Ocular Phaeohyphomycosis Caused by *Veronaea botryose*: A Novel Fungal Infection in Human Beings

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Purpose: To describe an aggressive, refractory case of *Veronaea botryosa*-associated mycokeratitis progressing to endophthalmitis.

Methods: Observational case report and review of relevant literature.

Results: An 80-year-old man with a history of lung cancer and diabetes mellitus type 2 presented as an emergent referral to the corneal service with a corneal ulcer and associated endothelial plaque that responded initially to topical steroid and antiviral therapy but subsequently progressed to fungal endophthalmitis. The patient underwent an emergent penetrating keratoplasty and pars plana vitrectomy. Despite multiple negative Grocott methenamine silver smears, gram stains, eye cultures (aerobic, anaerobic, and fungal), and inconclusive confocal microscopy, the host corneal tissue pathology revealed melanin-containing fungi (phaeohyphomycosis). Further speciation of the pathology specimen revealed mold and phenotypic characterization and DNA sequencing confirmed *V. botryose*.

Conclusions: *Veronaea botryose* is a rare fungal infection with previously reported human cutaneous, subcutaneous, and submucosal infections. This is the first documented case of phaeohyphomycosis caused by *V. botryosa* infection in human ocular tissue.

Key Words: fungal keratitis, ocular infection, phaehyphomycosis, *Veronaea botryose*

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Infectious keratitis can be progressive with devastating consequences, including corneal scarring, perforation, endophthalmitis, and loss of vision.[1] Identifying the underlying pathogen is important for the management and prognosis of surgical intervention. For instance, bacterial corneal infections tend to be more aggressive but tend to have better outcomes after corneal transplants; however, fungal infections can be indolent but have poor surgical outcomes. Fungal keratitis is caused by 2 subtypes: filamentous and yeast. The most common yeast to cause fungal keratitis is candida, whereas *Aspergillus* and *Fusarium* are the predominant filamentous agents.[2]

Phaeohyphomycosis is caused by the class of dematiaceous (darkly pigmented) fungi, sometimes called black yeast, and are defined by the presence of melanized yeast-like cells or hyphae in tissues.[3] The melanin is possibly the virulence factor. The production of melanin significantly enhances the virulence of many important human pathogenic fungi.[4] Fungal melanin is important in human disease and melanin's contribution to the ability of the fungi to survive in diverse hostile environments. Melanin has the capacity to alter cytokine responses, decrease phagocytosis, and reduce the toxicity of microbicidal peptides, reactive oxygen species, and antifungal drugs and to play a significant role in fungal cell wall mechanical strength.[4]

Veronaea botryose is a known species in this class of mycoses and has been rarely shown to be the cause human infection, and these have all been superficial and mild in course. Patients diagnosed with phaeohyphomycosis are often immunocompromised (diabetic patients, transplant recipients, and patients on immunosuppressive drugs or steroids).[5] In the literature, 12 cutaneous phaeohyphomycosis induced by *V. botryose* have been reported since 1990,[5] all of which resulted in cutaneous or subcutaneous infections. A review of the literature did not identify any previous known infections involving the ocular tissue. We present the first case of phaeohyphomycosis due to *V. botryosa* in human ocular tissue of an immunocompromised 80-year-old man with no associated cutaneous or subcutaneous findings.

CASE REPORT

An 80-year-old man was referred emergently to the corneal service at a university ophthalmology practice with progressive worsening of vision in the left eye. His medical history was significant for a history of lung cancer in remission treated with lobectomy and chemotherapy, essential hypertension, and well-controlled diabetes mellitus type II. Two months before presentation, he woke up with acute onset left eye pain and foreign body sensation and was unsuccessfully treated by an outside ophthalmologist with topical prednisolone, ketorolac, and artificial tears. He denied any injury, trauma, contact lens wear, fishing, or agricultural work before his symptom onset but did receive a varicella-zoster virus (VZV) vaccine 2 weeks before the onset of ocular symptoms. His ocular history included mild glaucoma, controlled on latanoprost, and bilateral pseudophakia. On presentation, visual acuity in the left eye was hand motion, intraocular pressure was 20 mm of Hg (symmetric with other eye), and there was a normal pupillary response. Slit-lamp biomicroscopy showed a large nasal stromal infiltration measuring 6 × 5.5 × 4 mm³ (Fig. 1A), with associated underlying mutton-fat keratic

