Case Series: Unusual Presentation of *Acanthamoeba* Coinfection in the Cornea

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SIGNIFICANCE: The cases illustrate *Acanthamoeba* coinfection with *Pseudomonas aeruginosa* or microsporidia in the cornea.

PURPOSE: This case series aimed to alert clinicians toward considering *Acanthamoeba* coinfection in the cornea when unusual presentation such as perineuritis or epitheliitis was observed in clinical images. Increased suspicion of *Acanthamoeba* coinfection may facilitate early diagnosis and prompt management, eventually leading to good vision outcomes.

CASE SERIES: An 11-year-old boy wearing orthokeratology lens for myopia control complained of pain in the right eye for 1 week. A paracentral corneal ulcer with perineuritis was observed. Culture from corneal tissue revealed *P. aeruginosa*, and an *in vivo* confocal microscopic examination showed highly reflective and oval-shaped structures indicating *Acanthamoeba* coinfection. Corneal lesions gradually improved under 0.02% polyhexamethylene biguanidine, 0.1% propamidine isethionate, and 0.3% ciprofloxacin. At 1 year, the final best-corrected visual acuity was 20/25 with residual paracentral corneal opacity. Another 20-year-old man complained of pain in the right eye for 2 weeks. Multiple raised corneal lesions associated with epitheliitis were found. Moreover, 1% acid-fast staining showed oval-shaped spores, and microsporidia infection was inferred. In addition, polymerase chain reaction results obtained after subjecting the patient to corneal debridement revealed positivity for *Acanthamoeba*. Polyhexamethylene biguanidine (0.02%) and 0.5% moxifloxacin were prescribed, and the lesions subsided. At a 2-year follow-up, the final best-corrected visual acuity was 20/25.

CONCLUSIONS: Perineuritis in orthokeratology lens wearers and epitheliitis without any predisposing factor are unusual presentations of *Acanthamoeba* coinfection in the cornea. These corneal findings should arouse the suspicion of coinfection and enable the clinicians to conduct the appropriate workup and initiate adequate treatment. This case series demonstrated that early diagnosis and prompt treatment can improve visual prognosis.

Optom Vis Sci 2022;99:605–611. doi:10.1097/OPX.0000000000001906

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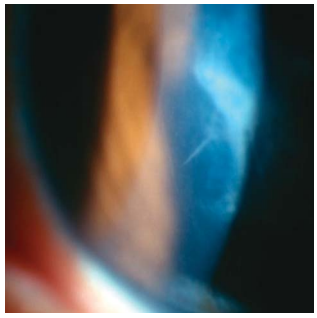

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Acanthamoeba keratitis is a severe and vision-threatening disease of the cornea, which is caused by the free-living, ubiquitous protozoa *Acanthamoeba.*[1,2] The main risk factors include wearing contact lenses, exposure to contaminated water or tap water, and damage of the corneal surface.[3] Diagnosis of this disease tends to be challenging and can often be delayed.[3] Depending on different stages of the disease, it may resemble herpetic, bacterial, or mycotic keratitis.[2] Because of the frequent misdiagnosis of *Acanthamoeba* keratitis, its clinical outcome is often unsatisfactory.[4–7]

In recent years, *Acanthamoeba* coinfection with other pathogens, such as bacteria or fungus, has been widely reported.[8,9] It could contribute to an even more complicated clinical picture and a further delay in diagnosis, leading to a poor clinical prognosis.[9,10] Common clinical features at the presentation of *Acanthamoeba* coinfection include central dense infiltrates, epithelial defects, and hypopyon.[4–7,9] However, these signs are often seen as late-stage changes with unpromising visual prognosis.[10]

Here, this cases series presents *Acanthamoeba* coinfection with unusual manifestations. The presentations of the perineuritis in orthokeratology lens wearers and epitheliitis in cases without any predisposing risk factor should raise the suspicion of another pathogen beyond *Acanthamoeba*. A high index of suspicion, early workup, and appropriate treatment would be crucial for achieving a good vision outcome.

CASE 1

An 11-year-old boy complained of experiencing pain in the right eye and tearing for 1 week (Fig. 1). He had been wearing overnight orthokeratology lens for myopia control for 2 years. He cleaned his orthokeratology lens with commercial contact lens solution daily but occasionally stored and irrigated the contact lens in tap water. Best-corrected visual acuity at presentation was counting fingers 10 cm. Slit-lamp examination showed a paracentral stromal infiltration (4 × 3 mm) with feathery borders. The epithelial defect was found at the center of the ulcer. Prominent corneal edema was observed, along with perineuritis around the lesion (Fig. 2A). Hypopyon was detected in the anterior chamber. Corneal scraping for culture analysis was performed. The patient was administered topical 0.3% ciprofloxacin on an hourly basis along with gentamicin.

