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Reactive uveitis, retinal vasculitis and scleritis as ocular end-stage of *Acanthamoeba* keratitis – a histological study

Running title: Uveitis and vasculitis of *Acanthamoeba* keratitis

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Abstract

We analysed histologically two *Acanthamoeba* keratitis eyes with anterior and posterior segment inflammation and blindness. Two enucleated eyes of 2 patients (age 45 and 51 years) with *Acanthamoeba* keratitis (PCR of epithelial abrasion positive) were analysed. Histological analysis was performed using haematoxylin-eosin, periodic acid-Schiff and Gömöri-methenamine silver staining. We could not observe *Acanthamoeba* trophozoites or cysts neither in the cornea nor in other ocular tissues. Meanwhile, we found uveitis, retinal vasculitis and scleritis in these eyes, due to the long-standing, recalcitrant *Acanthamoeba* keratitis. So in this stage of *Acanthamoeba* keratitis, systemic immune suppression may be necessary for a longer time period.

KEY WORDS: *Acanthamoeba* keratitis, enucleation, uveitis, retinal vasculitis, scleritis

INTRODUCTION

Acanthamoeba keratitis (AK) is a progressive, sight-threatening disease, occurring mostly in contact lens wearers. Its annual incidence was 17.53 to 21.14 per one million contact lens wearers in the UK ^[1]. In Germany, with about 80 million inhabitants, about 150 new cases have been reported in a 10-year-period ^[2].

Studies showed that 68%-92.3% of AK patients are contact lens wearers. ^[1, 3, 4] Expression of mannosylated glycoproteins on corneal epithelial cell surface is upregulated in contact lens wearers ^[3]. This plays an important role in AK pathogenesis. The *Acanthamoeba* trophozoite binds to these proteins through its mannose-binding site in order to release the so-called mannose-induced protease 133 (MIP-133) and *Acanthamoeba* plasminogen activator (aPA). MIP-133 and aPA give rise to lysis of epithelial, stromal cells and stromal matrix, leading to corneal erosions and ulceration ^[4]. Presence of bacteria or fungi also supports *Acanthamoeba* growth, often resulting in co-infection ^[5]. Although contact lens wear is considered as a risk of AK development, most interestingly, not each contact lens wearer tends to develop AK, implying that the individual immune response may play a crucial role. In many aspects, the immunology of *Acanthamoeba* keratitis needs further research to better understand its pathogenesis and to find potential intervention points to prohibit its development and optimize the human immune response ^[6-11].

Acanthamoeba keratitis patients at the early stage of the disease suffer from tearing and ocular pain. At this

time-point, the ophthalmologists observe a relative mild ophthalmological status, compared to the pronounced discomfort of the patient. A pseudodendritiform epitheliopathy, “dirty epithelium”, typically spot-like multifocal stromal infiltrates and radial perineuritis can be observed at this stage. Some days later, a Wessely immune ring around the infected area is observed. In case of bacterial or mycotic coinfection, a dense stromal infiltrate and hypopyon may also be present. In later stages secondary glaucoma, iris atrophy, mature cataract, scleritis and chorioretinitis may occur.

Until now, there is no standardized treatment of AK and there is no topical or systemic drug which could explicitly eliminate *Acanthamoeba* cysts from the human cornea. Topically, diamidines, biguanides and neomycin are most often used. In some cases penetrating keratoplasty, amniotic membrane transplantation and crosslinking treatment are applied as surgical therapy, but the removal of the eye through enucleation may also be necessary.^[12]

The purpose of this study was to histologically analyze two *Acanthamoeba* keratitis eyes with anterior and posterior segment inflammation and blindness.

SUBJECTS AND METHODS

Ethical Approval

This retrospective study was performed in accordance with the Declaration of Helsinki Guidelines for Human Research and the Health Insurance Portability and Accountability Act. The research project was approved by the Ethics Committee of Saarland (Number 213/18).

Patient History

We performed a retrospective record review between January 2006 and December 2017, at the Department of Ophthalmology of Saarland University Medical Center, Homburg/Saar searching for patients with the diagnosis of *Acanthamoeba* keratitis (polymerase-chain reaction (PCR) positive) and subsequent enucleation. During this time period, there were 30 PCR positive AK patients and 2 of them underwent enucleation.