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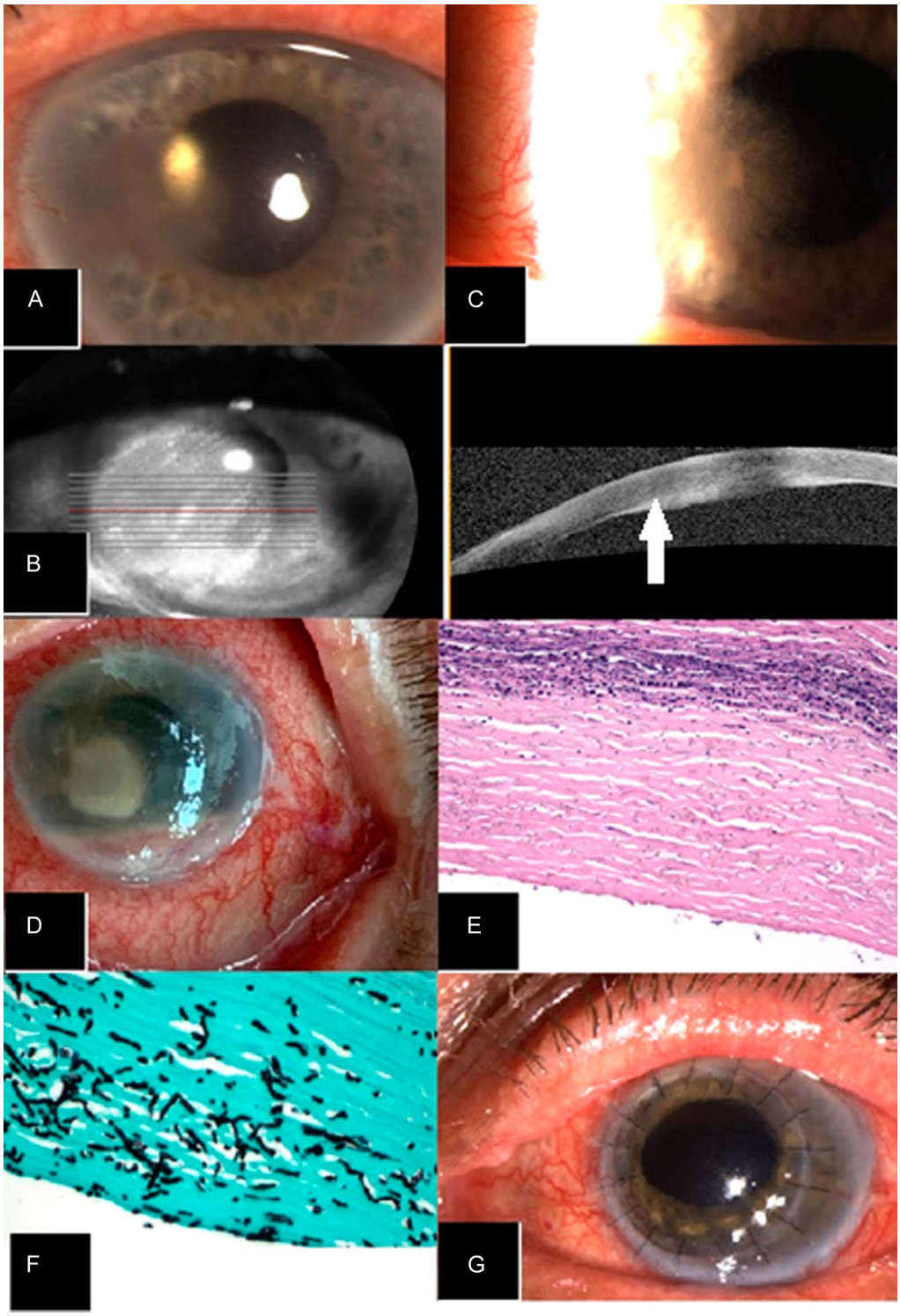

FIGURE 1. A, Large nasal stromal infiltration measuring 6 × 5.5 × 4 mm with no hypopyon or epithelial defect at presentation. B, AS-OCT shows a clear boundary between the corneal endothelial surface and the plaque at presentation. C, One month after tapering topical steroids and antivirals, a round dense endothelial plaque measuring 3.1 (height) × 4.1 (width) with feathery borders was observed. D, A large corneal infiltrate with hypopyon formation 2 weeks after increasing the dose of steroid for the second time. E, Hematoxylin and eosin, 20× magnification, the corneal stroma demonstrates a robust, band-like infiltrate of acute neutrophilic infiltration and the posterior aspect of the corneal stroma shows extensive involvement by fungal hyphae. F, GMS stain, 40× magnification, fungal hyphae stain avidly for silver-based histochemical tissue staining. G, Final vision was 20/40 and without infection flare up in 6 months post-op.

