Acanthamoeba Keratitis

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9.1 Introduction

In Europe, *Acanthamoeba* keratitis (AK) mostly occurs in contact lens wearers. However, in China and India, it may also occur due to direct trauma in rural areas. *Acanthamoeba* keratitis is often misdiagnosed and treated as herpetic, bacterial, or mycotic keratitis, as clinical signs and symptoms may be similar to other kinds of keratitis. In addition, AK is a rare clinical entity. Therefore, diagnosis is often delayed and ophthalmologists tend to observe a heterogeneous and protracted clinical course. Nevertheless, an early diagnosis is essential for the success of the treatment [1, 2].

9.2 Physiology and Life Cycle

Acanthamoeba are ubiquitous, free-living protozoa, present in air, soil, dust, drinking water, and also seawater. There is a dormant resilient cyst and an infective trophozoite form. The so-called vegetative form or trophozoite has a size of 25–40 μm and it is fed from bacteria, algae, and yeasts. Enterobacteria are especially preferred through *Acanthamoeba* but some *Acanthamoeba* species house bacteria as endosymbionts [3].

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The double-walled cysts have a 13–20 μm size and survive antibiotics, low temperatures (for example, 15 months at −15 °C), high doses of UV-light, and γ-radiation. In case of adverse conditions, *Acanthamoeba* trophozoites form cysts which may survive over 24 years.

Acanthamoeba are classified through their rDNA-sequence-types (T1–T12) (Stothard). AK most often occurs through the T4 genotype. Nevertheless, AK due to genotypes T2, T3, T5, T6, T8, T9, T10, T11, and T15 has also been described [4–10].

9.3 Pathophysiology

In case of a corneal infection, *Acanthamoeba* are first attached to the corneal epithelial cells through the Mannose-binding Protein. This binding supports secretion of metalloproteinase, serin- and cysteinproteinase through *Acanthamoeba*, which results in cytotoxic effects on human corneal epithelial cells and keratocytes and supports deeper corneal penetration of *Acanthamoeba* [11–13]. *Acanthamoeba* may also migrate along corneal nerves and damage these [14–15].

9.4 Epidemiology, Risk Factors, and Prevention

The first reports on *Acanthamoeba* keratitis were published in the 70s [16–17]. With increasing use of contact lenses, AK incidence increased in the 80s [18–20] and it was 1/30,000 contact lens wearer in the 90s (Great Britain, Hong Kong) [21]. Nowadays about 5% of contact lens-associated keratitis is caused by *Acanthamoeba* [22–23].

Main risk factors are extended use of contact lenses (therefore, daily lenses have a lower risk) [24–26], use of contact lenses during bath, and cleaning them with tap water [27]. Additional risk factors are corneal surface damage, exposition to contaminated water, and low socioeconomic status [28–29]. A study has proven that only hydrogen-peroxide containing contact lens cleaners are effective against all *Acanthamoeba* strains [30].

9.4.1 Clinical Symptoms

In early stages of the disease, about 75–90% of all patients are misdiagnosed, as typical *Acanthamoeba* keratitis symptoms are difficult to associate [5, 9]. Analysis of the German *Acanthamoeba Keratitis Registry* has shown that in 47.6% herpetic, in 25.2% mycotic, and in 3.9% bacterial keratitis was erroneously diagnosed by ophthalmologist, in *Acanthamoeba* keratitis patients [31]. Patient had the correct AK diagnosis not before 2.8 ± 4.0 months (range 0–23 months) after appearence of the first clinical symptoms, in Germany from 2001 to 2011 [31]. In about 23% of the cases, a mixed infection with virus, bacteria, or fungi is present [2, 32, 33–35]. Clinical signs of *Acanthamoeba* keratitis are the following: [33–46].

