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

Lei Shi
Department of Ophthalmology, Saarland University Medical Center and Department of Ophthalmology, The First Affiliated Hospital of University of Science and Technology of China https://orcid.org/0000-0001-5105-8374

Tobias Hager T
Department of Ophthalmology, Saarland University Medical Center

Fabian Norbert Fries
Department of Ophthalmology, Saarland University Medical Center

Loay Daas
Department of Ophthalmology, Saarland University Medical Center

Leonard Holbach
Department of Ophthalmology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

Carmen Hofmann-Rummelt
Department of Ophthalmology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

Elena Zemova
Department of Ophthalmology, Saarland University Medical Center

Berthold Seitz
Department of Ophthalmology, Saarland University Medical Center

Nóra Szentmáry
Department of Ophthalmology, Saarland University Medical Center and Department of Ophthalmology, Semmelweis University

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Abstract

Background: Histological analysis of two Acanthamoeba keratitis eyes with anterior and posterior segment inflammation and blindness. Methods: Two eyes of 2 patients (age 45 and 51 years) with acanthamoeba keratitis (PCR of epithelial abrasion positive) were analysed. Patients underwent triple-topical therapy (polyhexamethilen-biguanide, propamidin-isethionat and neomycin) with failed recovery, subsequent crosslinking, corneal cryotherapy, repeat penetrating keratoplasties, amniotic membrane transplantations and phacoemulsification with posterior chamber lens implantation. The patients developed ocular hypotony with central vein/artery occlusion, retinal/choroidal detachment and had no light perception, therefore, the inflamed eyes were enucleated. Histological analysis was performed using haematoxilin-eosin, periodic acid- Schiff and Gömöri-methenamine silver staining. Results: We could not observe acanthamoeba trophozoites or cysts neither in the cornea nor in other ocular tissues. Anterior synechiae was detected in the chamber angle of both globes and lymphocytic infiltration was observed around central retinal artery and vein, associated with fibrous metaplasia of the retinal pigment epithelium. We found perivascular inflammatory cell infiltration (mainly lymphocytes) in the episclera and around ciliary nerves, when analysing the first globe. This was associated with non-granulomatous uveitis, cilioschisis and tractional retinal detachment. Cross sections of the optic nerve revealed gliosis and optic nerve atrophy. Histopathologic studies of the second globe revealed a multifocal, non-granulomatous choroiditis with lymphocytic infiltration. Conclusions: In long-standing, recalcitrant acanthamoeba keratitis, uveititis, retinal vasculitis and scleritis may occur and result in blindness, even without further persistence of acanthamoeba trophozoites or cysts. In this stage of acanthamoeba keratitis, systemic immune suppression may be necessary for a longer time period.

Background

Acanthamoeba keratitis (AK) is a progressive, sight-threatening disease, occurring mostly in contact lens wearers. It is reported about 33 cases within one million contact lens wearers in the United States, which means an occurrence of about 150 new cases there, per year [1, 2, 3]. In Germany, with about 80 million inhabitants, about 150 new cases have been reported in a 10-year-period [4].

Expression of mannosilated glycoproteins on corneal epithelial cell surface is upregulated in contact lens wearers [5]. This plays an important role in AK pathogenesis. The acanthamoeba throphozoite binds to these proteins though 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 [6]. Presence of bacteria or fungi also supports acanthamoeba growth, often resulting in co-infection [7].

In case of inappropriate contact lens hygiene, AK may occur. However, most interestingly, not each contact lens wearer tends to develop AK, implying the individual immune response may play a crucial role.

Through mucosal immune system, IgA is produced in human tears, which prohibits acanthamoeba trophozoite binding to the ocular surface. Some contact lens wearers do not have this mucosal immune response and, therefore, have higher risk to develop AK [8,9]. This suggests that acanthamoeba patients may have an immunological “blind-spot”, which prevents them from mounting an effective immune response to acanthamoeba antigens.

