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Case Report

Non-Contact-Lens-Related *Acanthamoeba* Keratitis Caused by *Acanthamoeba* sp. Group T4D/T4e

Morgane Vander Eecken [1], Anne-Sophie Messiaen [2], Hannelore Hamerlinck [2], Stien Vandendriessche [2], Jerina Boelens [2,3] and Dimitri Roels [1,*]

[1] Department of Ophthalmology, Ghent University Hospital, 9000 Ghent, Belgium; morgane.vandereecken@ugent.be
[2] Laboratory of Medical Microbiology, Ghent University Hospital, 9000 Ghent, Belgium; anne-sophie.messiaen@uzgent.be (A.-S.M.); hannelore.hamerlinck@uzgent.be (H.H.); stien.vandendriessche@uzgent.be (S.V.); jerina.boelens@uzgent.be (J.B.)
[3] Department of Diagnostic Sciences, Ghent University, 9000 Ghent, Belgium
* Correspondence: dimitri.roels@uzgent.be; Tel.: +32-9-332-0223

Abstract: *Acanthamoeba* keratitis (AK) is a rare but serious infection of the cornea, typically associated with contact lens wear. Here, we present a case of AK caused by the *Acanthamoeba* genotype T4D/T4e in a patient without identifiable risk factors: a 34-year-old woman who initially presented with signs and symptoms suggestive of herpetic keratitis, and who did not respond to conventional treatment. Corneal culture and targeted metagenomic analysis (*18S rRNA*, *16S-like rRNA*) revealed the presence of an *Acanthamoeba* species closely related to the 'Nagington' strain. Despite intensive anti-*Acanthamoeba* therapy, complications arose necessitating penetrating keratoplasty. In conclusion, this case underscores the importance of considering *Acanthamoeba* as a causal agent of keratitis in non-contact-lens wearers. The identification of *Acanthamoeba* genotype T4D/T4e challenges the previous understanding of its pathogenic potential. Furthermore, it emphasizes the need for ongoing research into the pathogenicity of different *Acanthamoeba* subtypes. Early diagnosis and treatment are essential for preventing vision-threatening complications associated with AK.

Keywords: *Acanthamoeba*; keratitis; genotype T4D

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1. Introduction

Acanthamoeba spp., a group of free-living amoebae (FLA), are widely distributed throughout the natural environment. They can be found in diverse habitats such as soil, water, dust, air conditioning filters, and domestic tap water, as well as on contact lenses and lens cases [1–3]. The protist group of *Acanthamoeba* is one of the most widespread genera in the natural environment [4]. Certain strains of *Acanthamoeba* have the potential to act as opportunistic pathogens in humans, leading to various systemic infections, including the notable *Acanthamoeba* keratitis (AK). Previous studies have shown that Acanthamoeba genotypes differ in their susceptibility to common anti-acanthamoebic drugs [2]. While their geographical and environmental distributions are generally widespread and homogeneous, a few rare genotypes seem to be localized. However, this apparent localization could be influenced by sampling bias due to the limited number of strains available. Predominant among the pathogenic species responsible for AK are *A. polyphaga* (genotype T4E/T4h) and *A. castellani* (genotype T4A/T4a). Additionally, *A. culbertsoni* (genotype T10), *A. rhysodes* (genotype T4D/T4e), *A. griffini* (genotype T3), *A. quina* (genotype T4A/T4a), and *A. lugdenensis* (genotype T4A/T4d) have also been implicated in causing this infection [2]. Most cases of *Acanthamoeba* keratitis are linked to contact lens wear, although several cases of *Acanthamoeba* keratitis in non-contact-lens wearers have been described [5–8]. In this report, we describe a pioneering case of *Acanthamoeba T4D/T4e* as the causative agent of AK in a patient without predisposing factors.

2. Case Report

A 34-year-old woman was referred to our ophthalmology department for suspected herpetic keratitis in her left eye, persisting for 9 weeks. Despite undergoing treatment with topical ganciclovir ointment at night, topical dexamethasone eyedrops five times daily, and oral valacyclovir (3 g per day), only limited improvement in signs and symptoms was observed. At presentation, the best corrected visual acuity (BCVA) was limited to counting fingers. Biomicroscopy showed a circular corneal stromal edema with an intact overlying epithelium and a calm anterior chamber (Figure 1A,B). No perineuritis or keratic precipitates were noticed. Intraocular pressure was normal (13.5 mmHg) and fundoscopy showed no abnormalities. There was no history of surgery, trauma, contact lens wear, or other predisposing factors. Given the intact epithelium and calm anterior chamber, there was no added value in performing a corneal swab or anterior chamber tap for viral PCR. Herpetic stromal keratitis was suspected. The use of topical steroids and antivirals was slowly tapered, as were oral antivirals.