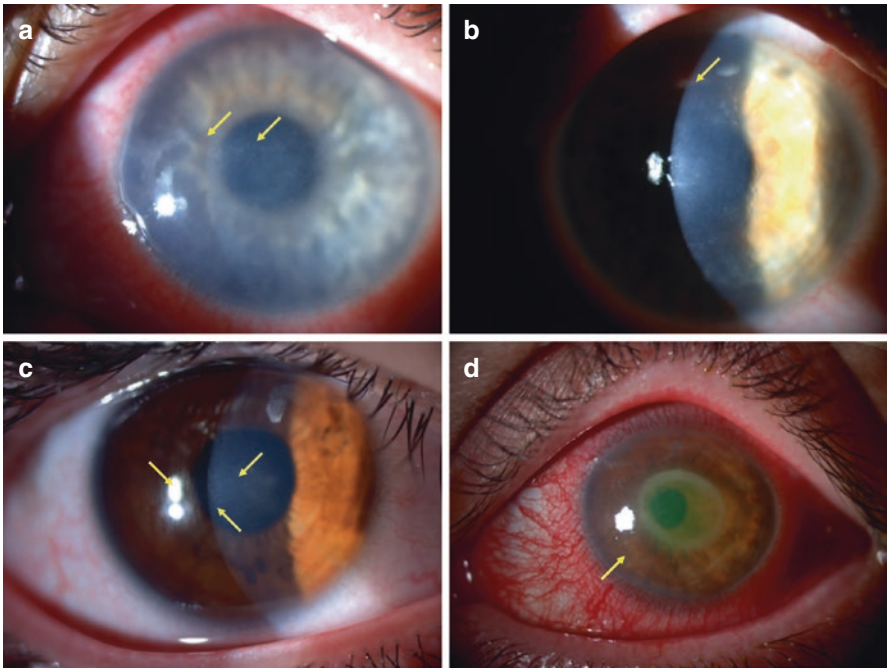

Fig. 9.1 Clinical signs in *Acanthamoeba* keratitis: (**a**) dirty epithelium, (**b**) multifocal stromal infiltrates, (**c**) *Acanthamoeba* dust, and (**d**) ring infiltrate with central epithelial defect

- Chameleon-like epithelial changes ("dirty epithelium," pseudodendritiformic epitheliopathy, epithelial microerosions, and microcysts) (Fig. 9.1a).
- Multifocal stromal infiltrates (Fig. 9.1b) or "dust-like" changes in the corneal stroma (Fig. 9.1c).
- Ring infiltrate ("Wessely immune ring") (Fig. 9.1d).
- Peripheral perineural infiltrate (Fig. 9.2).
- Common complications: broad-based anterior synechiae, secondary glaucoma, iris atrophy, mature cataract (Fig. 9.3), persistent epithelial defect.
- Rare complications: sterile anterior uveitis, scleritis (Fig. 9.3).
- Very rare complications: chorioretinitis and retinal vasculitis.

9.5 Diagnostics

- In case of clinical signs of *Acanthamoeba* keratitis, additional (laboratory) diagnostics always have to be performed. Confocal microscopy is used as in vivo diagnostics, in vitro diagnostics are polymerase chain reaction (PCR), histopathological examination, or microbiological culture [31, 32, 47–49]. The most important is to recognize clinical signs of *Acanthamoeba* keratitis, so that we use the appropriate diagnostic methods, as timely as possible.

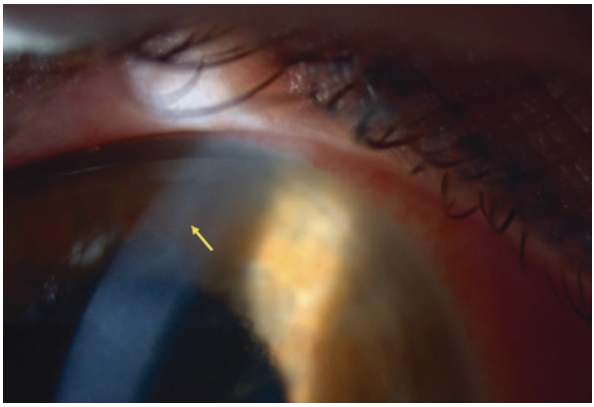

Fig. 9.2 Perineuritis in *Acanthamoeba* keratitis (arrow), 4 weeks after first symptoms (contact lens wearer)