About 90-100% of the adult populations have blood antibodies against acanthamoeba antigens, but the adaptive immune system cannot eradicate acanthamoeba trophzoites and cysts. Its activation through acanthamoeba increases IL-17 protein expression, which leads to neutrophil activation and migration and only moderate symptoms of AK [10]. 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.

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 pseudodendritiformic 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 trophozoites and 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.

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

Methods

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.

This retrospective study was performed in accordance with the Declaration of Helsinki Guidelines for Human Research and the Health Insurance Portability and Accountability Act.

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, 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).

The patients underwent triple-topical therapy (polyhexamethilen-biguanide, propamidin-isethionat and neomycin) and with failed recovery (2 and 5 months after first AK symptoms), surgical therapies followed.

The first patient received crosslinking (CXL) with amniotic membrane transplantation as patch 2 months, corneal cryotherapy with PKP 3 months (8.0/8.1 mm excimer laser trephination) (Figure 1B) and repeat PKP in combination with phacoemulsification and posterior chamber lens implantation and amniotic membrane transplantation as patch 4 months (triple-procedure, 10.0/10.5 mm hand-held trephination, repeat PKP for AK recurrence and host calcification, along the interface) (Figures 1C-D), after first symptoms of AK.

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), phacoemulsification with posterior chamber lens implantation 7 months and repeat PKP (8.0/8.1 mm excimer laser trephination, for non-healing epithelial defects) 9 months after presentation of the first AK symptoms. Thereafter, with non-healing epithelial defects, amniotic membrane transplantations as patch were performed 5 times.

Following PKPs, best corrected visual acuity was hand movement and 0.1 and 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 in the first patient 5 months and with central vein occlusion in the second patient 6 months after first AK symptoms.

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 none of the patients had light perception (7 and 12 months after the first symptoms of AK) and subsequently, 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/abcence of a mycotic infection of the enucleated eyes. Further on we will name the first patient's eye globe „first globe", and the second patient's eye globe „second globe".

Results

Images of the histological analysis are shown at figures 3-7.

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 globe.

There were anterior synechiae in the chamber angle of both globes and lymphocytic infiltration around the central retinal artery and vein, associated with fibrous metaplasia of the retinal pigment epithelium (Figures 4-7).

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

Histopathologic studies of the second globe revealed a multifocal, non-granulomatous choroiditis with lymphocytic infiltration (Figure 6).

Discussion

Discussion

In 2007, Awwad et al. [11] 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. [12] 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 throphozoites 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.[13] 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 [14,15]. 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” [16,17].

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

Hamrah [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 at 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. [11] and Burke at al. [12].

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 three 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 throphozoites 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, chrorioiditis and retinal vasculitis were present. Moshari et al. [22] found Acanthamoeba cysts and throphozoites in the human retina, without chronic choroidal and retinal perivascular inflammation.

Interestingly, Clarke et al. [23] 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 an animal immune response.

Previous studies have shown that polyhexamethilen-biguanide and propamidin-isethionat may be cytotoxic for human corneal cells in clinically relevent concentrations [24]. 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 [25,26].

In our opinion, 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.

Declarations

Acknowledgments There are none to acknowledge.

Funding None to declare.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The investigation was performed according to the tenets of the Declaration of Helsinki. The Ethics Committee of the Medical Association of Saarland gave permission for the study

Authors' contributions

LS-Design, drafting, data acquisition, analysis, and interpretation. TH-Design drafting, data acquisition, analysis, and interpretation. FNF-Drafting, data acquisition, analysis, and interpretation. LD-Design, drafting, and analysis. LH- Design, data acquisition, and interpretation. CHR-Drafting, data acquisition, and analysis. EZ-Drafting, and data acquisition. BS-Design drafting, data acquisition, analysis, and interpretation. NS-Design drafting, data acquisition, analysis, and interpretation. All authors read and approved the final manuscript.

Consent for publication Both patients signed consents for publication.

Competing interests

The authors declare that they have no competing interests.