Three months after the initial presentation, the biomicroscopic examination revealed a prepupillary corneal ring infiltrate in the left eye, accompanied by an overlying epithelial defect and minimal anterior chamber inflammation (Figure 1C,D). In vivo confocal microscopy (IVCM) revealed double-walled cysts distributed across the anterior stroma and a central absence of epithelium (Figure 2A). Corneal scraping was performed and microscopic examination after calcofluor-white staining of the corneal material revealed a suspicion of *Acanthamoeba* (Figure 2C,D). The treatment plan was adjusted to topical desomedine 0.1% eight times per day, chlorhexidine 0.02% eight times per day, ofloxacin three times per day and ofloxacin ointment at night. Oral doxycycline 100 mg twice per day and paracetamol 1g four times per day were started. The subsequent culture of the cornea confirmed the presence of *Acanthamoeba.* In the exploration of *Acanthamoeba* taxonomy at the molecular level, amplicon-based sequencing on the MinION platform was employed. Initial steps included the use of *18S rRNA* primers, namely G3F1/G3R1, G4F3/G4R3, and G6F1/G6R1 [9]. Preliminary analysis using the Kraken2 EuPathDB46 database suggested a potential match with *A. mauritaniensis*, which is a T4D genotype [10,11]. Recognizing the limitations of this approach, further phylogenetic analyses and blasting were conducted to confirm the classification. A distinct association with the T4D subtype became apparent, reinforcing confidence in the subtype assignment. Subsequent efforts to specify the taxonomic identity involved applying *16S-like rRNA* primers targeting the mitochondrial DNA of the pathogen, following the protocol by Ledee et al. [12]. The generated consensus sequences are available in the NCBI GenBank under the accession codes PP067180 (*16S-like rRNA*) and PP068260 (*18S rRNA*). Confirmation of the T4D/T4e subtype persisted, yet further advancements in taxonomization have proven challenging due to discrepancies in species nomenclature within existing databases, originating from inconsistencies between morphological and taxonomic classification methods. Phylogenetic trees were constructed for both fragments using the Maximum Likelihood method with the Tamura-Nei model and 100 bootstrap replicates, incorporating sub-genotype references as described by Corsaro et al. [10,13] (Figure 3). The patient strain can be identified as *Acanthamoeba* sp. group T4D/T4e and is a close relative to the Nagington strain, which was first described in 1974 [14].

Over a span of 12 days, alternating applications of topical desomedine 0.1% and chlorhexidine 0.02% were administered eight times daily. After one week, the patient displayed increased sensitivity to the topical antiseptics, leading to the discontinuation of desomedine 0.1%. Consequently, adjustments were made to the topical treatment, involving the administration of chlorhexidine 0.02% six times per day, dexamethasone once per day, ofloxacin three times per day, and ofloxacin ointment at night, accompanied by intensive lubrication. Oral doxycycline 100 mg was administered twice per day for a duration of ten days. After two weeks, clinical improvement was observed. The administration of topical chlorhexidine 0.02% gradually tapered to four times per day over a 5-week period.

Subsequently, further tapering was initiated, with a reduction of one drop every two weeks. Apart from these modifications, the local treatment protocol remained unchanged.

