosis. Future prospective investigations studying the impact of treatment duration and resistance on the success of medical therapy, and the effects of coinfection on treatment outcomes, are indicated.

5. Conclusions

In this series, patients with AK achieved excellent visual outcomes but often failed medical therapy. Misdiagnosis was common in AK patients, which resulted in delayed AK diagnosis. AK patients who received corticosteroids had a significant delay in diagnosis and required therapeutic procedures more frequently to control infection. Ophthalmologists should have a heightened suspicion of *Acanthamoeba*, particularly when herpes or microbial keratitis is suspected in the setting of contact lens associated keratitis. Our data suggest that obtaining an accurate diagnosis of AK quickly, holding topical corticosteroid use until AK is ruled out, and testing for other microbial infections may be the best opportunities to improve outcomes of *Acanthamoeba* keratitis.

Funding

The authors acknowledge the support of an unrestricted grant to the University of Iowa Department of Ophthalmology and Visual Sciences from Research to Prevent Blindness, Inc. New York, NY. Support was also received from the Heed Foundation.

Meeting presentation

American Academy of Ophthalmology (AAO) Annual Meeting in October 2018.

Author contributions

Brittni A. Scruggs: Conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing.

Tyler S. Quist: Conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing.

M. Bridget Zimmerman: Conception and design, collection and/or assembly of data, data analysis and interpretation.

Jorge L. Salinas: Conception and design, collection and/or assembly of data, data analysis and interpretation.

Mark A. Greiner: Conception and design, collection and/or assembly of data, financial support, administrative support, data analysis and interpretation, manuscript writing, final approval of manuscript.

Declaration of competing interest

No conflicting relationship exists for any author.

Acknowledgements

We thank the Iowa Lions Eye Bank, the M.D. Wagoner & M.A. Greiner Cornea Excellence Fund, the UIHC Cornea Research Fund, Mr. and Mrs. Lloyd and Betty Schermer, and Mr. and Mrs. Robert and Joell Brightfelt for generous support. We also thank Drs. Kenneth M. Goins, Anna S. Kitzmann, John E. Sutphin, and Michael D. Wagoner.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajoc.2022.101372.