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Figures

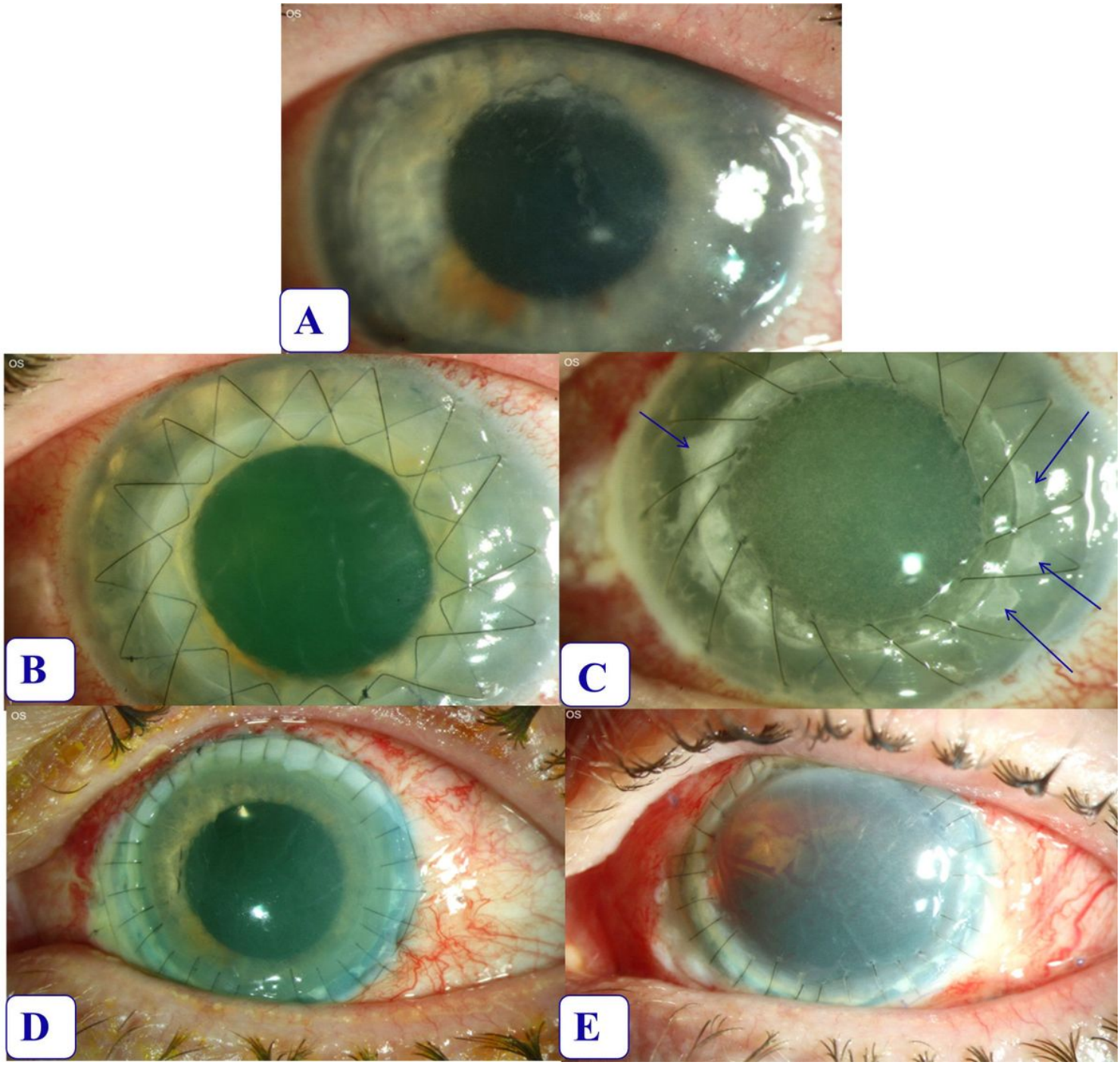

Figure 1