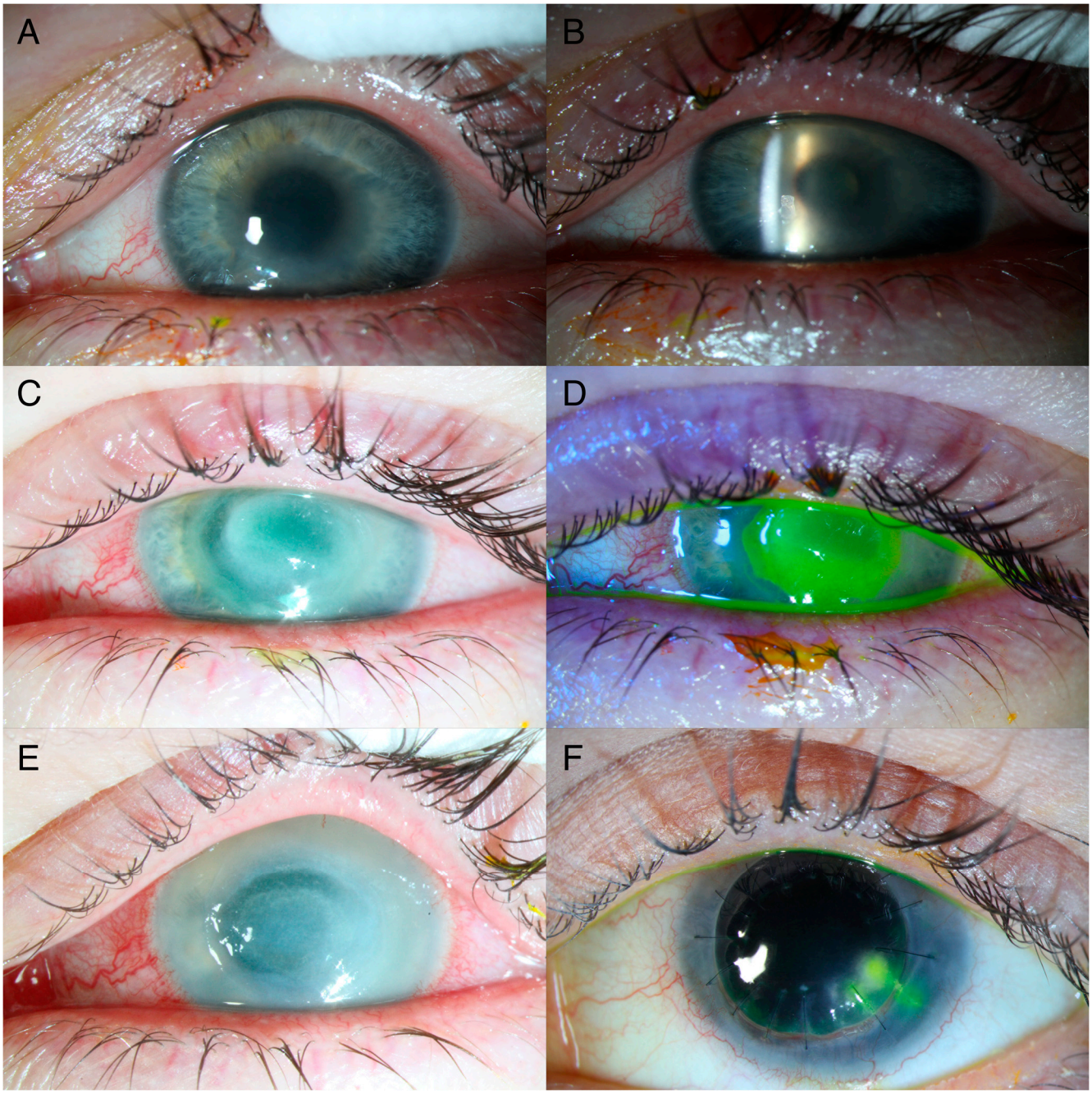

Figure 1. Slit lamp photography capturing the initial presentation of suspected herpetic stromal keratitis, revealing a circular stromal edema with an intact overlying epithelium (**A**,**B**). Subsequent images exhibiting a prepupillary corneal ring infiltrate and an associated overlying epithelial defect, three months later (**C**,**D**). Ten months after initial presentation\; a flare-up of *Acanthamoeba* keratitis with corneal infiltrate, stromal edema, and ciliary injection (**E**). Eight months after penetrating keratoplasty, the corneal graft was clear. Notice a small epithelial defect at 4 o'clock after loose suture removal (**F**).