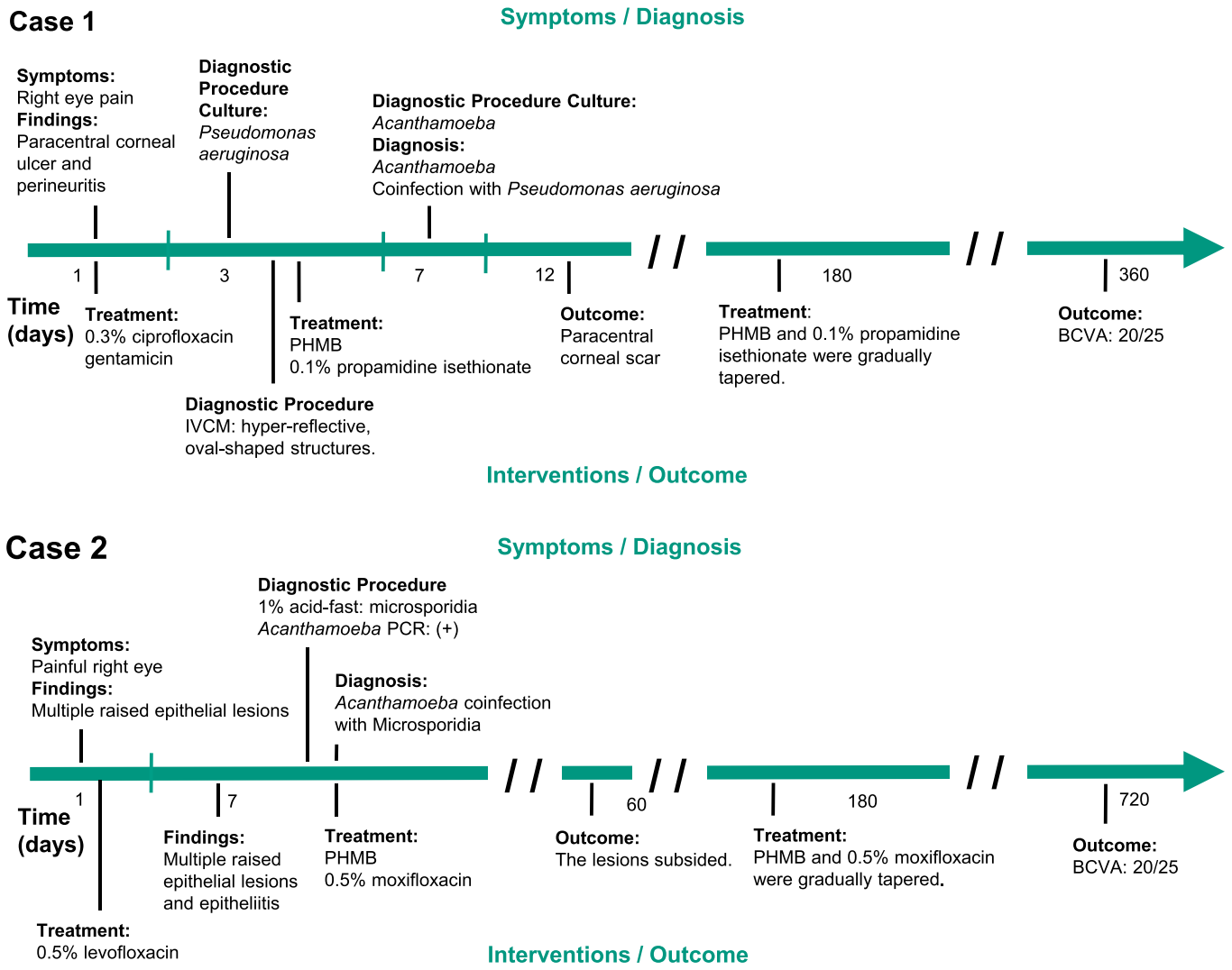

FIGURE 1. Clinical timeline. Case 1: an 11-year-old boy complained of pain in the right eye and presented with paracentral corneal ulcer and perineuritis. Bacterial culture results showed positivity for *Pseudomonas aeruginosa* after 3 days. *In vivo* confocal microscopy showed hyperreflective oval-shaped structures, which suggested *Acanthamoeba* coinfection. Consequently, 0.02% polyhexamethylene biguanidine and 0.1% propamidine isethionate were administered. Positivity of *Acanthamoeba* culture results subsequently confirmed the diagnosis. Perineuritis subsided in 2 weeks, and paracentral corneal opacity continued to persist. Polyhexamethylene biguanidine (0.02%) and 0.1% propamidine isethionate were gradually tapered in 6 months. His best-corrected visual acuity was 20/25 at 1-year follow-up. Case 2: a 20-year-old man complained of pain in the right eye and presented with multiple raised, whitish epithelial lesions. Epitheliitis presented several days later. Furthermore, 1% acid-fast stain showed oval-shaped spores, indicating microsporidia infection. Polymerase chain reaction results showed positivity for *Acanthamoeba*. Polyhexamethylene biguanidine (0.02%) and 0.5% moxifloxacin were administered. Corneal lesions subsided in 2 months, and the final best-corrected visual acuity was 20/25 at 2-year follow-up.

Three days later, bacterial culture results revealed positivity for *Pseudomonas aeruginosa* growth. Ciprofloxacin was effective against *P. aeruginosa* according to the sensitivity test. Stromal infiltrates and perineuritis continued to persist (Fig. 2B). His anterior chamber reaction gradually resolved, but paracentral corneal ulcer with perineuritis did not improve. *In vivo* confocal microscopy showed hyperreflective, oval-shaped structures, which are compatible with *Acanthamoeba* cysts (Fig. 2C). Repeated corneal debridement was carried out for culture analysis, which showed positivity for *Acanthamoeba* growth at day 7. Under a combination treatment of 0.3% ciprofloxacin, 0.1% propamidine isethionate, and 0.02% polyhexamethylene biguanidine, perineuritis and stromal infiltration eventually subsided (Fig. 2D). Topical medication was gradually tapered over 6 months. The patient's best-corrected visual acuity was 20/30 at 7 months and consequently recovered to 20/25 at 1 year.

CASE 2

A 20-year-old college student without any underlying disease visited the outpatient department with a complaint of pain in the right eye and increased ocular discharge for 2 weeks (Fig. 1). He reported no contact lens use and did not have a history of any other trauma or exposure to dust, soils, and other fine particles. At first presentation, his initial best-corrected visual acuity in the right eye was 20/100. Multiple whitish, raised epithelial lesions with