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

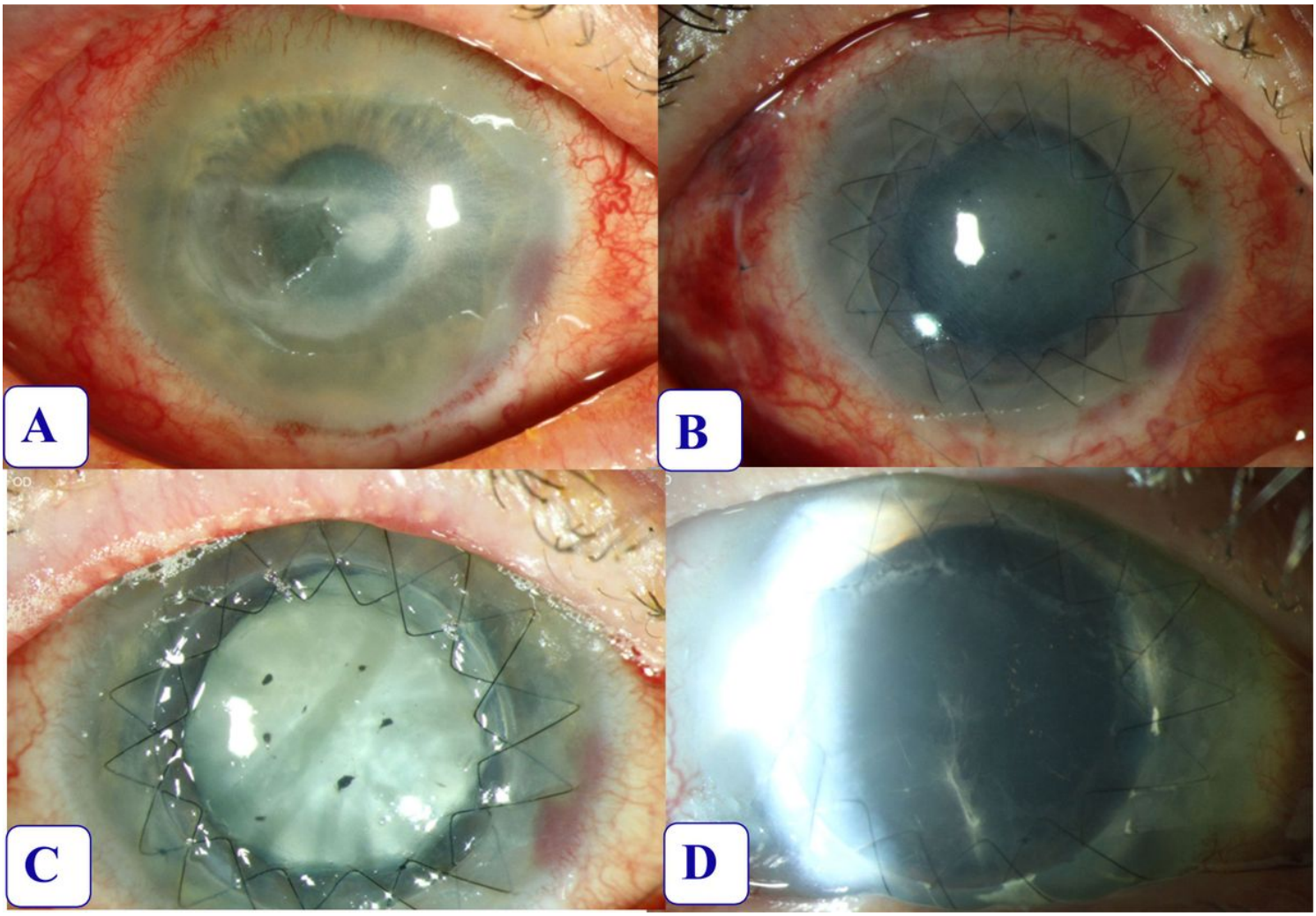

Figure 2

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).