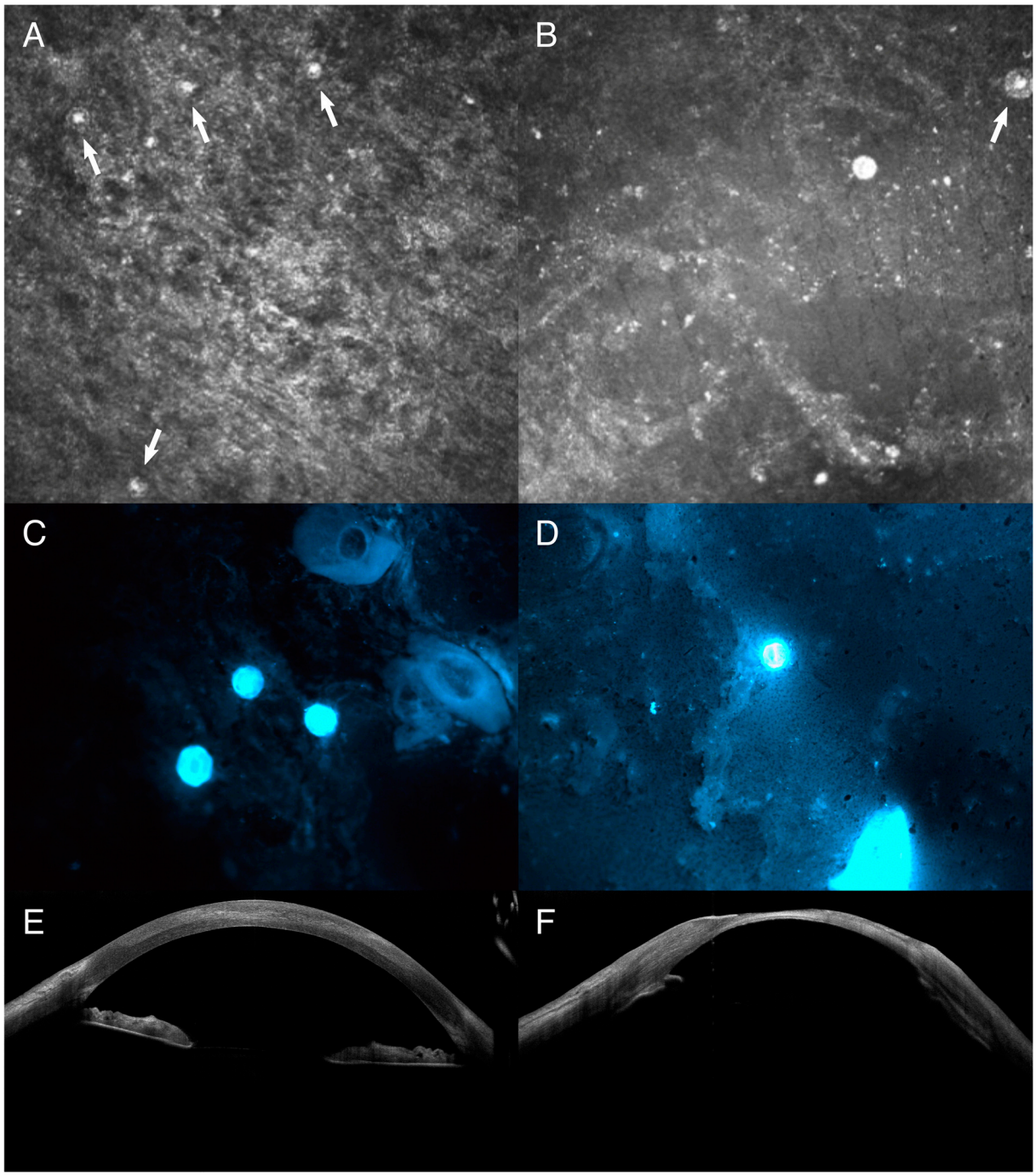

Figure 2. In vivo confocal microscopy displaying double-walled *Acanthamoeba* cysts (white arrows) distributed across the anterior stroma at diagnosis (**A**) and after flare-up (**B**). Calcofluor-white staining of corneal scrapings, illustrating *Acanthamoeba* cysts (**C**,**D**). Anterior segment OCT at presentation (**E**) and ten months later, illustrating severe corneal thinning and scarring (**F**).

