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Diagnosis and Treatment of *Pythium Insidiosum* Corneal Ulcer in a Chinese Child: A Case Report and Literature Review

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Patient: Male, 7
Final Diagnosis: Pythium insidiosum
Symptoms: Painful vision loss • red eye
Medication: —
Clinical Procedure: Application of multiple antifungal medicines followed by the penetrating keratoplasty
Specialty: Ophthalmology

Objective: Rare disease
Background: *Pythium insidiosum* keratitis is a rare but sight-threatening disease with a high morbidity rate. It can be misdiagnosed as fungal keratitis in clinic settings. We report a case of severe *Pythium insidiosum* keratitis in a Chinese child, treated with combined approaches.
Case Report: A 7-year-old boy from Hainan province in the south of China developed a suppurative corneal ulcer after being in a forest. A mass of hyphae was detected by confocal imaging *in vivo*, fungal smear test, and histochemical examination. Treatment with Natamycin, fluconazole, and Voriconazole for 1 month was unsuccessful, and a penetrating keratoplasty with anterior vitrectomy was performed. The infection reappeared 1 day after surgery, immediately after which anterior chamber irrigation with 0.02% Fluconazole and amphotericin B solution was performed. Finally, the globe was saved and there was no further recurrent infection. A cultured mycelial organism, which was hard to identify by biomorphology, was confirmed by PCR to be *Pythium insidiosum*. The zoospores were observed in water environments. The imaging characteristics of *P. insidiosum* in confocal microscopy are described herein.
Conclusions: This is the first case of *Pythium insidiosum* keratitis reported in China. It can be misdiagnosed as fungal keratitis in the clinic. Improving the awareness of clinicians, promoting early diagnosis, and a multidisciplinary approach, especially early surgery, improve the prognosis.

MeSH Keywords: Corneal Ulcer • Microscopy, Confocal • Oomycetes • Pythium

Abbreviations: ***P. insidiosum*** – *Pythium insidiosum*; **PK** – penetrating keratoplasty

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Background

Pythium insidiosum keratitis is an uncommon but sight-threatening disease with high morbidity [1–3]. It occurs when the cornea is infected by *Pythium insidiosum* (*P. insidiosum*). *P. insidiosum*, belonging to the kingdom Stramenopila, is a fungus-like, aquatic oomycete found in tropical, subtropical, and temperate climates [4]. It was long misrecognized as a fungus due to its fungus-like morphologic characteristics. The organism usually presents in 2 forms: one is the hyphae characterized by right-angle branching or broad filaments, and the other is the aquatic motile biflagellate zoospore, which is the infective propagule and only presents in aquatic environments [5]. Clinical presentation of human pythiosis can be classified into 4 types: subcutaneous [6], vascular [7–11], ocular [3,12,13], and systemic [4,14,15]. Consistent with other forms, ocular pythiosis has been proved to have extremely poor prognosis. In retrospect, there are 3 papers that described the rates of patient undergoing enucleation/evisceration. The investigations in 2003 and 2006 revealed the rate to be 87.5% and 79%, respectively [14,16]. Recently, Nitingpong et al. [2] indicated that the rate can reduced to 55% by combination therapy including surgery, antifungal agents, and *P. insidiosum* antigen (PIA). *P. insidiosum* keratitis in younger patients seems to have a better prognosis than in older ones. Aside from enucleation/evisceration, penetrating keratoplasty (PK) is the most effective means for removing infected tissue while protecting the globe and vision. Although cases of *P. insidiosum* keratitis have been discovered in several countries, including Thailand [1,17], India [18], Australia [19], Haidi [3], New Zealand [13], Israel [20,21], Malaysia [22], and Canada [23], there has been no previous case reported originally from China. Here, we present a case of P. *insidiosum* keratitis in a Chinese child.

Case Report

A 7-year-old boy from a town in Hainan province with subtropical climate developed a suppurative corneal ulcer after being scraped by twigs while climbing a tree. That evening his right eye felt gritty, ans 5 days, later without any treatment, he was hospitalized in an eye hospital for examination and treatment.