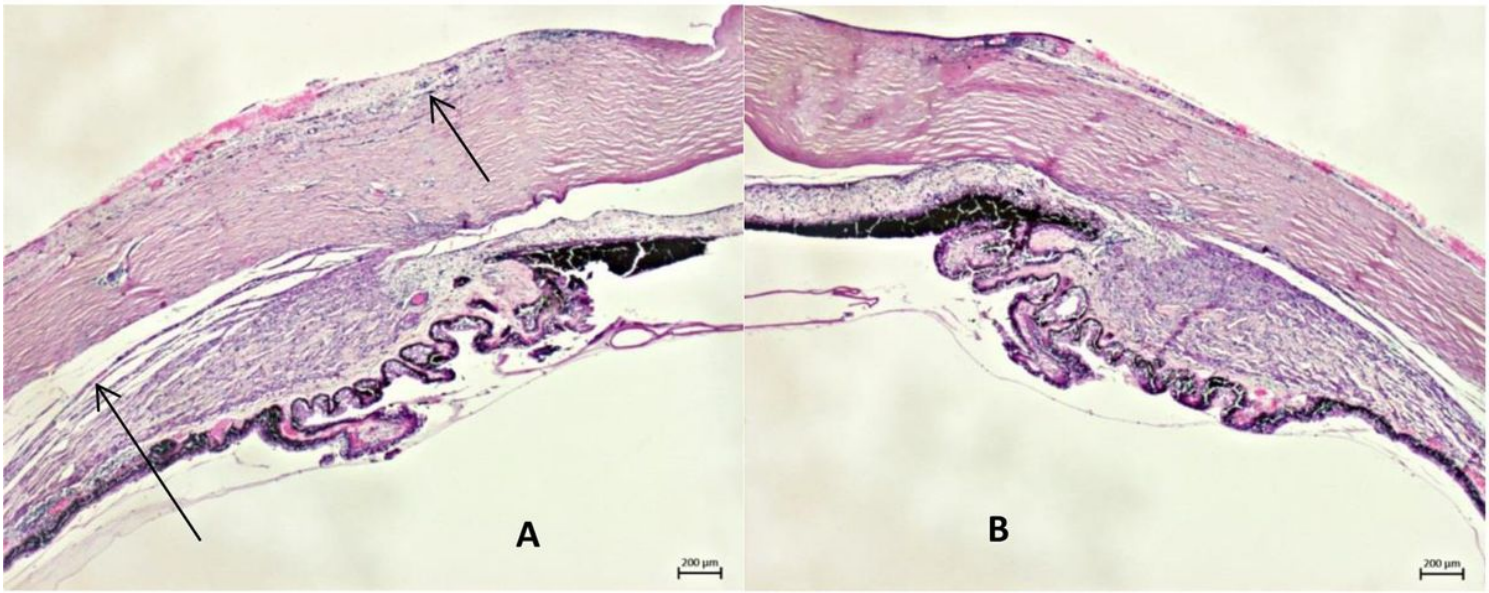

Figure 3

A and B Acanthamoeba trophozoites or cysts were not detectable in the corneal or other ocular tissues. Lymphocytic infiltration in the epi-sclera (arrow) and a choroidal detachment (long arrow) were present in sections of the first globe (haematoxylin-eosin).