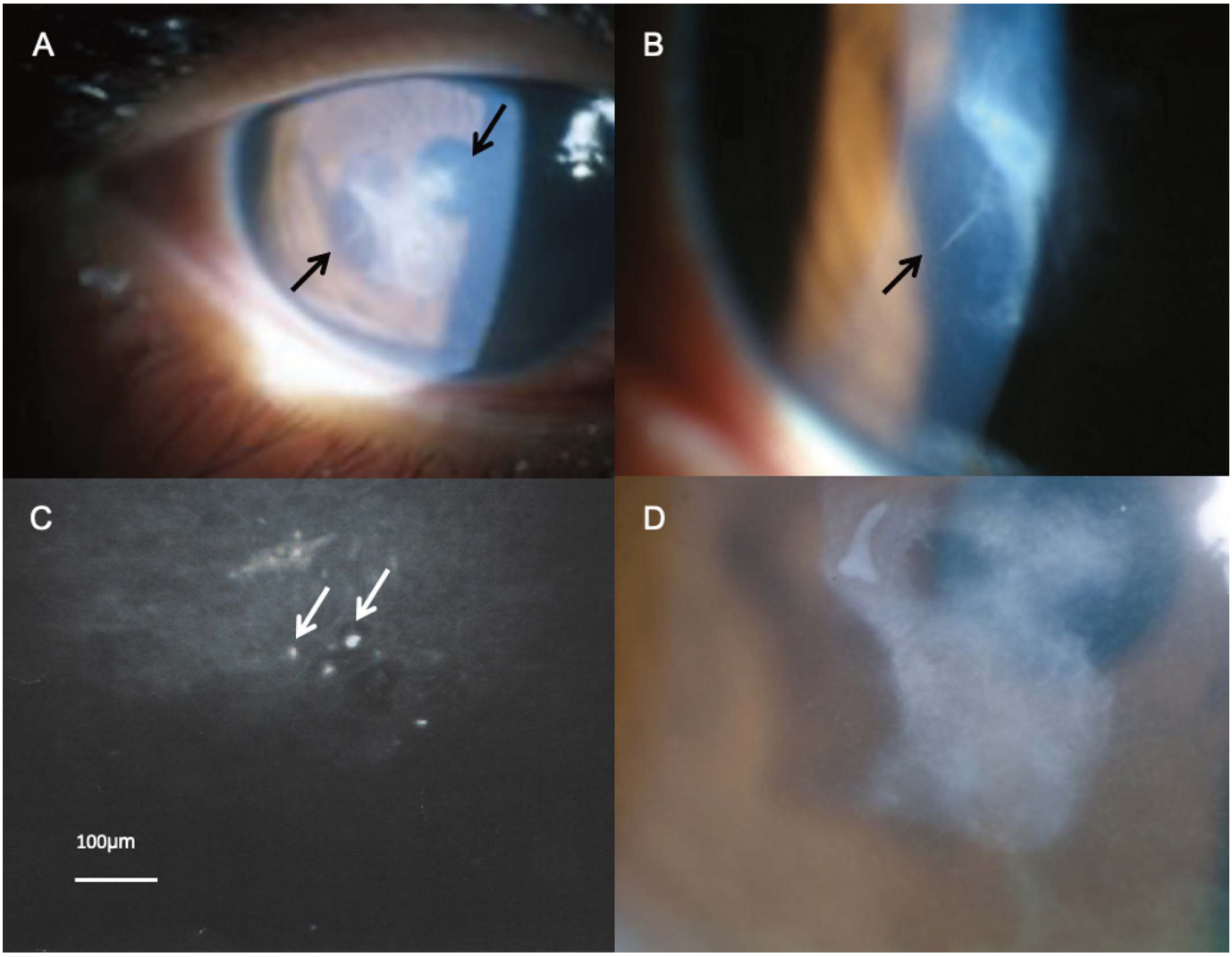

FIGURE 2. An 11-year-old orthokeratology lens wearer presented to clinic with a paracentral corneal ulcer. He reported ocular pain and tearing for 1 week. A, Besides paracentral corneal ulcer, perineuritis (black arrow) was observed. B, At a higher magnification, nerve inflammation was observed in a radial pattern (black arrow). C, *In vivo* confocal microscopy revealed *Acanthamoeba* cysts, which were oval or round in shape and were highly reflective structures (white arrow). Scale bar, 100 μm. D, After 0.02% polyhexamethylene biguanidine was added to the treatment besides topical antibiotics for 5 days, perineuritis gradually disappeared, and only a paracentral corneal scar remained.

diffuse superficial punctate keratitis and limbal injection were noted (Figs. 3A, B). Corneal lesions were observed to have a granular cystic appearance. Epitheliitis gradually appeared in a circular pattern several days later, and the lesions worsened under the treatment of 0.5% levofloxacin (Figs. 3C, D). Corneal debridement was carried out using a Merocel sponge (BVI Medical, Waltham, MA) (Fig. 4A). Corneal specimens were subjected to 1% acid-fast staining, polymerase chain reaction, Gram staining, Giemsa staining, and bacterial culturing. Corneal specimen showed oval spores in 1% acid-fast stain (Fig. 4B). Microsporidia infection was inferred based on clinical images and the results of acid-fast staining. In addition, polymerase chain reaction results revealed positivity for *Acanthamoeba*.

Under the indication of *Acanthamoeba* coinfection with microsporidia, 0.02% polyhexamethylene biguanidine was administered on an hourly basis. The lesions subsided within 2 months of 0.02% polyhexamethylene biguanidine administration and 0.5% moxifloxacin without any recurrence (Figs. 4C, D). The medication was slowly tapered for 6 months after initial presentation. His final best-corrected visual acuity was 20/25 at a 2-year follow-up.

