Persistently Vitreous Culture-Positive Exogenous Fungal Endophthalmitis

Short Title: Persistent Exogenous Fungal Endophthalmitis

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Introduction

Exogenous fungal endophthalmitis can occur from direct inoculation after surgery or trauma or extension from infectious keratitis. The incidence of fungal endophthalmitis is approximately 1-4% after trauma, 0.002-0.005% after cataract surgery, and less than 0.5% after infectious keratitis.¹⁻⁵ After trabeculectomies, the 5 year cumulative incidence of culture-proven endophthalmitis is approximately 0.5%; however, only 1% is fungal.^{6,7} Reports of fungal endophthalmitis after intravitreal injections are rare and primarily associated with contaminated triamcinolone.⁸ The symptoms of fungal endophthalmitis can occur acutely or insidiously, with chronic inflammation and infection leading to significant damage to intraocular structures.

The purpose of the study is to identify the clinical settings, fungal isolates, and best corrected visual acuities (BCVA) of patients with persistently vitreous culture-positive exogenous fungal endophthalmitis.

Methods

The retrospective, consecutive, case series was approved by the Institutional Review Board of the University of Miami Miller School of Medicine and was compliant with the Health Insurance Portability and Accountability Act of 1996. The research adhered to the tenets of the Declaration of Helsinki. The inclusion criteria were patients with the same exogenous fungal organism identified on at least 2 consecutive vitreous cultures obtained on separate days at the Bascom Palmer Eye Institute from January 1, 1981 to December 31, 2015. Patients with polymicrobial cultures, endogenous fungal endophthalmitis, and incomplete medical records were excluded.

The vitreous samples were plated on Sabouraud agar, incubated at 35° Celsius for 72 hours, then examined daily for fungal colonies for 2 weeks. Positive samples were stained with Giemsa or calcofluor white. Fungal identification and antifungal susceptibilities were determined by sending the samples to the Fungus Testing Laboratory in San Antonio, Texas. The fungal sensitivities were not repeated for the second set of positive vitreous cultures.

The treatment regimen was chosen by the individual physician based on the patient's clinical course. There were no predefined protocols for the timing and types of treatments. The indications for retreatment were clinically persistent or worsening endophthalmitis, as evidenced by increasing fibrin or hypopyon, persistent fungal infiltrates, worsening visual acuity, and/or persistent intraocular inflammation.

Statistical calculations were performed using the Statistical Package for the Social Sciences software (SPSS Inc, Chicago, Illinois, USA), with a *P* value less than 0.05 being considered statistically significant. Snellen visual acuity was converted to its logarithm of minimal angle of resolution (logMAR) equivalent, with counting fingers being assigned a value of 1.9, hand motion 2.3, light perception 2.7, and no light perception 3.0.⁹ The best corrected visual acuities (BCVA) are presented as the mean logMAR $\pm$ standard deviation, followed by the approximate Snellen chart equivalent. The visual acuities were analyzed using student's t-test and one-way analysis of variance with Tukey post-hoc analyses.

Results

Of the 165 patients with positive fungal vitreous cultures over the 35 year study period, eighteen patients had two positive fungal cultures (11%). Two cases from the 1980s were unavailable for review; therefore, sixteen eyes in 16 patients were included in the study. The mean age was 68.6 ± 18 years old; ten patients were males (63%), and half were right eyes (8/16). The most common past medical histories were hypertension (7/16, 44%) and diabetes mellitus (4/16, 25%). Excluding the 4 diabetic patients, none were systemically immunocompromised. Four patients (25%) were on topical steroids on presentation.

The most common past ocular histories were glaucoma (5/16, 31%), corneal transplants (2/16, 13%), and retinal detachment (1/16, 6%). The mean follow-up period was 42 months (range: 7-288 months). Table 1 summarizes the patient demographics.

The clinical settings included cataract surgery (9/16, 56%), glaucoma surgery (4/16, 25%), trauma (2/16, 13%), and corneal ulcer (1/16, 6%). Excluding the two patients whose previous surgical dates were unknown, the mean time from the predisposing event to the initial onset of symptoms and treatment was 2.0 ± 1.6 months (Figure 1).

The fungal isolates were *Candida* (4/16, 25%, including *C. albicans* (2), *C. parapsilosis* (2)), *Fusarium* (3/16, 19%), *Acremonium strictum* (2/16, 13%), *Curvularia* (2/16, 13%), *Paecilomyces lilacinus* (2/16, 13%), *Aspergillus nigricans* (1/16, 6%), *Phialophora richardsiae* (1/16, 6%), and *Helicomyces* (1/16, 6%). Fourteen patients (88%) were initially treated for presumed bacterial endophthalmitis with intravitreal injections of vancomycin and ceftazidime; two patients received concurrent intravitreal steroids before the diagnoses of fungal endophthalmitis were made (13%). Only two patients (13%) who presented with fungal infiltrates in the anterior chamber and vitreous cavity received intravitreal antifungal therapy as part of their initial therapy. A mean of 4.4 ± 2.8 antifungal injections and 1.1 ± 0.6 antibacterial injections were administered.