On arrival, the right eye was found to be very photophobic, with 1/20 vision. A 2×3 mm^2 nasal peripheral corneal white stromal ulcer with staining was surrounded by diffuse infiltration along with multiple radial keratoneuritis at almost 360° (Figure 1A, 1B). A set of corneal scrapings for wet smear and microbial culture, as well as confocal microscopy *in vivo,* were performed instantly. A mass of hyphae found in the wet smear stained positive for acridine orange hydrochloride and lactophenol blue, and was characterized with thick cell wall, sparsely septate, with vehicles inside (Figure 2A, 2B). Numerous filaments with high refraction were observed by confocal microscopy, and were found to be spread, involving the full corneal thickness, arranged in a sinuous, irregular branching pattern, forming "X"," Y", and" S" shapes (Figure 2C). Fungal keratitis was identified but there were no results from fungi culture on the first day after hospitalization. Therefore, an intensive trail of empirical antifungal therapy was performed, including topical Natamycin and Fluconazole eye drops every half hour, topical Fluconazole ointment every night, and oral Voriconazole 100 mg Bid. Topical Atropine ointment was applied for cycloplegia. Seven days later, the cultures of his corneal scraping tissue for fungi, bacteria, and Acanthamoeba showed negative results. However, the symptoms still persisted, the ulcer did not improve, and the area of diffuse infiltration extended progressively. At 12 days later, the corneal opacity was rescraped and recultured, but the results showed negative again. Twenty days later, the area of the ulcer grew to 5×4 mm^2 (Figure 1C, 1D). Surrounding this were subepithelial and superficial stromal opacities with dot-like and tentacle-like infiltrates. The third set of microbial cultures was performed, but with negative results. For private reasons, the patient insisted on discharge on the 27th day after hospitalization, against physician advice. The corneal ulcer expanded to about 7×7 mm^2 when the patient was leaving hospital. He was prescribed Natamycin, fluconazole, and Voriconazole to continue the antifungal therapy when discharged to his home.

On the 14th day after discharge, the young boy again presented with a corneal perforation of the right eye, and was hospitalized that same day. On examination, best corrected visual acuity was hand movement in the right eye and 20/20 in the left eye. The right eye showed a large central corneal ulcer about 9×9.5 mm^2, with an extremely shallow anterior chamber (Figure 1E). Partial keratocele close to the nasal limbus was detected. The infiltrates extended deeply into the anterior chamber, forming endothelial plaques, and occupied the central 8 to 9 mm of the cornea. PK was suggested. Due to more days needed for the cornea donor preparation, antifungal medicines combined with intraocular pressure-lowering agents were offered to the patient. However, iris incarceration was worsening because of the rapidly progressive corneal melting. The lens could not be observed via slit microscope examination (Figure 1F). Therapeutic PK was performed as soon as we obtained the donor cornea. Trephination was done using a Barron radial vacuum trephine (Baron Precision Instruments, LLC, GrandBlanc, MI) with a 9.75-mm diameter circular blade, trying to encompass all the infiltrates. Anterior chamber irrigation with Vancomycin (1 mg/0.1 ml) was also carried out. The fibrinoid membrane on the iris surface and the pupil area was carefully peeled off using forceps. The lens was still not observed during the surgery process. The cut-off cornea bottom was collected and divided into 2 parts for pathogen identification via PCR and microbial cultures. The donor-recipient