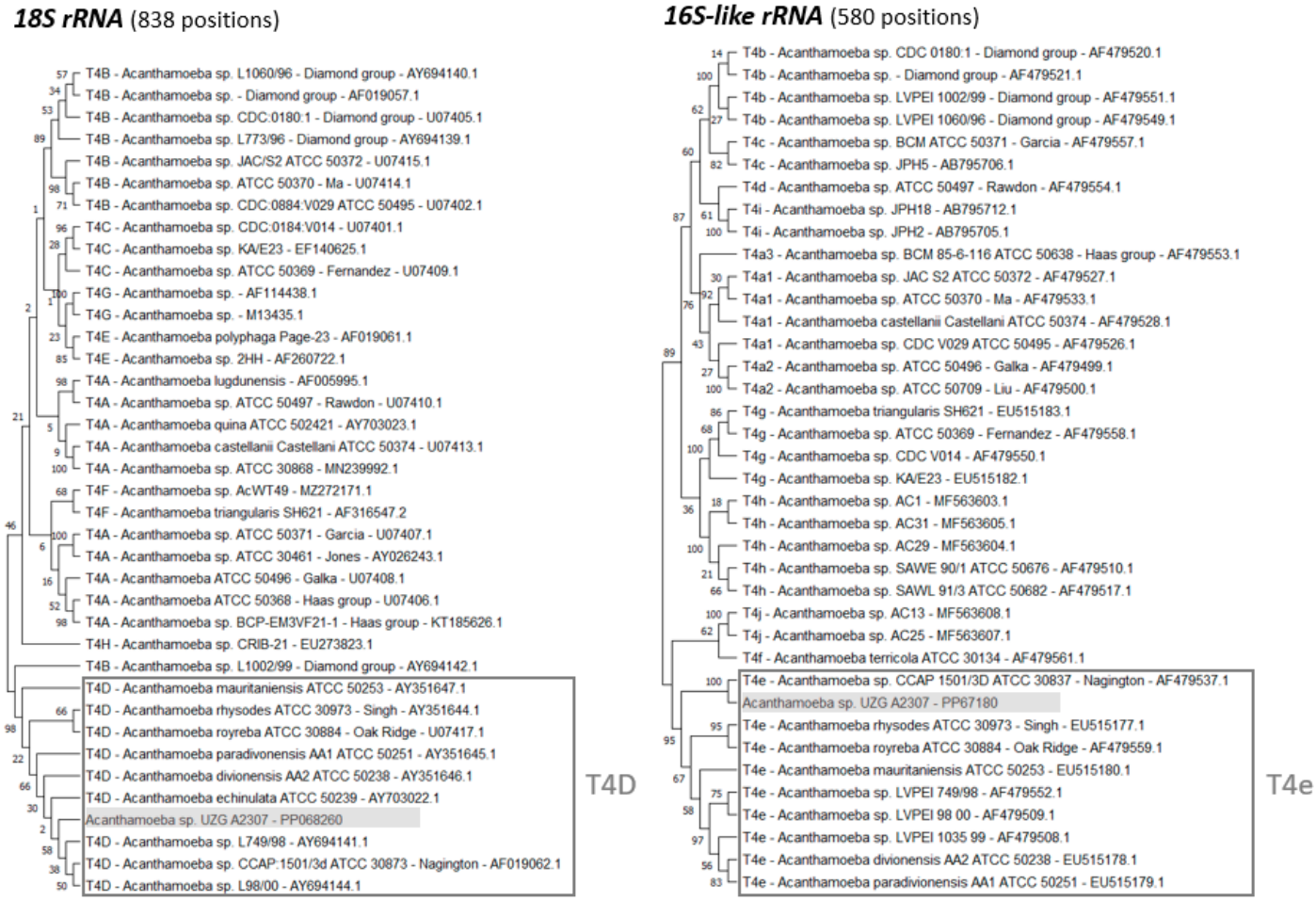

Figure 3. Phylogenetic trees (maximum likelihood, Tamura-Nei model) of *Acanthamoeba* T4 genotype based on nuclear DNA (*18S rRNA*; left) and mitochondrial DNA (*16S-like rRNA*; right) following the sub-genotyping established by Corsaro [10,13]. Bootstrap values (100 replicates) are indicated at the nodes. The investigated strain is highlighted in gray.

Two months later, a persistent large epithelial defect was observed, for which an amniotic membrane transplantation (AMT) was performed to facilitate corneal healing. Metagenomics performed on the corneal material once again identified *Acanthamoeba* sp. group T4D/T4e, this time at reduced abundance. Postoperatively, topical treatment included thorough lubrication and the application of ofloxacin three times per day, dexamethasone once per day, and chlorhexidine 0.02% twice per day for the first week and once daily for the subsequent week. However, due to a persisting epithelial defect observed three weeks postoperatively, the treatment approach was modified. In order to promote epithelial healing, hourly autologous serum 20% eye drops were started. Additionally, topical dexamethasone was stopped. In the following weeks, a gradual closure of the overlying epithelial defect was observed.