These two patients were both contact lens wearers and their clinical history is described below. In these two eyes of 2 female patients (age 45 and 51 years) PCR of epithelial abrasion confirmed the clinical diagnosis of *Acanthamoeba* keratitis (time to diagnosis after first symptoms 2 weeks and 3 months). These cases had been treated previously as herpetic or herpetic/bacterial keratitis in another hospital, respectively. There was no evidence of previous or subsequent systemic disease in any of the patients. Best corrected visual acuity at the time of diagnosis was 0.2 and 0.05 and clinical signs of AK were dirty epithelium and multifocal stromal infiltrates (Figure 1A) in the first and corneal ulcer, ring infiltrate, keratic precipitates, hypopyon, intrastromal bleeding and posterior synechiae in the second eye (Figure 2A).

Up-to date, there is no specific treatment for the *acathamoeba* isolates, causing keratitis. However, in Germany, mainly triple-topical therapy (polyhexamethilen-biguanide, propamidin-isethionat and neomycin) is used. Both patients underwent triple-topical therapy and with failed recovery (2 and 5 months after first AK symptoms and with continuous triple-topical therapy), surgical therapies followed. Before surgery, during continuous triple therapy there were persisting epithelial defects in both patients, with the size of about 4x5mms and 7x8mms. Repeat iatrogenic epithelial removals were not performed. Although persisting epithelial defects may also be related to the toxicity of the used triple-therapy itself, we interpreted their presence as lack of success with antiamoebic therapy and performed surgery.

The first patient received crosslinking (CXL) with amniotic membrane transplantation as patch (2 months after

first symptoms. All amniotic membranes have been prepared in our eye bank and were used following cold storage (-80 °C), for both patients. Corneal cryotherapy with PKP was performed 3 months (8.0/8.1 mm excimer laser trephination) (Figure 1B) after initiation of keratitis. One month later, repeat PKP in combination with phacoemulsification and posterior chamber lens implantation and amniotic membrane transplantation as patch has been performed (triple-procedure, 10.0/10.5 mm hand-held trephination, repeat PKP for AK recurrence and host calcification, along the interface) (Figures 1C-D). Histological analysis of both explanted corneal tissues (PKP and repeat PKP) revealed presence of trophozoites and cysts, verifying unsuccessful previous triple-therapy.

The second patient underwent CXL, subsequent corneal cryotherapy with PKP and amniotic membrane transplantation as patch (7.5/7.6 mm excimer laser trephination) 5 months (Figure 2B-C) after first symptoms. Two months later, she had phacoemulsification with posterior chamber lens implantation and repeat PKP (8.0/8.1 mm excimer laser trephination, for non-healing epithelial defects). Histological analysis of both explanted corneal tissues (PKP and repeat PKP) revealed presence of trophozoites and cysts, also referring to failed previous triple-therapy. Thereafter, with non-healing epithelial defects, amniotic membrane transplantations as patch were performed 5 times.

Beside our “standard” systemic immune modulatory treatment after PKPs (250-150-150-125-125-100-100-80-80-64-64-32-32-16-16-8-8 mgs methylprednisolon), no additional immune suppression have been used after keratoplasties. We took this decision, as at this time-point, our patients did not show signs of severe anterior and posterior segment inflammation or corneal vascularization.

Following PKPs, best corrected visual acuity was hand movement and 0.1. Triple-topical therapy was continued 5x daily with additional prednisolone-acetate eye drops 5x daily. However, the epithelial defects further did not heal and the patients developed secondary glaucoma 3 and 6 months after presentation of first AK symptoms, which was successfully cured with conservative therapy. This was followed by central artery occlusion (CRAO) in the first patient 5 months and with central vein occlusion (CRVO) in the second patient 6 months after first AK symptoms. CRAO and CRVO were diagnosed through fundus examination. Fluorescein angiography could not give us additional information through the deepithelialized and oedematous transplanted corneas.

The first patient ended up with ciliary body, choroid and retinal detachment 11 months after first keratitis symptoms and, therefore, sclerotomy, pars plana vitrectomy with silicon oil implantation was performed. The second patient, with subsequent central retinal vein occlusion, received intravitreal Bevacizumab 9 and 10 months after first AK symptoms.

Ocular hypotony became obvious in both patients 11 and 9 months, respectively, after the first AK symptoms (Figure 1E-F and Figure 2D). A “filamentous, spider-net-like” inflammatory reaction was detected in the anterior chamber of the second patient simultaneously with ocular hypotony, 9 months after first AK symptoms (Figure 2D).