References

1. Behnia M, Hatam-Nahavandi K, Hajialilo E, et al. Occurrence of acanthamoeba genotypes in wastewater samples in tehran, Iran. *Iran J Parasitol*. Oct-Dec 2017;12(4):516–521, 2017.
2. Booton GC, Joslin CE, Shoff M, Tu EY, Kelly DJ, Fuerst PA. Genotypic identification of Acanthamoeba sp. isolates associated with an outbreak of acanthamoeba keratitis. *Cornea*. Jul 2009;28(6):673–676. https://doi.org/10.1097/ICO.0b013e31819342a7.
3. Walochnik J, Scheikl U, Haller-Schober EM. Twenty years of acanthamoeba diagnostics in Austria. *J Eukaryot Microbiol*. Jan-Feb 2015;62(1):3–11. https://doi.org/10.1111/jeu.12149, 2015.
4. Lorenzo-Morales J, Lindo JF, Martinez E, et al. Pathogenic acanthamoeba strains from water sources in Jamaica, west indies. *Ann Trop Med Parasitol*. Dec 2005;99(8):751–758. https://doi.org/10.1179/136485905X65215.
5. Bagheri H, Shafiei R, Shafiei F, Sajjadi S. Isolation of acanthamoeba spp. from drinking waters in several hospitals of Iran. *Iran J Parasitol*. Jun 2010;5(2):19–25.
6. Schuster FL, Visvesvara GS. Opportunistic amoebae: challenges in prophylaxis and treatment. *Drug Resist Updates*. Feb 2004;7(1):41–51. https://doi.org/10.1016/j.drup.2004.01.002.
7. Davis MJ, Packo KH, Epstein RJ, Grostern RJ, Cohen JA. Acanthamoeba endophthalmitis following penetrating keratoplasty for Acanthamoeba keratitis. *Arch Ophthalmol*. Apr 2010;128(4):505–506. https://doi.org/10.1001/archophthalmol.2010.33.
8. Kitzmann AS, Goins KM, Sutphin JE, Wagoner MD. Keratoplasty for treatment of Acanthamoeba keratitis. *Ophthalmology*. May 2009;116(5):864–869. https://doi.org/10.1016/j.ophtha.2008.12.029.
9. Sacher BA, Wagoner MD, Goins KM, Sutphin JE, Greiner MA, Kitzmann AS. Treatment of acanthamoeba keratitis with intravenous pentamidine before therapeutic keratoplasty. *Cornea*. Jan 2015;34(1):49–53. https://doi.org/10.1097/ICO.0000000000000263.
10. Sarnicola E, Sarnicola C, Sabatino F, Tosi GM, Perri P, Sarnicola V. Early deep anterior lamellar keratoplasty (DALK) for acanthamoeba keratitis poorly responsive to medical treatment. *Cornea*. Jan 2016;35(1):1–5. https://doi.org/10.1097/ICO.0000000000000681.
11. Schaumberg DA, Snow KK, Dana MR. The epidemic of Acanthamoeba keratitis: where do we stand? *Cornea*. Jan 1998;17(1):3–10.
12. Cope JR, Collier SA, Rao MM, et al. Contact lens wearer demographics and risk behaviors for contact lens-related eye infections–United States, 2014. *MMWR Morb Mortal Wkly Rep*. Aug 2015;64(32):865–870.
13. Scruggs BA, Quist TS, Salinas JL, Greiner MA. Notes from the field: acanthamoeba keratitis cases - Iowa, 2002-2017. *MMWR Morb Mortal Wkly Rep*. May 2019;68(19):448–449. https://doi.org/10.15585/mmwr.mm6819a6.
14. Randag AC, van Rooij J, van Goor AT, et al. The rising incidence of Acanthamoeba keratitis: a 7-year nationwide survey and clinical assessment of risk factors and functional outcomes. *PLoS One*. 2019;14(9), e0222092. https://doi.org/10.1371/journal.pone.0222092.
15. Robaei D, Carnt N, Minassian DC, Dart JK. The impact of topical corticosteroid use before diagnosis on the outcome of Acanthamoeba keratitis. *Ophthalmology*. Jul 2014;121(7):1383–1388. https://doi.org/10.1016/j.ophtha.2014.01.031.
16. (CDC). CfDC. Acanthamoeba keratitis associated with contact lenses–United States. *MMWR Morb Mortal Wkly Rep*. Jun 1986;35(25):405–408.
17. Clarke DW, Niederkorn JY. The pathophysiology of Acanthamoeba keratitis. *Trends Parasitol*. Apr 2006;22(4):175–180. https://doi.org/10.1016/j.pt.2006.02.004.
18. Hargrave SL, McCulley JP, Husseini Z. Results of a trial of combined propamidine isethionate and neomycin therapy for Acanthamoeba keratitis. Brolene Study Group. *Ophthalmology*. May 1999;106(5):952–957. https://doi.org/10.1016/s0161-6420(99)00515-1.
19. Royer DJ, Echegaray-Mendez J, Lin L, et al. Complement and CD4. *Elife*. 08 15 2019. https://doi.org/10.7554/eLife.48378, 8doi:.
20. Mathers WD, Goldberg MA, Sutphin JE, Ditkoff JW, Folberg R. Coexistent Acanthamoeba keratitis and herpetic keratitis. *Arch Ophthalmol*. Jun 1997;115(6):714–718. https://doi.org/10.1001/archopht.1997.01100150716002.
21. Rumelt S, Cohen I, Rehany U. Spontaneous corneal graft ulcerative perforation due to mixed Acanthamoeba and herpes simplex keratitis: a clinicopathologic study. *Cornea*. Mar 2000;19(2):240–242. https://doi.org/10.1097/00003226-200003000-00021.
22. 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*. Apr 2006;113(4):538–547. https://doi.org/10.1016/j.ophtha.2005.12.022.
23. Muiño L, Rodrigo D, Villegas R, et al. Effectiveness of sampling methods employed for Acanthamoeba keratitis diagnosis by culture. *Int Ophthalmol*. Jul 2019;39(7):1451–1458. https://doi.org/10.1007/s10792-018-0958-3.
24. Höllhumer R, Keay L, Watson SL. Acanthamoeba keratitis in Australia: demographics, associated factors, presentation and outcomes: a 15-year case review. *Eye*. 2020;34(4):725–732. https://doi.org/10.1038/s41433-019-0589-6, 04.

25. Rodríguez-Zaragoza S. Ecology of free-living amoebae. *Crit Rev Microbiol*. 1994;20 (3):225–241. https://doi.org/10.3109/10408419409114556.
26. Nunes TE, Brazil NT, Fuentefria AM, Rott MB. Acanthamoeba and Fusarium interactions: a possible problem in keratitis. *Acta Trop*. May 2016;157:102–107. https://doi.org/10.1016/j.actatropica.2016.02.001.
27. Carnt N, Robaei D, Watson SL, Minassian DC, Dart JK. The impact of topical corticosteroids used in conjunction with antiamoebic therapy on the outcome of acanthamoeba keratitis. *Ophthalmology*. 2016;123(5):984–990. https://doi.org/10.1016/j.ophtha.2016.01.020, 05.