DISCUSSION

Diagnosis of *Acanthamoeba* keratitis coinfection can be challenging. It is often missed or delayed, critically affecting patient prognosis or outcome and ultimately their quality of life.[11] *Acanthamoeba* keratitis coinfection is initially diagnosed based on patient history, clinical presentation, and clinical suspicion. The timing of the case presentations (case 1, 1 week; case 2, 2 weeks) was uncommon for bacterial infections, which would develop more rapidly. A previous study has demonstrated that central dense infiltrates, epithelial defects, and hypopyon are prevailing corneal ulcer morphology in *Acanthamoeba* mixed infection.[9]

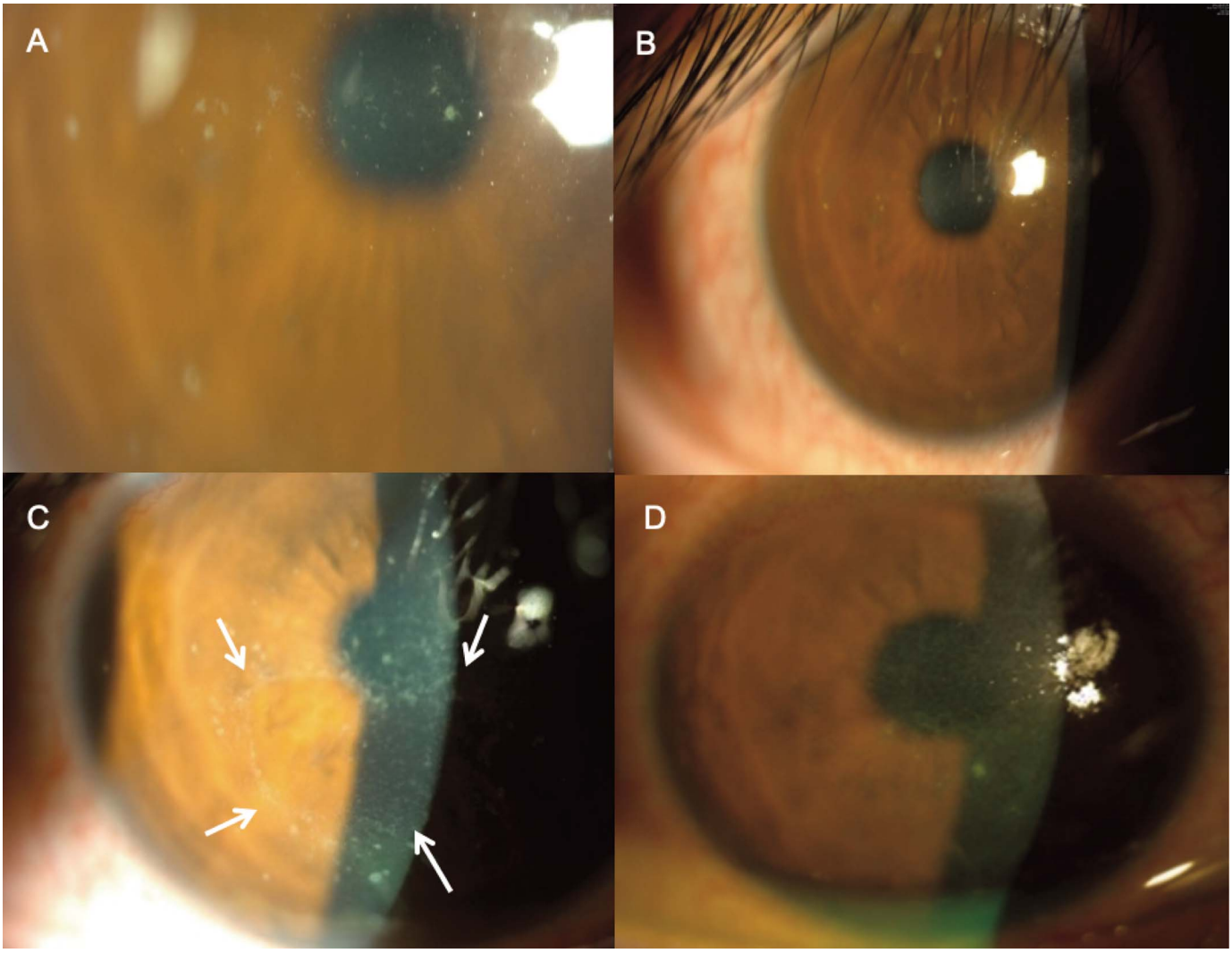

FIGURE 3. A 20-year-old male patient, without any trauma or exposure histories, had suffered from ocular pain for 2 weeks. A, Multiple raised lesions were placed dispersedly in the central cornea on day 1. B, Limbal injection was also shown on day 1. C, In addition, epitheliitis appeared in a circular shape at the paracentral area (white arrow). D, The epitheliitis soon became more obvious and filled up the circular space.