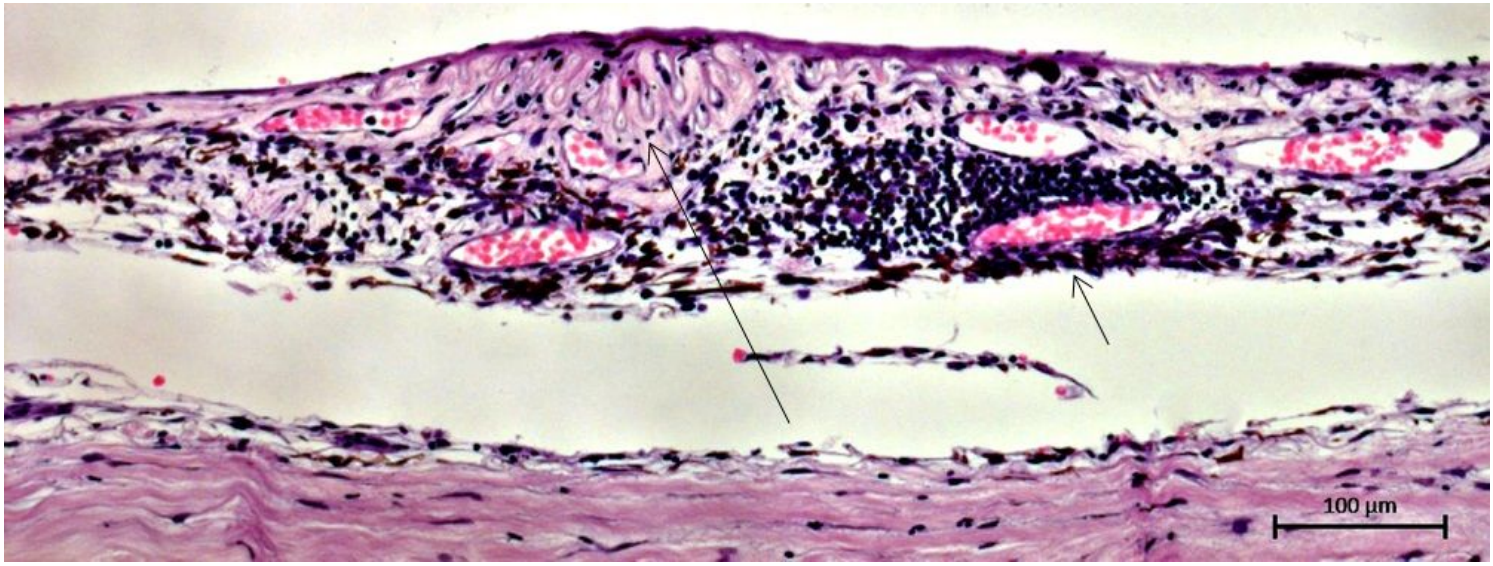

Figure 4

Perivascular lymphocytic infiltration (arrow) and retinal atrophy (long-arrow) in sections of the first globe (haematoxylin-eosin).