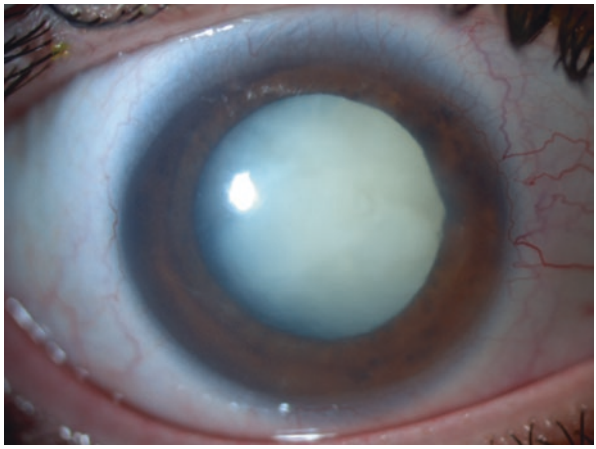

Fig. 9.3 Scleritis, persistent mydriasis, and mature cataract in severe *Acanthamoeba* keratitis

- Polymerase chain reaction (PCR) of corneal scrapings has with 84–100% the highest sensitivity and may give a result within 60 min [50–53].
- However, PCR may have the disadvantage that also not living *Acanthamoeba* genome may give a positive result [3].
- In vivo confocal microscopy has more than 90% sensitivity in experienced hands. However, only *Acanthamoeba* cysts are well recognized using this method [31, 48, 49].
- In vitro culture may have 0–70% sensitivity. This technique uses the fact that *Acanthamoeba* grows well on *Escherichia coli* (*E. coli*) and *Acanthamoeba* forms lines in an *E. coli* covered plate. This method has the disadvantage of giving results only within 3 weeks [54–56]. PCR or in vitro culture may also be used to analyze the contact lens case, which may add information to our diagnostics.
- Presence of *Acanthamoeba* may also be verified through *histopathological analysis*, with 31–65% sensitivity. Corneal scrapings or excision or explanted tissue from keratoplasty may be analyzed using periodic acid-Schiff, Masson, Gram, Giemsa, Grocott-methenamine-silver, or Calcofluor-white stainings. As sensitivity of this diagnostic method is lower than that of PCR, it is used rarely in clinical practice [33, 50, 57].

9.6 Differential Diagnosis

"Dirty epithelium" and pseudodendritiformic epitheliopathy have to be differentiated from an epithelial *herpetic* keratitis (dendritic or geographic). These do not have round spot-like widenings at the endings of the epithelial erosions, unlike herpetic epithelial keratitis.

In absence of bacterial or mycotic superinfection of an AK, the stromal infiltrates in AK are multifocal, dot-like (like unsharp-edged stromal stars), and in part transparent in an early stage of the disease. This may look like a "stromal dust," as it is mostly not accompanied by dense stromal infiltrates. In contrast, bacterial or mycotic stromal infiltrates are dense and typically monofocal and more whitish. Nevertheless, satellite infiltrates in fungal keratitis may imitate multifocal stromal infiltrates of AK.

The Wessely immune ring may be present in bacterial, myctotic, or *Acanthamoeba* keratitis.

9.7 Treatment

There are only case series on safety and effectivity of medical and surgical treatment of *Acanthamoeba* keratitis and there are up-to-date no completed randomized controlled clinical studies. Nevertheless, a first clinical trial is expected to be completed in 2021 (ClinicalTrials.gov Identifier: NCT03274895).

9.7.1 Conservative Treatment

9.7.1.1 Diamidine and Biguanide

Diamidines, such as propamidine-isethionate (Brolene), hexamidine-diisethionate (Hexacyl), and dibromopropamidine (Golden Eye) are used in 0.1% concentration [58–60]. Biguanides, such as polyhexamethylene-biguanide (polyhexanide) (Lavasept) and chlorhexidine (Curasept) are applied in 0.02% concentration [2]. Nevertheless, an actual clinical trial is analyzing the potential effect of 0.08% polyhexanide in AK (NCT03274895).

The concentration-dependent effect of diamidines and biguanides on human epithelial cells, keratocytes, and endothelial cells has already been described and propamidine-isethionate as diamidine and chlorhexidine as biguanide seem to be the least cytotoxic. However, these may reduce proliferation and migration of human corneal cells more than other diamidines and biguanides [61].

9.7.1.2 Antibiotics

Neomycin kills trophozoites, prevents bacterial superinfection [62], and reduces bacterial load, as food source for *Acanthamoeba* [2].

9.7.1.3 Povidone-Iodine and Miltefosine

An in vitro experiment reported on a better anticystic effect of 1% povidone-iodine as propamidine-isethionate or polyhexamid. However, up to now, clinical studies did not verify these results [63]. Miltefosine was effective against *Acanthamoeba* in vitro [64].