Three months after repeat PKP, both patients had no light perception (7 and 12 months after the first symptoms of AK) and subsequently, also due to pain, the inflamed blind eyes were enucleated (13 months after the first symptoms of AK in both patients).

Histological Analysis

Histological analysis of the enucleated eyes was performed at the Department of Pathology of Saarland University, Homburg/Saar, Germany and at the Department of Ophthalmology of the Friedrich-Alexander

University of Erlangen-Nürnberg, Erlangen, Germany.

After formaline-fixation and paraffin wax-embedding of the patients' enucleated eyes, 3 µm thickness sections were cut using a standard microtome and transferred onto microscope slides (SuperFrost, Menzel-Gläser, Braunschweig, Germany). We performed serial sections anteroposteriorly (parallel to the optical axis) and cross-sections of the optic nerves. The slides were dried at 37 °C overnight. Standard haematoxylin–eosin, periodic acid Schiff (PAS) and Gömöri-methenamine silver (GMS) stainings were then performed. Using PAS and GMS stainings, we analyzed presence/absence of trophozoites or cysts in the enucleated eyes. With GMS, we also aimed to determine presence/absence of a mycotic infection of the enucleated eyes. Further on we will name the first patient's eye globe as „first case”, and the second patient's eye globe as „second case”.



Figure 1. Images of the first case. *Acanthamoeba* keratitis with “dirty epithelium” and multifocal stromal infiltrates (A), after first PKP (B), recurrence of AK and calcification of recipient along interface (arrows)(C), repeat PKP with amniotic membrane transplantation as patch (D) and with ocular hypotony, retinal and choroidal detachment (E).



Figure 2. Images of the second case. *Acanthamoeba* keratitis with corneal ulcer, ring infiltrate, intrastromal bleeding and posterior synechiae (A), after first PKP and amniotic membrane transplantation as patch (B), with mature cataract (C) and with “filamentous, spider-net-like” inflammatory reaction in the anterior chamber (D).