Upon identification of fungal isolates, five patients (31%) received intravitreal antifungal injections while 9 (56%) had PPV with antifungal injections. One patient resolved his infection without intravitreal antifungal therapy; he had oral voriconazole and a PPV with removal of the IOL and capsular bag. The initial intravitreal antifungal therapies were amphotericin B (12/16, 75%), voriconazole (4/16, 25%), and miconazole (3/16, 19%). Twelve patients (75%) were placed on systemic antifungal treatment in order to augment local therapy; the medications included oral voriconazole (3/16, 19%), oral diflucan (3/16, 19%), oral ketoconazole (2/16, 13%), oral natamycin (2/16, 13%), intravenous voriconazole (1/16, 6%), and oral fluconazole (1/16, 6%).

All patients underwent a pars plana vitrectomy at least once during the course of their treatment. The 9 patients (53%) whose IOLs and capsular bags were removed had significantly better final visual acuities than those who did not (1.1 ± 1.4 logMAR, $\approx 20/260$, vs. 2.5 ± 0.8 logMAR, light perception, $P=0.011$) (Figure 2). Of the 7 patients whose IOLs and capsular bags were not removed, four (57%) proceeded to enucleation or alcohol ablation.

The fungal sensitivities were available in 9 patients (56%). The minimum inhibitory concentrations (MIC) for all isolates ranged from 0.06-2.1 $\mu\text{g}/\text{ml}$ for voriconazole, 0.2-8 $\mu\text{g}/\text{mL}$ for amphotericin B, and 0.25-6 $\mu\text{g}/\text{ml}$ for fluconazole. Molds

had higher median MICs compared to yeast. The sensitivities are summarized in Table 2.

In the current study, the mean visual acuity on presentation was 1.76 ± 0.9 logMAR (Snellen equivalent $\approx 20/1200$), which was similar to the mean vision at the last examination (1.84 ± 1.2 logMAR, $\approx 20/1400$, $P=0.83$). Two patients (13%) achieved BCVAs of 20/40 or better at the last examination, and half achieved $\geq 20/400$ (8/16). The final visual acuity was similar in mold and yeast ($P=0.37$). The pre-infection BCVA was known in 10 patients; four patients (40%) achieved a final visual acuity within 1 line of their pre-endophthalmitis BCVA. Patients who underwent initial vitrectomies had worse final vision than those who had initial vitreous tap and injects (2.2 ± 1.0 logMAR, $\approx 20/3000$, vs. 1.1 ± 1.0 logMAR, $\approx 20/220$, $P=0.040$). The two patients who received early intravitreal steroids had worse initial and final visual BCVAs than patients who did not (final BCVA: no light perception vs. 1.6 ± 1.1 logMAR, $\approx 20/800$, respectively, $P=0.0028$). There was no difference in the final BCVAs based on the type of antifungal therapy ($P=0.31$), early antifungal therapy ($P=0.14$), or the adjunctive use of systemic antifungal therapy ($P=0.63$). Table 3 summarizes the mean best corrected visual acuities at the initial and last follow-up examinations.

Discussion

Since fungal endophthalmitis is rare, many patients are initially treated for suspected bacterial infections. All but 2 patients with fungal endophthalmitis in the current series were initially treated for bacterial endophthalmitis. Delays in the identification and treatment of fungal infections can result in significant intraocular inflammation and tissue damage. Untreated, *Aspergillus* can cause diminution of the a-waves and b-waves on electroretinogram within 72 hours of infection in animal models.¹⁰ Furthermore, active persistent infection can be difficult to differentiate from residual non-infectious inflammation; in these cases, repeat vitreous cultures can help further treatments.

The most common clinical setting for persistent exogenous fungal endophthalmitis was after cataract surgery, which is one of the most frequently performed anterior segment surgeries. Glaucoma surgery was the second most common clinical setting. Artificial shunts and glaucoma drainage implants may be associated with bleb leakage, tube erosions, and infections.^{11, 12}

The most common fungal isolate in the current case series was *Candida* (4/16, 25%); however, mold was the most common type of fungal isolate (*Fusarium*, *Acremonium*, *Curvularia*, *Paecilomyces*, *Aspergillus*, *Phialophora*, and *Helicomyces*). *Aspergillus* comprised 6% (1/16). In contrast, the most commonly reported cause of exogenous fungal endophthalmitis in the literature was *Aspergillus* (54-74%).^{13, 14} The present study was conducted in an academic facility in South Florida, with referrals from tropical regions; therefore, the fungal isolates and the prevalence of persistent fungal infections in the current study may not reflect the organisms present in other regions of the world.