Central dense infiltrates are the initial presentation in 50% of *Acanthamoeba* coinfection cases.[9] In this cases series, perineuritis was observed in case 1, whereas epitheliitis was observed in case 2. Perineuritis and epitheliitis are known presentations in early *Acanthamoeba* keratitis infection, but these two presentations are relatively unusual in *Acanthamoeba* keratitis coinfection.[1,4–7,9] Perineuritis, a typical characteristic of *Acanthamoeba* keratitis, was also reported in contact lens–related microbial keratitis caused by *P. aeruginosa.*[12] Epitheliitis, a sign of *Acanthamoeba* keratitis, presenting in cases without any predisposing factor would imply existence of coinfection.

Diagnosis of *Acanthamoeba* keratitis coinfection is frequently delayed, which in turn may prolong treatment courses, eventually requiring surgical intervention to restore corneal integrity and visual acuity.[2] On average, patients were diagnosed after 40 days of symptom onset in a previous case series.[9] Delayed diagnosis may occur owing to unspecific signs, misdiagnosis as herpetic simplex keratitis or mycotic keratitis,[1,9] lack of cardinal symptoms such as pain,[4] or challenges in obtaining microbiological evidence.[13]

Table 1 shows documented coinfections of *Acanthamoeba* and *P. aeruginosa* in the literature. Initial presentation for most of the cases involving *Acanthamoeba* coinfection with *P. aeruginosa* is most often changes occurring in the later stages, such as ring infiltrates or deep central stromal infiltrate. Only one case (Table 1) presented with anterior stromal infiltrates with a final best-corrected visual acuity of up to 20/20.[1] A previous case report indicated that a late-stage change of corneal ulcer morphology may also be a poor prognostic factor for vision outcome in *Acanthamoeba* keratitis coinfection.[9] Therefore, it is important to identify the corneal morphology in an *Acanthamoeba* keratitis coinfection, such as perineuritis and epitheliitis. In an *Acanthamoeba* keratitis coinfection, perineural infiltrate presents as a radial-shaped lesion located from the limbus to middle stroma, which results from the inflammation and subsequent loss of corneal fibers.[3,14] Epitheliitis, a type of epitheliopathy, presents as gray epithelial opacity or microcysts, which needs to be differentiated from an epithelial herpetic keratitis.[3,14] Epitheliitis does not have round spot-like widenings at the ending of the epithelial erosions, unlike herpetic epithelial keratitis.[3]