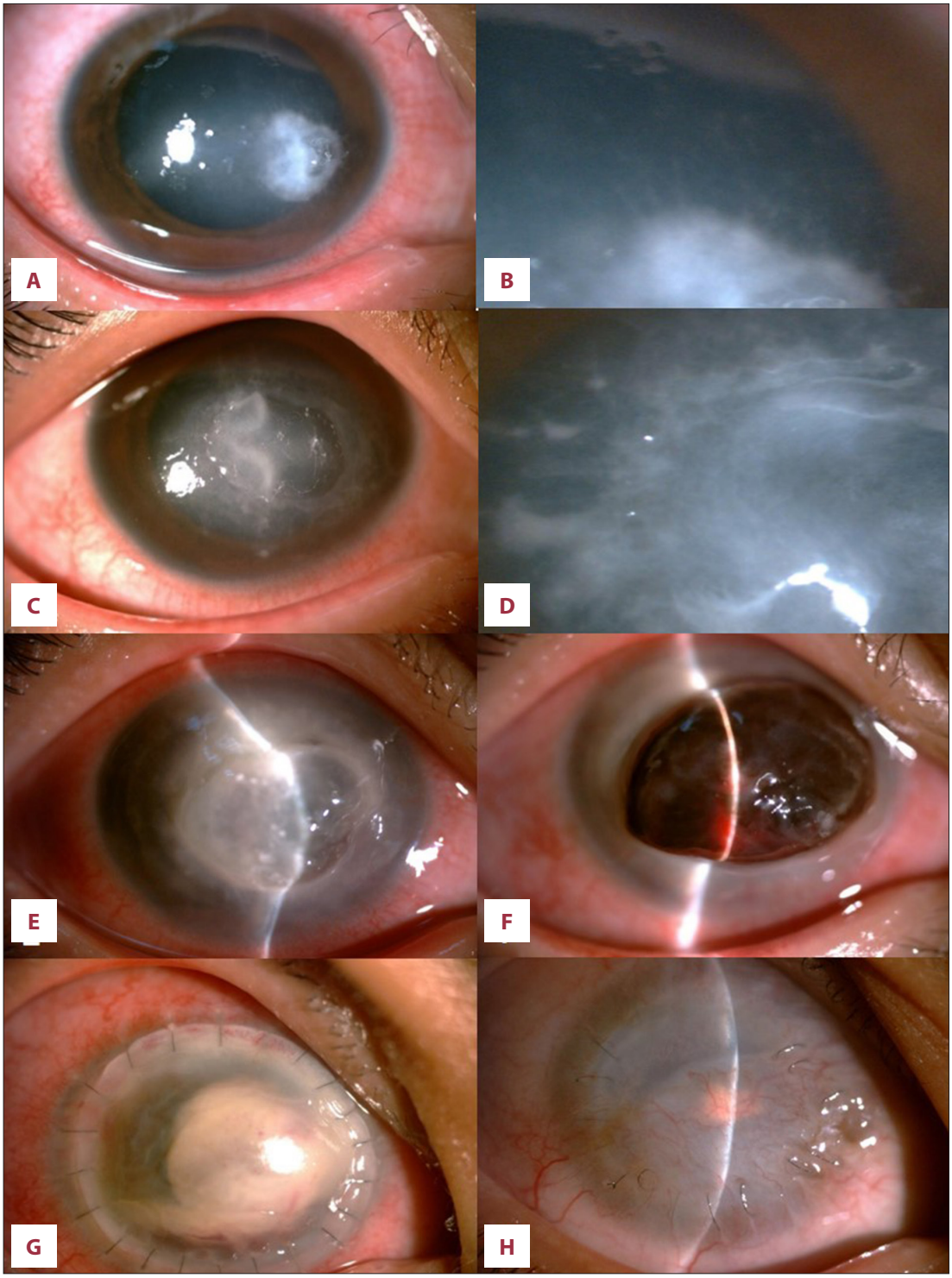

Figure 1. (**A**) Slit-lamp photograph shows a 2×3 mm^2 nasal peripheral corneal white stromal ulcer surrounded by diffuse infiltration. (**B**) Image magnification shows the radial keratoneuritis. (**C**) Worsening of clinical features at day 20. The necrotic ulcer surface with typical flocculent-like morphology appeared. (**D**) Image magnification shows the dot-like and tentacle-like infiltrates. (**E**) Melting of cornea and the formation of partial keratocele and endothelial plaques. (**F**) Corneal perforation with iris incarceration. (**G**) Appearance of anterior chamber white exudate after PK and anterior chamber irrigation. (**H**) Slit-lamp photograph 3 months after PK. Conjunctiva covers the cornea with neovascularization. The white plaque behind the cornea contracted significantly.