Four weeks later, however, the patient presented with an exacerbation of the corneal infiltrate, stromal edema, and ciliary injection (Figure 1E). A flare-up of the *Acanthamoeba* keratitis was suspected. In vivo confocal microscopy displayed numerous hyperreflective cysts (Figure 2B). The culture of a corneal swab on a non-nutrient agar with *E. coli* confluent growth reaffirmed the presence of *Acanthamoeba.* The sample was stored; however, the isolated strain was not. Topical treatment was changed to chlorhexidine 0.04% and propamidine 0.1%, alternating hourly.

Ten months after the initial presentation, the patient's BCVA remained limited to hand movements. Biomicroscopic and anterior segment optical coherence tomography

(OCT) examination revealed a significant central epithelial defect, marked corneal thinning (Figure 2F, compared to presentation, Figure 2E) with impending perforation, and a hypermature osmotically swollen lens. Additionally, there was evidence of peripheral anterior synechiae spanning 360°, leading to closed-angle glaucoma with elevated intraocular pressure. Given these critical findings, an urgent corneal transplantation of penetrating keratoplasty (PKP) type was performed, combined with extracapsular cataract extraction and total iridectomy. It is noteworthy that no intraocular lens implant was inserted in this particular case. Postoperatively, topical treatment included thorough lubrication, the thrice-daily application of ofloxacin, the once-daily administration of dexamethasone, chlorhexidine 0.04% twice daily, a combination of a beta-blocker and carbonic acid anhydrase inhibitor twice daily, and ofloxacin ointment at night. Additionally, oral prednisolone at a dosage of 60 mg was introduced in a gradually tapering schedule. Microbiological analysis of the cornea/iris/lens material obtained during surgery yielded negative results for *Acanthamoeba*. Eight months after PKP, BCVA was 20/200 with a clear graft and no evidence supporting the reactivation of *Acanthamoeba* (Figure 1F). Because of refractory glaucoma, our patient was scheduled for glaucoma filtration surgery.

3. Discussion

Acanthamoeba keratitis is a rare yet severe vision-threatening parasitic infection of the cornea caused by *Acanthamoeba* species, accounting for 2% of microbiology-confirmed keratitis cases [2,5,15]. The incidence of AK varies between developed and developing countries [2,3,5,8,16]. In developed countries, soft contact lens wear is a primary risk factor, while in developing nations, ocular trauma, surgery, and exposure to *Acanthamoeba*-contaminated water, soil, dust, stone, mud, or vegetation are the predominant causes.

Infectious keratitis is one of the leading causes of visual impairment and blindness worldwide. The swift identification of the causative pathogen is crucial for optimal visual outcomes [16]. Diagnosing AK is challenging because of the unspecific signs and symptoms, particularly in non-contact-lens wearers. Sharma et al. reported that in such instances, AK often presents at an advanced stage without pathognomonic features [7]. The condition can be mistaken for herpes simplex keratitis or other viral, bacterial, or fungal keratitis forms unresponsive to initiated therapy, as observed in our patient [8,17].

A comprehensive diagnostic approach is essential for diagnosing AK. Biomicroscopy remains pivotal for detecting corneal abnormalities and clinical signs such as a corneal ring ulcer, as was observed in our patient [17]. The tentative diagnosis of AK can be made by IVCM, a noninvasive technique providing real-time images of *Acanthamoeba* double-walled cysts. The reported sensitivity and specificity of IVCM in AK for diagnosing AK are 100% and 84%, respectively, depending on the observer's expertise [5,18,19]. Nonetheless, the gold standard remains the identification of *Acanthamoeba* through corneal scraping specimen culture [5,8,15]. The culture has a specificity of 100% and a sensitivity of 67% [15]. Direct detection via the microscopic examination of calcofluor-white or Gram-stained smears is also crucial. PCR-based techniques can rapidly identify *Acanthamoeba* with a sensitivity ranging from 65% to 90% and a specificity ranging from 96 to 100% [15]. In this particular case study, the diagnosis of AK was confirmed through the culture analysis of a corneal specimen obtained by scraping. Remarkably, this diagnosis was established in a patient with no history of contact lens wear or other associated risk factors. Non-contact-lens-related *Acanthamoeba* keratitis has been reported previously and is predominantly linked to ocular surface disorders and trauma caused by substances such as vegetative matter, sand, dust, or contaminated water [5,7]. Sharma et al. proposed the potential existence of Acanthamoeba pathotypes specifically associated with non-contact-lens-related keratitis. However, this is debatable, as non-contact-lens-related AK cases are primarily observed in developing countries, suggesting that hygienic and socioeconomic conditions may be more influential than a distinct amoeba pathotype [7]. This led to the decision to conduct sequencing identifying the presence of the *Acanthamoeba* T4D/T4e genotype. The necessity for a more cohesive and standardized nomenclature approach is evident and