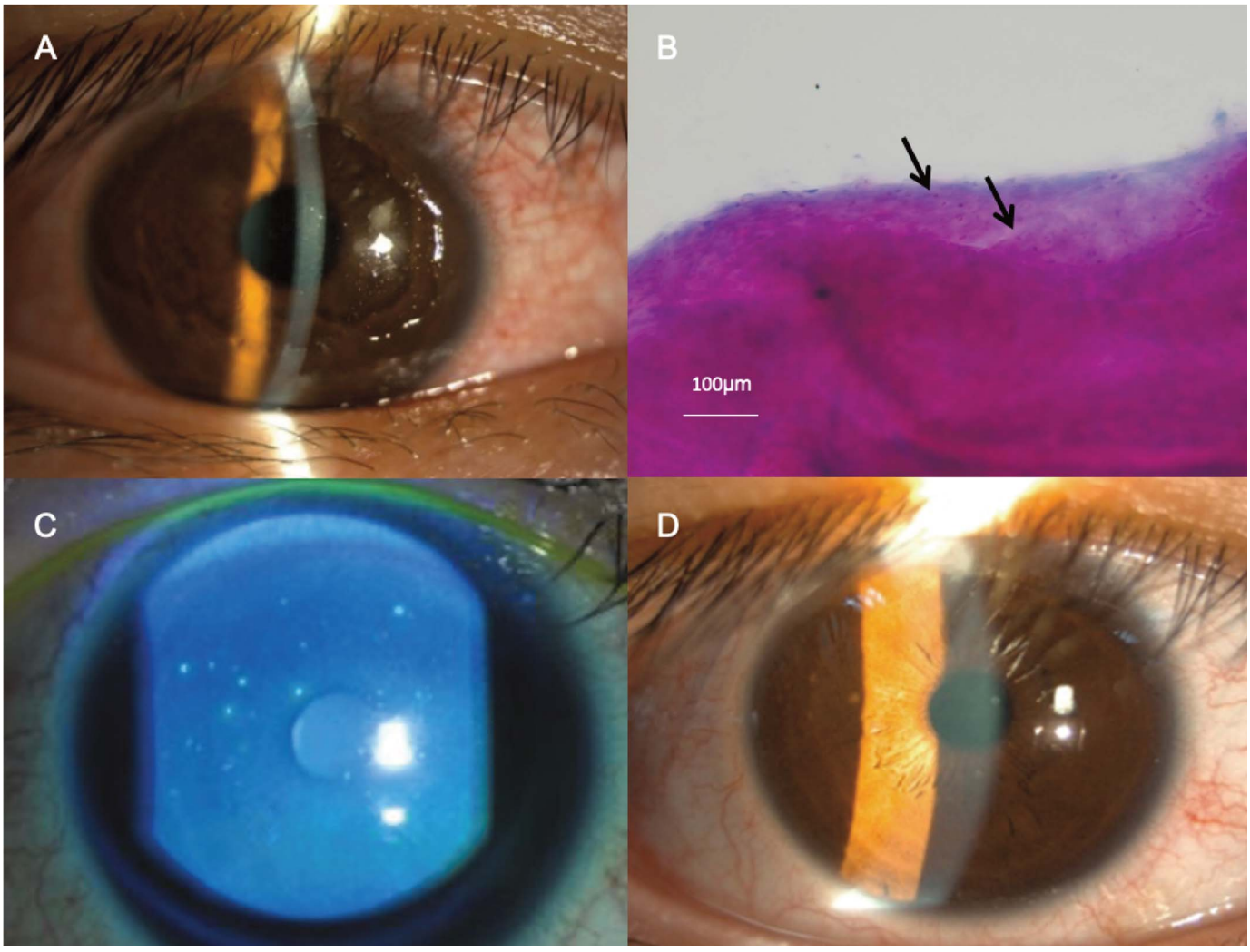

FIGURE 4. Topical antibiotic treatment in case 2 was in vain. Corneal debridement for 1% acid-fast stain and polymerase chain reaction analysis were performed. (A) After debridement, central corneal epithelial defects were observed. (B) The specimen sent for 1% acid-fast staining analysis exhibited multiple oval-shaped spores (black arrow), indicating microsporidia infection. Scale bar, 100 μm. (C) Polymerase chain reaction showed positivity for *Acanthamoeba*; 0.02% polyhexamethylene biguanidine was initiated. After a 2-month administration of 0.02% polyhexamethylene biguanidine, slit-lamp examination and fluorescein staining of this patient revealed an improvement. (D) Complete resolution was observed in this patient after 0.02% polyhexamethylene biguanidine and 0.5% moxifloxacin administration for 10 weeks. Visual acuity eventually improved to 20/25.

Acanthamoeba is well documented to be a copathogen with other viruses, bacteria, fungi, and protozoa.[1,9] In a retrospective study conducted at a tertiary hospital in India, approximately 40% of culture-positive *Acanthamoeba* keratitis patients test positive for another bacterial species.[8] Coinfection with *Acanthamoeba* is not an uncommon occurrence and is becoming an important issue. Documented risk factors for *Acanthamoeba* coinfection include contact lens use, trauma, exposure to tap water, and prior topical steroid use.