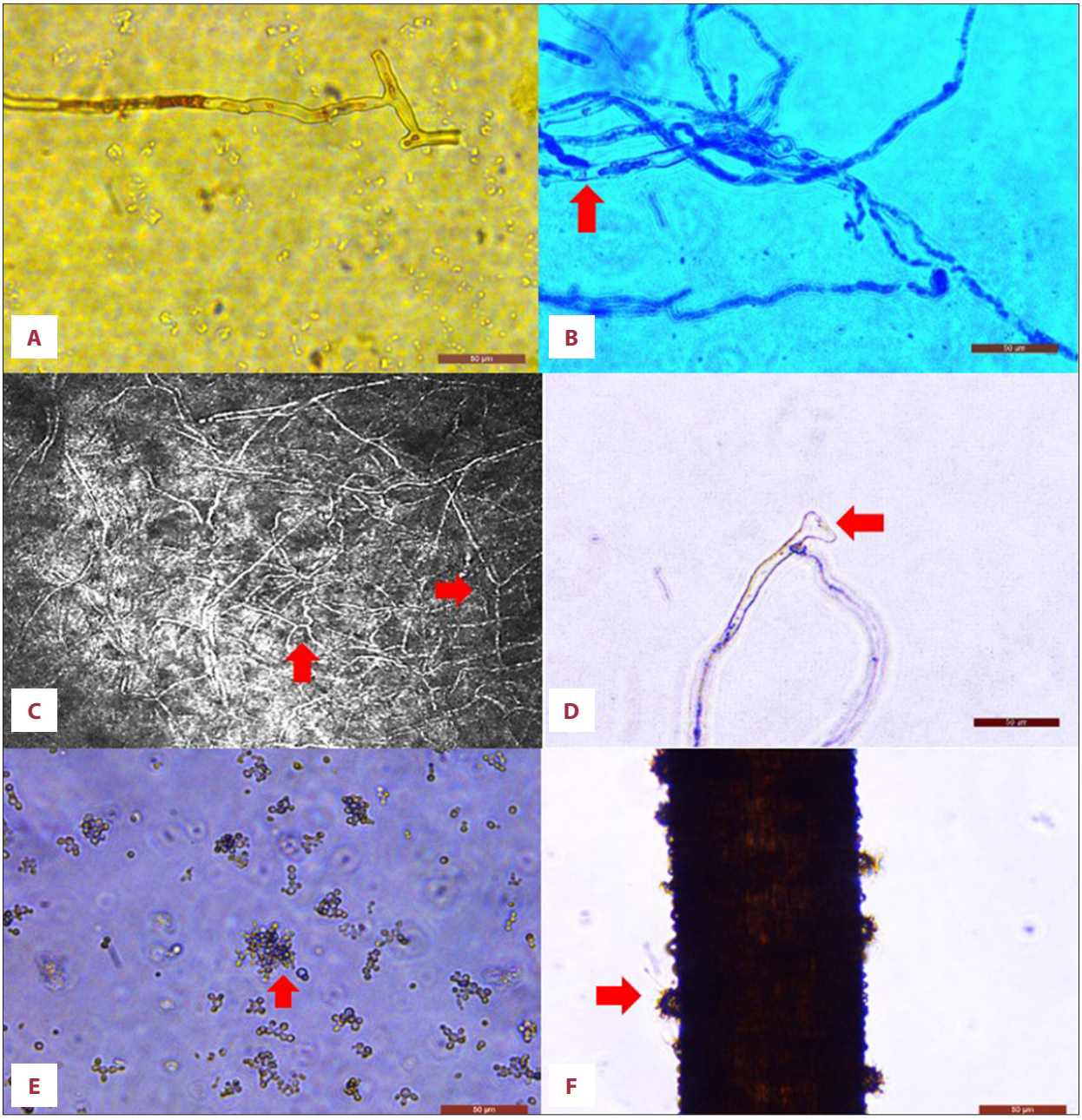

Figure 2. (**A**) "T"- shaped filament with a typical 90-degree angle is positively stained by acridine orange hydrochloride. (**B**) The lactophenol blue positive staining filaments are characterized by thick cell wall, a few septate (red arrow), and mass of vehicles inside. (**C**) The morphology of filaments in infected cornea in confocal microscopy *in vivo*. The irregular filaments arrange in various patterns. The red arrows show the "X"- and "Y"- shaped filaments (red arrow). (**D**) The "end-plate"- like structure in the filament of *P. insidiosum* observed by the microscopy (red arrow). (**E**) The formation and distribution of zoospores (red arrow) in the general broth culture. (**F**) The zoospores adhere to the surface of the hair in water (red arrow).

junction was sutured using 10-0 nylon interrupted sutures. Postoperatively, the patient was treated with topical antifungal medicines, including Natamycin and Fluconazole eyedrops. Two days after the operation, infection recurred, with white hypopyon about 2 mm. Therefore, B ultrasonic scanning was carefully performed to assess the situation of the vitreous (data not shown). There was no direct evidence from B ultrasonic scanning supporting fungal endophthalmitis. Then, anterior chamber irrigation with 0.02% Fluconazole and amphotericin B solution (5 μg/0.1 ml) was sequentially performed. The hypopyon were collected for microbial culture. The white-yellowish clusters succeeded growing out in the potato dextrose agar petri