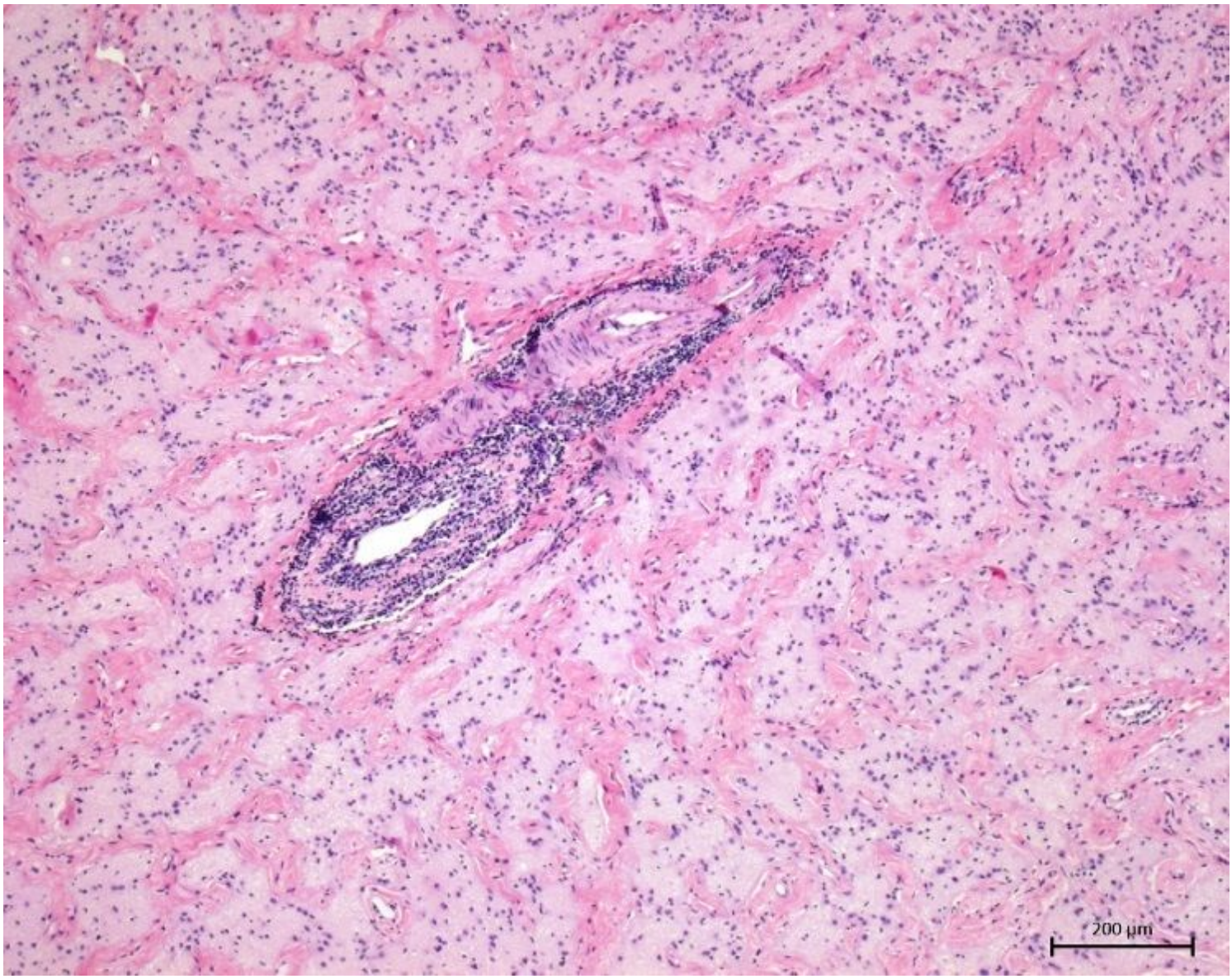

Figure 5

Perivascular lympocytic infiltration around central retinal artery in the first globe (haematoxylin-eosin).

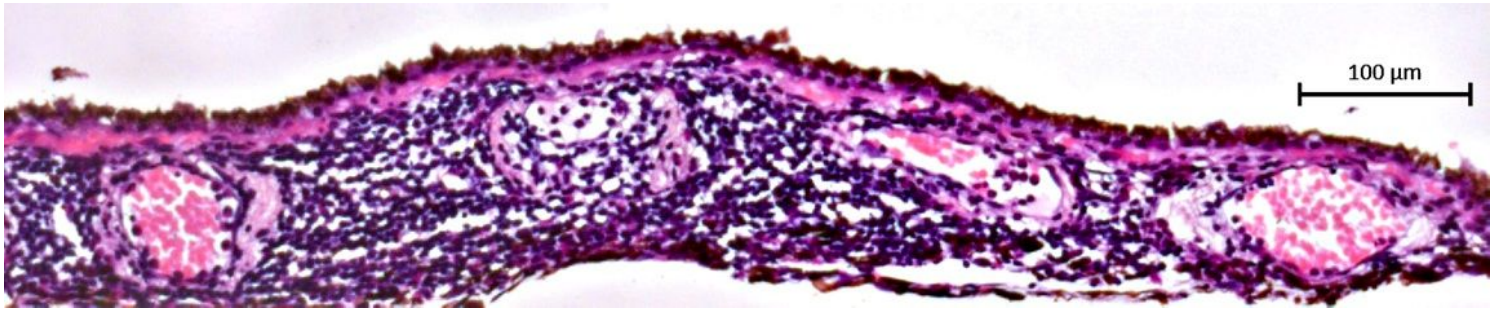

Figure 6

Lymphocytic infiltration within the choroid in sections of the second globe (haematoxylin-eosin).