9.7.1.4 Steroids

Topical use of steroids may masquerade clinical signs of *Acanthamoeba* keratitis, as long as these are used. In addition, they support excystment and an increase in number of throphozoites. On contrary, a patient with *Acanthamoeba* keratitis and severe inflammation may benefit from their use. Steroids should never be used without additional topical antiseptics and should never be applied at the early stage of *Acanthamoeba* keratitis treatment (never in the first week even after appropriate diagnosis) [65, 66]. In case of abrupt stopping topical steroids, a Wessely immune ring may develop within 2 days in patients with AK [67].

9.7.1.5 Antifungals

Miconazole and Clotrimazole have been previously used as topical treatment of AK [68, 69]. In addition, there are reports on local and systemic voriconazole used in these patients [68–70]. An in vitro study described better anticystic effect using natamycin in contrast to propamidine-isethionate or polyhexamethylene-biguanide [63]. However, data on clinical use of natamycin in AK patients is not available.

In Germany, we suggest topical application of polyhexamethylene-biguanide, propamidine-isethionate, and Neomycin as triple-therapy in case of AK [2]. During the first two days a "surprise attack" or "flash war" is initiated with polyhexamethylene-biguanide and propamidine-isethionate every quarter to half an hour day and night. Then, until the sixth day, polyhexamethylene-biguanide and propamidine-isethionate are applied every hour and only over the day (6^{00}–24^{00}). The following 4 weeks eye-drop use is reduced to every 2 h. Additionally, neomycin five times a day is also applied [62]. In therapy-resistant cases, we may change polyhexamethylene-biguanide to chlorhexidine or increase concentration (for polyhexamethylene-biguanide to 0.06%, for chlorhexidine to 0.2%).

To the best of our knowledge, combination therapy using diamidine, biguanide, and antibiotics should be continued in descending doses until 1 year. However, in case of nonhealing epithelial defects after penetrating keratoplasty, we may reduce the use of diamidine and biguanide with 1 drop every 2 months.

In our opinion, a specific treatment, following isolation of the pathognomic *Acanthamoeba* strain should be clinically applied in the future, following in vitro culture and testing.

9.8 Surgical Treatment

Through diagnostic and therapeutic epithelial abrasion we remove microorganisms and get a better penetration of topical medication [71]. If topical conservative treatment does not improve clinical signs and symptoms, corneal cryotherapy, amniotic

membrane transplantation, or penetrating keratoplasty may be performed. In therapy-resistant cases, a cross linking treatment as photodynamic therapy may be used, in some cases repeatedly.

Corneal cryotherapy is an adjuvant treatment of topical therapy. The infected corneal areas or the recipient area before penetrating keratoplasty will be treated using a Cold Cryoprobe 2–3 times ("freeze-thaw-freeze") until ice crystals are formed in the corneal stroma [72]. As part of a penetrating keratoplasty, cryotherapy is circularly used (about 2–3 s at −80 °C to the recipient bed) before recipient trephination. The effect of this type of cryotherapy on limbal epithelial stem cells is up-to-date not clarified.

An Amniotic Membrane Transplantation (AMT) may be used, for persistent epithelial defects or ulcers as "Patch," "Graft," or "Sandwich" and may help to reach a quiet stage of the eye [73]. In many cases, AMT has to be repeated several times, to reach epithelial closure.

Photodynamic Therapy (PDT) may be an alternative treatment option in therapy-resistant infectious keratitis [74]. The successful use of Riboflavin-UVA cross-linking in Acanthamoeba keratitis has been summarized in a case series in 2011 [75]. Nevertheless, in case of stromal infiltrates, UVA-light penetration to the corneal stroma may be reduced. An accelerated cross linking in *Acanthamoeba* keratitis is not suggested as primary treatment [76].

In case of *Acanthamoeba* keratitis expansion in direction of the corneoscleral limbus, an early penetrating keratoplasty has to be considered, in order to perform the excision in uninfected corneal tissue. In case of progressive, therapy-resistant ulceration over weeks and months, with peripheral reparative neovascularization, we suggest an early (<5 months disease course) penetrating keratoplasty [77] (Figs. 9.4 and 9.5). The origin of frequent therapy-resistant epithelial defects at the transplanted tissue after PKP, is not clarified, yet. Potential treatment options of these epithelial defects are (1) autologous serum, (2) AMT, (3) Cacicol, or (4) Neurotrophic Growth Factor (NGF).