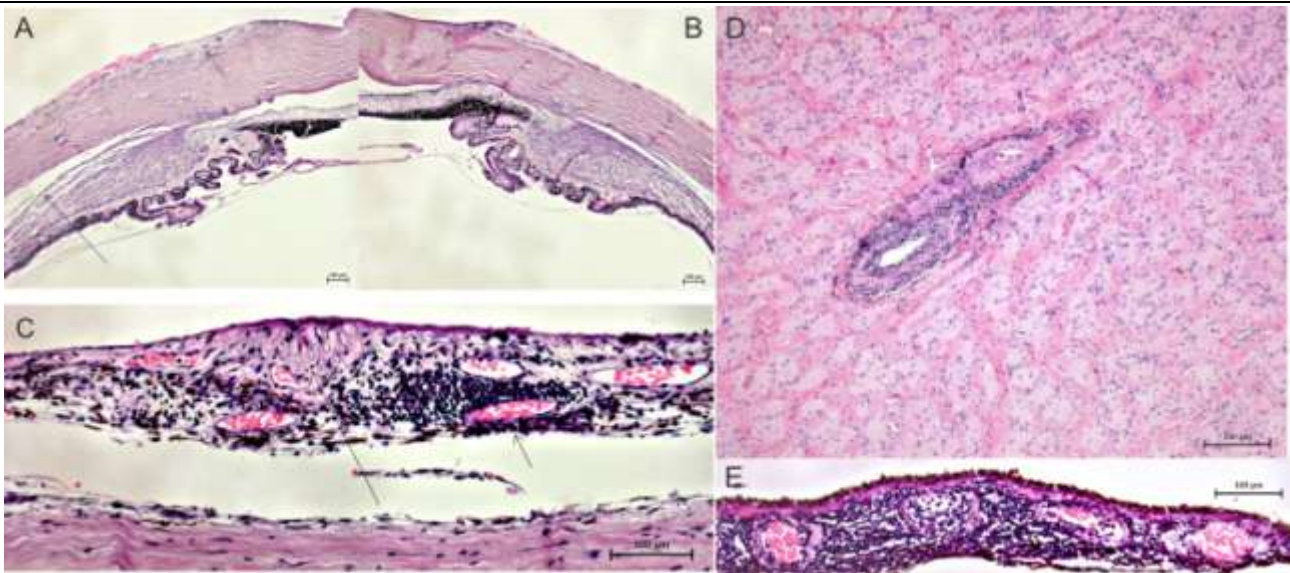


Figure 3. Histological images of both cases. In the first case (A and B, haematoxylin-eosin), *Acanthamoeba* trophozoites or cysts were not detectable in the corneal or other ocular tissues, but lymphocytic infiltration of the episclera (arrow) and choroidal detachment (long arrow) were shown. In the first case (C, haematoxylin-eosin), perivascular lymphocytic infiltration (arrow) and retinal atrophy (long-arrow) and perivascular lymphocytic infiltration around central retinal artery was detectable (D, haematoxylin-eosin). In the second case, there was lymphocytic infiltration of the choroid (E, haematoxylin-eosin).

RESULTS

Images of the histological analysis are shown at figure 3.

There was no central corneal epithelium on the analysed globes. We could not observe *Acanthamoeba* trophozoites or cysts neither in the cornea (Figures 3 A and B) nor in other ocular tissues. There was one corneal endothelial cell per field of view (original magnification 40x) analyzing the first and no corneal endothelial cells examining the second case.

There were anterior synechiae in the chamber angle of both cases and lymphocytic infiltration around the central retinal artery and vein, associated with fibrous metaplasia of the retinal pigment epithelium (Figures 3 C-E).

Additionally, we observed perivascular inflammatory cell infiltration (mainly lymphocytes) in the episclera and around ciliary nerves, analysing the first case (Figure 3A). This was associated with non-granulomatous uveitis, ciliosthisis and tractional retinal detachment. Cross sections of the optic nerve revealed gliosis and optic nerve atrophy.

Histopathologic studies of the second case revealed a multifocal, non-granulomatous choroiditis with lymphocytic infiltration (Figure 3E).

DISCUSSION

In 2007, Awwad et al.^[13] reported chronic chorioretinal inflammation with perivascular lymphocytic infiltration and diffuse neuroretinal ischemia as a new potentially blinding syndrome in 4 of 5 enucleated eyes after AK. In 4 of these patients, there were *Acanthamoeba* cysts in the cornea. Nevertheless, the posterior segment of the eye failed to demonstrate *Acanthamoeba* cysts or throphozoites. Burke et al.^[14] had reported similar results in one

patient in 1992.

Most interestingly, we observed episcleritis, non-granulomatous uveitis with choroidal and central retinal artery/vein lymphocytic infiltration (vasculitis) and neuroretinal degeneration, without presence of *Acanthamoeba* trophozoites or cysts in the cornea or other ocular tissues, in two enucleated eyes of two patients.

Extracorneal invasion of *Acanthamoeba* had only been described in 8 patients between 1975 and 2013, in the literature. In three of these cases, scleral invasion and in 5 others *Acanthamoeba* sclerokeratitis have been described. Iovieno et al. ^[15] reported 18.5% occurrence of sclerokeratitis in their case series with presence of degraded necrotic cysts in scleral nodule biopsy of these patients. They considered sclerokeratitis as a T-cell-mediated immune response, which requires systemic immunosuppression ^[10, 16]. *Acanthamoeba* antigens elicit an immune response that leads to generation of T cell clones. These T cell clones then cross-react with antigens expressed in the normal eye, which may lead to the generation of additional T cell clones by a process called “epitope spreading” ^[17].

We hypothesize that lymphocytes are more efficient than neutrophils and macrophages to chemoattract *Acanthamoeba*. But on the other hand, it can induce an immune response, which may also destroy other structures of the eye.

Lee ^[18] has reported, that corneal antigen presenting cells can reside in the central cornea, migrate to the cervical lymph nodes and activate T-cells. These T-cells then trigger an inflammatory reaction in the vascularized ocular tissues, such as uvea and retina. Interestingly, Johns et al. ^[19] reported on chorioretinitis without vitritis in the contralateral eye of an immunocompetent AK patient, which might have been a regional immune-related inflammation, induced by local tissue infection through *Acanthamoeba*.

There is another hypothesis that *Acanthamoeba* may induce a state of autoimmunity through molecular mimicry via corneal antigen presenting cells or a type III immune reaction, which may target vascular receptors leading to vasculitis and thrombosis.