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precipitates localized to the site of stromal infiltration with an endothelial plaque, a small likely long-standing localized small Descemet membrane tear temporal to the area of stromal infiltration and a significant anterior chamber (AC) 3+ reaction. There was no epithelial defect, hypopyon, or anterior vitreous cell; however, early microcystic edema was noted. The posterior examination was unremarkable.

Owing to the concern for infectious etiology, cultures and gram staining as well as anterior segment imaging were performed. Confocal microscopy was inconclusive for any fungal elements. Anterior segment optical coherence tomography (AS-OCT) showed a clear boundary between the corneal endothelial surface and plaque (Fig. 1B). Smear and cultures did not show any organisms. Because of his recent history of VZV vaccination and corneal edema, a working diagnosis of zoster endotheliitis was entertained, and the patient was treated with oral valacyclovir, one gram 3 times daily and readministered on topical steroids 6 times per day. His vision improved to 20/80 after 4 weeks of treatment, and the AC reaction improved tremendously. In addition, the corneal infiltrate was less dense and the plaque was rounder after herpetic treatment. Unfortunately, the patient presented emergently one month later after tapering his topical steroids and oral valacyclovir with counting fingers vision and a round dense endothelial plaque measuring 3.1 (height) × 4.1 (width) with feathery borders nasally (Fig. 1C). Assuming that the patient's infection had rebounded on the prednisolone taper, the topical steroids were readminsitered and the patient's clinical course deteriorated rapidly. After 2 weeks, his vision decreased to hand motion and he developed a large corneal infiltrate with hypopyon (Fig. 1D) and increased intraocular pressure. B scan at this time did not show vitreous involvement. An AC tap was performed and sent for viral polymerase chain reaction (PCR), which was negative for VZV. Corneal scraping for smear and culture was repeated. Antifungal therapy including topical amphotericin and oral voriconazole were started to cover patient for possible fungal etiology. The repeated smear and culture results were negative. Two days later, the patient's vision had decreased to light perception and vitritis was present on examination; therefore, progression to endophthalmitis in the setting of corneal ulcer was diagnosed. The patient underwent emergent temporary keratoplasty, penetrating keratoplasty, pars plana lensectomy, and removal of intraocular lens with intravitreal, subconjunctival, and intrastromal amphotericin B. We were able to remove all the visible infection at the time of surgery, and the intrastromal injection was delivered in the residual part of the recipient cornea.

The host corneal tissue and vitreous sample were sent for pathology evaluation. Hematoxylin and eosin demonstrated a robust, band-like acute neutrophilic infiltration. It additionally showed extensive involvement by melanized fungal hyphae (hematoxylin and eosin, 25×) on the posterior aspect of the corneal stroma (Fig. 1E). Grocott methenamine silver stain also found abundant fungal hyphae for silver-based histochemical tissue staining (Fig. 1F). The cultures grew mold that was identified as *V. botryosa* and confirmed with panfungal polymerase chain reaction, combined phenotypic characterization, and DNA sequencing.

After surgery, the patient was given topical 1% cyclosporine, 0.15% amphotericin B, and 0.5% moxifloxacin 4 times a day and 100 mg oral voriconazole twice per day. Topical 1% prednisolone acetate was started 1-month postoperatively. 0.15% amphotericin B, 1% cyclosporine, and 1% prednisolone were tapered down gradually over 4 months. The patient's best-corrected visual acuity improved to 20/40 at 6 months postoperatively, and the patient did not have any infectious flare up (Fig. 1G).

DISCUSSION

A review of the literature in the past decade illustrates a trend of *V. botryose* infection-associated fungal infections in aquatic animals, including cultured sturgeon (*Acipenser* spp.), captive amphibians, and wild reptiles.[6] Currently, *V. botryosa* ocular phaeohyphomycosis has been reported in fishes in Florida and California.[7] Fungal keratitis of the eye in fish was confirmed to be *V. botryose* by PCR testing. In addition, Kumar et al[8] reported a case of phaeohyphomycosis in a dermal cyst measuring 3.2 cm of right supraorbital region. The occupational, clinical, and histomorphological features after biopsy of this lesion suggested the diagnosis of phaeohyphomycosis, subgroup *V. botryosa*; however, confirmatory PCR testing was not performed in this case report. Our patient denied any traumatic and contact history with organic matter, swimming, or fishing.