crucial for advancing our comprehension of *Acanthamoeba* taxonomy and its implications for broader scientific research.

Coronado-Velazquez et al. demonstrated that *Acanthamoeba* genotype T4D exhibits behavior akin to the pathogenic *A. castellanii* strain [1]. Their in vitro experiments showed that T4D can generate and release serine proteases, which contribute to corneal epithelial damage and the alteration of tight-junction proteins, facilitating amoebic invasion.

Notably, cases of AK in non-contact-lens wearers have been documented in India (Sharma et al.) and Turkey (Ertabaklar et al.) [20,21]. Sharma et al. reported cases of *Acanthamoeba* keratitis in India among non-contact-lens wearers, later identified as T4D, suggesting a regional pathogenicity of this genotype. Similarly, Ertabaklar et al. identified T4D in AK cases in Turkey, further highlighting its potential to cause severe keratitis outside typical risk factors.

The resemblance of T4D/T4e to the Nagington strain, first described in 1974, emphasizes its pathogenic potential. This case underscores a significant shift in our understanding of the *Acanthamoeba* sp. group T4D/T4e, previously considered nonpathogenic, which is now recognized as a potential threat causing human disease. This report presents a pioneering case of *Acanthamoeba* T4D/T4e as the causative agent of AK in a patient without predisposing factors.

To obtain a good visual prognosis, early diagnosis and precise treatment are imperative, although the risk of recurrence remains high. The management of AK is multifaceted, aiming to eradicate all pathogens from the corneal tissue and to resolve the inflammatory response [8,17]. Topical polyhexamethylene biguanide (PHMB) and chlorhexidine are the most important components in AK treatment, exhibiting efficacy against both trophozoites and cysts. Subsequent adjustments in the frequency of drops are made in accordance with the patient's clinical progress. Performing epithelial debridement early in the disease is thought to remove pathogens from the corneal tissue, aiding medication penetration [17]. Toxic keratopathy, a known side effect, could require substantial treatment adjustments. In cases of severe infection, lasting epithelial defects may necessitate the use of amniotic membranes [22]. They aid in restoring ocular surface integrity and offer metabolic and mechanical support for corneal healing. As was the case with our patient, the literature indicates that a PKP may be necessary in the presence of (potential) corneal perforation, extensive scarring, or if topical treatments prove ineffective [8].

The existing literature has documented a limited number of cases attributing progressive iris atrophy and cataract formation to the toxic side effects of topical treatments, specifically involving chlorhexidine and propamidine [23,24]. Our patient presented with notable corneal thinning, hypermature cataract, peripheral anterior synechiae, and glaucoma, consistent with these documented adverse effects. Notably, the absence of *Acanthamoeba* trophozoites and/or cysts in the removed ocular material led us to consider toxicity as the likely etiology. This finding suggested that the rapid progression of cataracts is unlikely to be directly caused by the invasion of *Acanthamoeba* trophozoites and/or cysts through the lens capsule.

In conclusion, we present a pioneering case of *Acanthamoeba* genotype T4D/T4e keratitis in a patient without predisposing factors. Until recently, this subtype was considered to be a nonpathogenic amoeba, not known to cause disease in humans. This case underscores the importance of early and accurate diagnosis in AK, combining IVCM, culture, and sequencing, and the potential challenges and complications associated with long-term topical treatments. The implications of this discovery highlight the need for further research to elucidate the mechanisms and factors contributing to the pathogenicity of *Acanthamoeba* T4D/T4e subtypes and their role in AK among individuals devoid of risk factors.

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Informed Consent Statement: Written informed consent has been obtained from the patient to publish this paper.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

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