In our cases, there was retinal artery occlusion in one patient and retinal vein occlusion in the second patient, before enucleation. Histopathological examination found lymphocytic infiltration of these vessels. This may indicate a possible local immune-mediated vasculitis with secondary thrombosis and occlusion. We hypothesize that the peripheral vasculitis might be rather related to reactive inflammation than to the *Acanthamoeba* itself. This could have happened similarly in three patients reported by Awwad et al. ^[13] and Burke et al. ^[14, 18]. In our two patients, conservative and surgical treatment even could have been successful. However, the immune reaction to the *Acanthamoeba* seemed to generate an ocular inflammatory disease leading to blindness.

Interestingly, necrotizing vasculitis, leukocytoclastic vasculitis, thrombosis of small vessels and thrombo-occlusive vasculitis have also been described in systemic *Acanthamoeba*-related diseases, such as cutaneous *Acanthamoeba* infections and *Acanthamoeba* encephalitis.

There are only 4 case reports on *Acanthamoeba* in the posterior part of the eye. Jones et al. ^[20] described a case in a 7-year-old boy with meningoencephalitis, with trophozoites in the ciliary body. Heffler et al. ^[21] reported on *Acanthamoeba* cysts in the aqueous humor and in the vitreous in a patient with acquired immune deficiency syndrome. In both patients, chorioiditis and retinal vasculitis were present. Moshari et al. ^[22] found *Acanthamoeba* cysts and trophozoites in the human retina, without chronic choroidal and retinal perivascular inflammation. Mammo et al ^[23] report a recurrent *Acanthamoeba* infection presenting initially as keratitis, followed by retinitis and histopathology-confirmed endophthalmitis.

Interestingly, Clarke et al. [24] showed that the clearance of the anterior chamber happens within 15 days following injection of *Acanthamoeba* trophozoites to the anterior chamber of hamster eyes. This was supported through a robust neutrophilic reaction in these eyes. This also supports the hypothesis, that choroid and retinal inflammation is rather immune-mediated and not related to the presence of the *Acanthamoeba*. However, there might be a difference in human and animal immune response.

Iovieno et al. described [15], that in case of AK-related mild scleritis/limbitis, treatment with topical steroids and oral NSAIDs may be sufficient. However, moderate/severe scleritis requires systemic immunosuppressive therapy (cyclosporine or mycophenolat-mophetil) over months (about 7 months) [16]. Monitoring scleritic pain may help to decide on the length of the immunosuppressive treatment [16]. *Acanthamoeba* sclerokeratitis is associated with poor clinical outcomes, but management of ASK with anti-inflammatory/immunosuppressive treatment is usually effective in reducing scleral inflammation and symptoms and the number of enucleations [15, 16].

Previous studies have shown that polyhexamethilen-biguanide and propamidin-isethionat may be cytotoxic for human corneal cells in clinically relevant concentrations. [25] It has also been suggested, that posterior segment inflammation may be related to toxicity of topical treatment used in AK, however, previous studies also reported AK patients with long-lasting topical treatment and absence of posterior pole inflammation, which contradicts this theory. Nevertheless, mature cataract formation in both patients could be related to toxicity of biguanides. These can then disrupt the lens surface, provoke lenticular oxidative or osmotic stress, and contribute to cataract formation by altering lipid membranes, damaging lens fibers, and inducing electrolyte imbalance. [26, 27]

In our study, enucleation was performed at the end of patient histories with repeat (intraocular) surgeries. The most conspicuous finding of the histological analysis is that there were no trophozoites or cysts in both enucleated eyes. Although there were *Acanthamoeba* trophozoites and cysts in the explanted corneal buttons of PKPs and repeat PKPs previously, these were not persisting in corneal and ocular tissues subsequently. However, intraocular inflammation with central retinal artery/vein occlusion developed. Therefore, we hypothesize that *Acanthamoeba* or the long lasting triple-therapy triggered an immune response, which was persisting without microorganisms.

In case of uveitis or retinal vasculitis in AK patients, a systemic immune-suppression for a longer period of time should be initiated in order to avoid the potentially blinding syndrome of the posterior part of the eye, most probably related to immune-mediated processes.

CONCLUSION

In summary, in long-standing, recalcitrant *Acanthamoeba* keratitis, uveitis, retinal vasculitis and scleritis may occur and result in blindness, even without further persistence of *Acanthamoeba* trophozoites or cysts. The etiology of these inflammatory complications is unclear, but may be explained with molecular mimicry or type III immune-reaction. Therefore, in late stage of *Acanthamoeba* keratitis, systemic immune suppression may be necessary for a longer period of time.

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