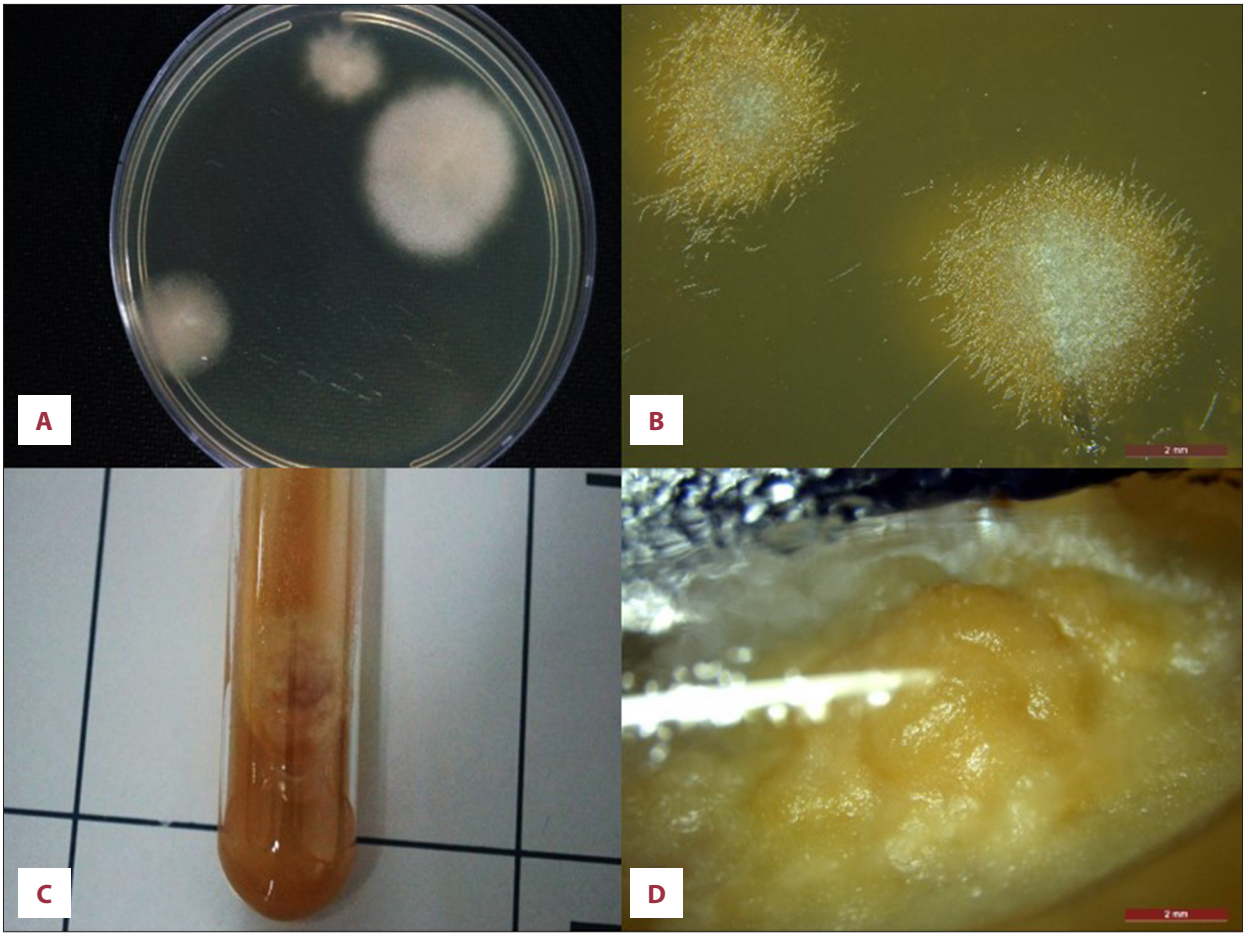

Figure 3. The morphologies of *P. insidiosum* cultured in a petri dish (**A, B**) and a tube (**C, D**) containing potato dextrose agar. (**B**) The white-yellowish clusters in the dish show flat and filamentous form. (**D**) The *P. insidiosum* in tube presents convex and circular forms.