[1,14] Whereas the patient described in case 1 wore orthokeratology lens, the patient described in case 2 reported no contact lens use and no trauma but had a history of exposure to muddy water. The use of contact lenses appears to be a strong risk factor for *Acanthamoeba* coinfection with *P. aeruginosa*.[1,4–7] Table 1 showed five cases of concurrent *P. aeruginosa* and *Acanthamoeba* infection. All patients reported a history of contact lens use.

In case 1, both *Acanthamoeba* and *P. aeruginosa* were identified through culture results, which were also the most common pathogens causing infectious keratitis in orthokeratology lens wearers.[15] As for an interaction between these copathogens, *in vitro* studies have revealed that the survival of *Acanthamoeba* might be enhanced on being cocultured with *P. aeruginosa* and other bacteria.[15–17] Moreover, an enhancement of *Acanthamoeba* toxicity toward the cornea after acquisition of bacterial endosymbionts has been previously described.[18] *Acanthamoeba* containing *P. aeruginosa* endosymbionts isolated from corneal specimens of *Acanthamoeba* keratitis coinfection patients showed a higher level of corneal toxicity.[18] Microsporidia and *Acanthamoeba* coinfection was detected in case 2. No previous cases of infectious keratitis caused by coinfection with these two pathogens have been reported in the literature. Microsporidia, as obligate intracellular parasites, have increasingly started causing ocular infection in subtropical areas, such as Singapore and India,[19] and such cases have been believed to be underreported owing to misdiagnosis with adenovirus keratoconjunctivitis and nonspecific signs.[10] Furthermore,