Following penetrating keratoplasty, we continue the use of the above described topical treatment for up to 1 year [2, 78]. However, there are also no controlled

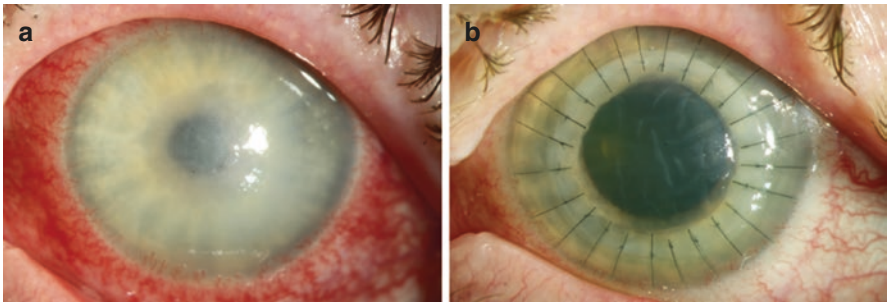

Fig. 9.4 (**a**) Multifocal stromal infiltrates (interstitial keratitis) in *Acanthamoeba* keratitis and (**b**) 2 days after central excimer laser penetrating keratoplasty with interrupted sutures

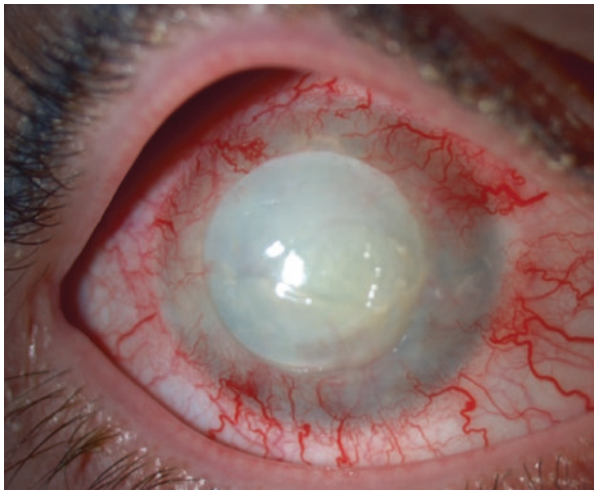

Fig. 9.5 Central, nonhealing epithelial defect, scleritis, iris atrophy, and mature cataract 14 months after penetrating keratoplasty in *Acanthamoeba* keratitis. There is no light perception. PKP has been performed 10 months after first symptoms of the contact lens-associated disease

clinical trials related to this topic. Perhaps local therapy may be stopped earlier, in order to avoid persistent epithelial defects, peripheral anterior synechiae, and mature cataract. Confocal microscopy may be useful in diagnosis of AK recurrences [32].

In case of perforated corneal ulcers, a nonmechanical, excimer laser keratoplasty is best performed [78]. Using an elliptical excimer laser trephination with metal masks, we may remove the infected corneal area with a more homogeneous distance from the limbal vessels, especially in typically elliptical-shaped acanthamoeba keratitis [79].

Some authors suggest at least a 3-month-long observation period without inflammatory signs, following discontinuation of conservative therapy, before planning an elective penetrating keratoplasty, following AK. In such elective PKPs, transplant survival may be 100% following 5 years and 67% after 10 years [80–82].

9.9 Conclusion

In summary, *Acanthamoeba* keratitis presents in early stages with grey-dirty epithelium, pseudodendritiformic epitheliopathy, perineuritis, multifocal stromal infiltrates, ring infiltrates and in later stages with scleritis, iris atrophy, anterior synechiae, secondary glaucoma, mature cataract, and chorioretinitis. As conservative treatment, we use up to 1 year triple-topical therapy (polyhexamethylene-biguanide, propamidine-isethionate, neomycin). In therapy-resistant cases, surgical treatment options such as corneal cryotherapy, amniotic membrane transplantation, riboflavin-UVA cross linking, and penetrating keratoplasty may be applied.

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