Veronaea botryosa can induce chronic diseases of the skin, subcutaneous, submucosal tissue, and also ocular involvement by the presence of fumagoid cells and phaeohyphomycosis. It requires both histopathological and mycological analyses. Histology shows brown hyphae, septate vesicular dark brown thickened wall accompanied by yeast-like elements and also pigmented cell wall in Gomori-Grocott staining. However, melanin was not clinically visible in our eye examination, but it was obvious in pathology.

We present the first report of ocular phaeohyphomycosis caused by *V. botryosa* in human beings. Our patient had a corneal ulcer with an insidious course, mimicking a herpetic endotheliitis with temporary response to antiviral therapy and topical prednisolone. After 5 months of symptoms, we realized that this patient had an occult fungal keratitis with a more typical presentation that rapidly progressed at that point to an endophthalmitis. The infection required emergent surgical intervention with a therapeutic keratoplasty, with pars plana vitrectomy and intravitreal, subconjunctival, and intrastromal injection of amphotericin B. After 6 months, best-corrected visual acuity improved to 20/40 with rigid gas permeable refraction and no flare up of the infection was observed.

Veronaea botryosa belongs to a small genus of widely distributed but poorly understood saprobic fungi typically found in soil and on plant materials. Since 1990, 12 human *V. botryosa* phaeohyphomycoses have been identified in the literature.[5,9–18] Lesions involving of soft tissue, specifically dermis, submucosa, or subcutis of the head and extremities were reported. Patients diagnosed with phaeohyphomycoses are often immunocompromised (diabetic patients, transplant recipients, and patients on immunosuppressive drugs or steroids); however, it is not a predisposing factor for this infection.[5,9,11,18,19] We report *V. botryosa* keratitis, which progressed to endophthalmitis, without any associated dermatological involvement. Our patient's immune status was compromised secondary to multifactorial comorbidities; however, his diabetes mellitus was well controlled and lung cancer had been in remission for 18 months before presentation.

Our initial impression was viral endotheliitis because of the presence of an endothelial plaque. Endothelial plaques are nonspecific and commonly observed in fungal, bacterial, and herpetic keratitis.

In 2017, a study characterizing endothelial plaque using AS-OCT showed that a clear boundary between the corneal endothelial surface and the plaque was most consistent with

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herpetic keratitis.[20] By contrast, in patients with fungal keratitis, the AS-OCT images showed an unclear boundary between the corneal endothelial surface and plaque. The endothelial plaque in our patient did not reflect the findings of this study because our patient's AS-OCT demonstrated a clear boundary between the corneal endothelial surface and plaque.

The patient's keratitis rapidly progressed to endophthalmitis. Progression of infectious keratitis to endophthalmitis is relatively uncommon. In a large study of 9934 patients with infectious keratitis, 0.5% progressed to culture-proven endophthalmitis.[21] This study suggested that the patients at a higher risk for progression to endophthalmitis include patients using topical corticosteroids, fungal keratitis, corneal perforation, and infectious keratitis adjacent to a previous surgical wound. Patients with sequential keratitis and endophthalmitis have generally poor visual outcomes. Our patient's progression to endophthalmitis responded significantly to prompt treatment and surgery. We strongly recommend aggressive diagnostic and surgical management of patients with presumed fungal endophthalmitis. Testing with cultures and smears including Grocott methenamine silver stains, confocal microscopy, AS-OCT, aqueous cultures, and corneal biopsy should all be considered in cases where diagnostic microbiology is difficult. In addition, early surgical intervention to reduce microbial load with therapeutic keratoplasty and pars plana vitrectomy with concurrent intravitreal, subconjunctival, and intrastromal injections of amphotericin B (5 μg/0.05 mL) should be considered to decrease the fungal load. The role of oral antifungals with good ocular penetration, such as voriconazole 200 mg orally daily, can also help decrease the fungal burden, especially if the fungal infection has penetrated into the AC. Postoperatively, topical steroids should be avoided until surgical pathology has excluded fungal infection, and antifungal drops should be continued for 1 month. In cases with confirmed fungal hyphae, we typically wait for 1 month after surgery before starting topical steroids. Topical cyclosporine A 1% four times per day should be considered immediately postoperatively to maintain graft viability if topical steroids are not used.

To our knowledge, this is the first report of human ocular infection of *V. botryosa*.

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