TABLE 1. Details of previous reports on mixed infection of *Acanthamoeba* and *Pseudomonas aeruginosa* in the literature (PubMed)

Reference	Initial BCVA	Risk factor	Clinical presentation	Treatment	Final BCVA
Dini et al. (2000)[7]	Counting fingers	Soft contact lens, tap water	Central corneal ulcer, hypopyon with ring infiltrate	Ciprofloxacin, polyhexamethylene biguanide neomycin	Counting fingers
Sharma et al. (2013)[6]	Hand movements	Contact lens	Dense ring infiltrate, hypopyon with perineuritis	Gentamicin, cefazolin, chlorhexidine, polymyxin B, propamidine isethionate	Counting fingers
Hong et al. (2014)[5]	Counting fingers	Soft contact lens, tap water	Central corneal ulcer, hypopyon with ring infiltrate	Tobramycin, levofloxacin, polyhexamethylene biguanide, metronidazole	1/20
Sızmaz et al. (2016)[4]	Counting fingers	Contact lens, tap water	Large corneal ulcer, hypopyon with ring infiltrate	Amikacin, vancomycin, chlorhexidine, propamidine isethionate	Not applicable
Hsu (2019)[1]	Not applicable	Orthokeratology lens	Anterior dendritic stromal infiltration	Voriconazole, levofloxacin, chlorhexidine	20/20

BCVA = best-corrected visual acuity.

the mechanism of microsporidia-related corneal disease remains unclear. Infection through exposure to muddy water, contact lens, ocular trauma, and swimming pool has been reported.[19,20]

In clinical settings, the diagnosis of *Acanthamoeba* keratitis coinfection is based on the results of cytological staining of corneal scraping performed using Giemsa or calcofluor white, *in vivo* confocal microscopy, culture analysis of corneal scrapings, polymerase chain reaction, and history of corneal biopsies, all of which help confirm the clinical suspicion.[11] *In vivo* confocal microscopy with a sensitivity and specificity of more than 90% is an invaluable technique in the investigation of patients with clinical presentations of *Acanthamoeba* keratitis coinfection.[5] The use of polymerase chain reaction for diagnosing *Acanthamoeba* keratitis coinfection is becoming more common and has a high sensitivity of 84% and a specificity of 100%.[3]

In summary, this cases series showed that, in orthokeratology lens wearers, the presentation of perineuritis should raise the suspicion of coexistent pathogens besides *Acanthamoeba*. In cases without any predisposing risk factor (such as contact lens wearing), the presentation of epitheliitis should lead the clinicians to consider that *Acanthamoeba* may not be the only pathogen in the corneal infection. The timing of the case presentation and the particular features on the presentations in contact lens wearers (e.g., perineuritis) and non–contact lens wearers (e.g., epitheliitis) would suggest the possibility of *Acanthamoeba* coinfection. Early diagnosis and prompt treatment will help achieve good visual outcomes.

CONCLUSIONS

Acanthamoeba coinfection is not uncommon and a potentially vision-threatening problem. Recognition of the particular signs of *Acanthamoeba* keratitis coinfection, such as perineuritis in contact lens wearers and epitheliitis in cases without any predisposing factor, may help clinicians make the correct diagnosis as early as possible. Late-stage presentation, such as ring infiltrates and deep stromal infiltrates, may predispose patients to a poor visual outcome. Detailed ocular history, ophthalmic examination, and appropriate laboratory workups should be carried out for detecting signs of *Acanthamoeba*-related keratitis. As demonstrated in this cases series, early diagnosis and prompt management would lead to a good vision outcome.

ARTICLE INFORMATION

Submitted: October 12, 2021

Accepted: March 16, 2022

Funding/Support: This work was supported in part by the Taipei City Hospital (110XDAA00124).

Conflict of Interest Disclosure: None of the authors have reported a financial conflict of interest.

Author Contributions and Acknowledgments: Conceptualization: C-CC; Data Curation: Y-CW, C-YY, C-CC; Investigation: C-CL; Resources: C-CL; Supervision: C-YY, C-CC; Visualization: C-CL; Writing – Original Draft: Y-HC, Y-CW, C-CC; Writing – Review & Editing: Y-HC, Y-CW, C-CC.

Y-HC and Y-CW contributed equally to this work and are co–first authors. No identifiable health information was included in this case series.

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