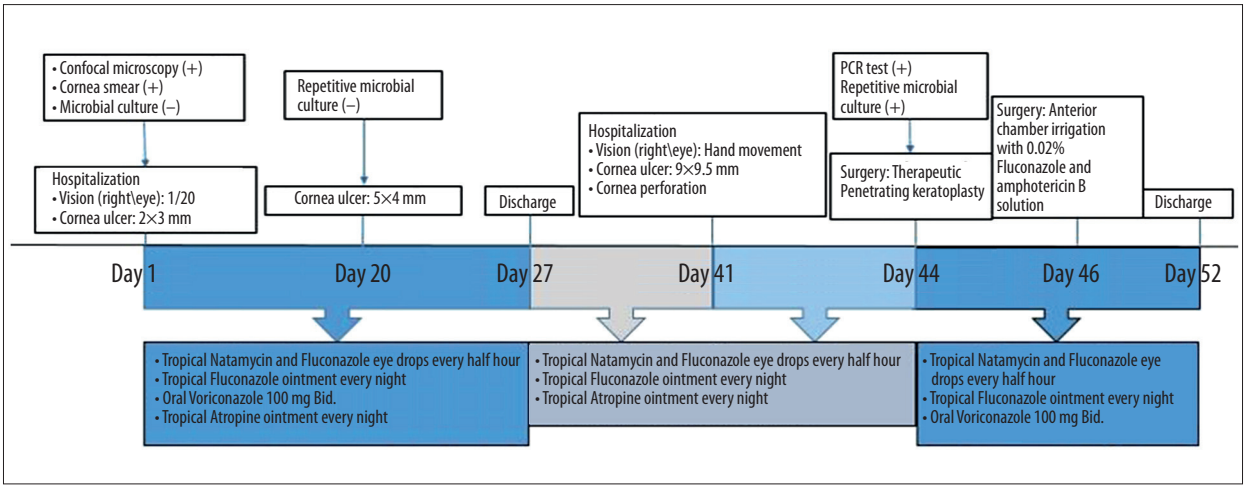

Figure 4. Timeline of interventions and outcomes.

dish and culture tube containing the corneal bottom and/or hypopyon collected in surgery (Figure 3), and confirmed to be *P. insidiosum* by using PCR. Topical antifungal medicines were applied hourly, and oral Voriconazole 100 mg bd was administered after the operation. However, the anterior chamber white plaque occupying the pupillary area reappeared 2 days after anterior chamber irrigation, revealing a suspicious recurrent infection (Figure 1G). Fortunately, the plaque contracted, and the ocular irritation symptom gradually improved. At 3 months after PK, the whole cornea was covered by conjunctiva with neovascularization (Figure 1H). The white plaque inside the anterior chamber significantly contracted. The right eye ball was saved, with hand movement visual acuity. The timeline for hospital course and treatment is presented in Figure 4.

Discussion

We believe this paper is the first report on *P. insidiosum* keratitis originating in China. As a rare but destructive infection disease, most ocular pythiosis cases are officially published from Thailand. Recently, India has been highlighted due to the 9 cases reported in 2015. The researchers from Thailand and India demonstrated that ocular pythiosis may be not as rare as was previously considered, but simply goes unrecognized [2,18]. Briefly, the main reasons for the misdiagnosis of pythiosis include lack of characteristic clinical features, unawareness among clinicians, and a lack of early diagnosis in some regions.

In our case, although multiple laboratory approaches had been carried out, until the performance of PCR, the causative agent had been erroneously assumed to be a fungus. There is scant evidence to use in accurately distinguishing *P. insidiosum* from fungi, and the contributions of medical history, clinical symptoms and signs, and the biomorphology evidences from wet smear, microbial culture, histochemical staining, and confocal microscopy are limited. However, some valuable clues were still discovered by reviewing this case.

The first clue that is easy for clinicians to notice is the necrotic ulcer surface with typical flocculent-like morphology. It appeared at the intermediate stage, along with the presence of dot-like and tentacle-like corneal infiltrates described in Savitri Sharma's report [18]. The flocculent-like sphacelus covered the ulcer surface and became easier to be observed after application of Natamycin. The second clue is the properties of filaments revealed by histochemical staining and confocal microscopy. *P. insidiosum* is an oomycota with filaments resembling fungi. In accordance with previous reports [19,24], the hyphae of *P. insidiosum* stain positive for acridine orange hydrochloride and lactophenol blue, showing a thick cell wall, sparse septate, and numerous vehicles inside. At the end of some filaments, an "end-plate"- like structure was discovered in this case, and was assumed to be associated with sporangias release (Figure 2B). Confocal microscopy has been used for *P. insidiosum* detection in several previous studies [17,20], but few relative characteristics have been described in affected human corneas. In this case, the filaments in the cornea, as recorded by confocal microscopy, were irregularly arranged, and were 3–5 μm wide and 200–400 μm long (some as large as >400 μm). Compared with fungus hyphae, the filaments of *P. insidiosum* displayed more flexible patterns. Some were broad without branches, but most were branching at a right angle, showing a "Y" pattern. Occasionally, parts showed an interlocking pattern characterized by an "X" pattern.

In retrospect, *P. insidiosum* can be cultured in various types of agar, including potato dextrose agar, Sabouraud dextrose agar, and chocolate agar [5,24]. In this case, the pathogen was cultured successfully on potato dextrose agar at 37°C (Figure 3). The colony grown in a petri dish was flat, opaque, and white-yellowish, with filiform margin (Figure 3A, 3B). However, the clusters formed in the culture tube containing potato dextrose agar were convex with light yellowish color (Figure 3C, 3D). The colony was characterized by smooth surface and adhered to the agar media. Zoospores are a valuable sign of *P. insidiosum*, but cannot be detected using the agar media culture system, but can be recorded in either the general broth culture or the water containing antibiotic agent and human hairs (Figure 3E, 3F). The vehicles on the top of filaments formed the sporangias and were released as zoospores, which moved forward and attached to the hair surface (Figure 3F). However, it cannot be ignored that the formation of biflagellate zoospores is not specific for *P. insidiosum*, and is a characteristic shared by other Pythium and Lagenidium species [4,25].

Molecular tools based on the PCR and gene blast technique are the main methods used for identification of the pathogen. In this case and most previous reports, ITS1, ITS2, and 5.8 S rRNA gene are the main target gene amplified and sequenced by PCR [26–29]. Besides, Exo-1,3-β-Glucanase was promoted as a novel target for *Pythium insidiosum* detection recently [30].

Ocular pythiosis usually has an exceptionally high morbidity rate due to the poor response to maximal medical therapy, even with a prompt diagnosis [31]. In this case and in most of previously reported cases, debridement with disease-free surgical margins is considered to be the only effective treatment. The combination of antifungal drugs, antibacterial drugs, iron chelation, and PIA therapy without debridement surgery has been demonstrated to be ineffective in the patients with ocular pythiosis. However, previous *in vitro* experiments revealed the synergistic effects of antifungal drugs against *P. insidiosum* strains. Recently, azithromycin as an antibacterial agent was highlighted in Jesus FP's report due to its effects against subcutaneous pythiosis in a rabbit model. Since 1998, when the

use of PIA first succeeded in curing a patient with a surgical unresponsive vascular pythiosis, it was used as an adjunctive treatment in ocular pythiosis, with inconclusive effect [32–34]. In 2013, a case series report from Thailand described 4 *P. insidiosum* keratitis cases. Recurrent infections appeared in 3 of them after the primary surgery. All 3 patients received reoperation followed by a PIA injection trail [1]. No recurrence was described then, indicating the promising effect of *P. insidiosum* vaccine. However, 1 investigation involving 9 patients with ocular pythiosis, also from Thailand, revealed a controversy on the PIA effect. In Nitingpong's report, all 9 patients received a routine PIA trail before and after surgery, but only 4 of them saved their globes [2]. In our case, immunotherapy was not offered to the patient due to the lack of PIA.

Conclusions

P. insidiosum keratitis is still a challenge to clinicians. A well-designed prospective study is needed to determine the sensitivity and specificity of typical clinical features in making a preliminary diagnosis. Increasing consciousness and use of molecular tools will help make a prompt diagnosis. No standard treatment progress for ocular pythiosis has been proposed, but a multidisciplinary approach, including early surgery, antifungal medicine, and PIA, seems to be a good strategy for patients.

Commercial relationships

None.

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