Antifungal sensitivities were obtained in 56% (9/16) of the persistent fungal endophthalmitis patients. The MICs of isolates to voriconazole and amphotericin B were similar between persistent cases and all cases of fungal endophthalmitis.^{14, 15} Intravitreal voriconazole causes less retinal toxicity than amphotericin B, but the

concentrations of voriconazole remain above the MIC for most fungal organisms for only about 8 hours. In contrast, amphotericin B has a half-life of 1.8 days in aphakic, vitrectomized eyes and 7-15 days in phakic eyes.^{15, 16} Repeat injections of antifungal therapy may be necessary to adequately treat fungal endophthalmitis.

The pathogenesis of persistent exogenous fungal endophthalmitis is unclear but may occur through antifungal resistance, poor drug penetrance, intraocular fungal sequestration, and/or host immunosuppression.¹⁴⁻¹⁷ Resistance to azoles and amphotericin B have been reported due to genetic mutations that increase azole efflux, upregulate P450 drug metabolism, alter the ergosterol biosynthesis pathway, overexpress sterole binding sites, and cause chromosomal non-disjunction.¹⁷ In addition, ketoconazole has poor oral absorption, and intravenous amphotericin B does not reach therapeutic levels in the vitreous.¹⁴ Spores from molds may also sequester inside the eye and are more resistant to azole antifungals.¹⁷ Certain species of *Candida*, *Aspergillus*, and *Fusarium* can form biofilms, which can withstand up to 1000 times the concentration of antifungals required to treat non-encased, planktonic fungus.^{18,19} Methods to overcome antifungal resistance include injecting intravitreal medications, increasing drug concentrations, using lipid formulations of amphotericin B, combining antifungal therapies, and surgically excising fungal infiltrates.¹⁷

Pars plana vitrectomy with removal of the intraocular lens and capsular bag may eliminate the nidus for infection. Irrigation during vitrectomy may remove inflammatory and infectious debris.²⁰ In two case series with a total of 18 patients with fungal endophthalmitis, the infections resolved after PPV with intravitreal antifungal therapy and IOL explantations.^{20, 21} All patients in the current series underwent a PPV during the course of their treatment. One patient (6%) resolved his infection without intravitreal antifungal therapy by undergoing a PPV with removal of the IOL and capsular bag and oral voriconazole.

The overall final visual acuity was poor in patients with persistent fungal endophthalmitis and was similar between eyes with mold or yeast. Only 13% of patients (2/16) achieved 20/40 or better, half were $\geq 20/400$, and 25% of eyes (4/16) were enucleated. A study that included both persistent and non-persistent cases of fungal endophthalmitis found similar visual acuity outcomes; approximately 54% achieved 20/400 or better, and 24% were enucleated.²² The 9 patients (57%) who underwent IOL/capsular bag removals had better final visual acuities than those who did not (20/260 vs. light perception, $P=0.011$), which may have been partly due to improved clearance of infections after the vitrectomies and IOL/capsular bag removals.

Patients who underwent initial PPV and initial intravitreal steroids had worse final visual outcomes, likely reflecting selection biases. Patients with worse clinical presentations were more likely to undergo PPVs than tap and injects. The two patients who received steroids presented with light perception vision and were eventually enucleated. Steroids decrease inflammation, but when administered without concurrent antifungal therapy, *in vitro* studies have demonstrated decreased effectiveness of macrophages and monocytes in suppressing fungal growth.¹⁹ When dexamethasone is given with amphotericin B, however, several case series reported faster clearance of endophthalmitis and not worse clinical outcomes.^{19, 23} The use of intravitreal steroids in fungal endophthalmitis remains controversial.

The limitations of the current study include the retrospective nature, relatively small number of patients, selection bias, and difficulty in obtaining positive fungal cultures. The ability to identify fungal growth with positive cultures has been reported to be between 25-70%.²⁴ Although not used in the current study, polymerase chain reaction could increase early detection of fungal infections.²² Furthermore, it was not possible to demonstrate a difference in clinical outcomes between patients treated with voriconazole compared to amphotericin B in the current study.

In conclusion, persistent vitreous culture-positive exogenous fungal endophthalmitis is uncommon. The most common clinical setting was cataract surgery, and the most common isolate was mold. Multiple intravitreal antifungal injections may be necessary to resolve the fungal endophthalmitis. Patients who underwent pars plana vitrectomies with removal of the IOLs/capsular bags had better final vision. Regardless of the etiology, visual acuity outcomes at the last follow-up were generally poor.

Acknowledgements:

- a. **Funding/Support:** The research was supported in part by the National Eye Institute Center Core Grant (P30EY014801), a Department of Defense Grant (W81XWH-09-1-0675, Washington, D.C.), and an unrestricted grant from the Research to Prevent Blindness, Inc., New York, NY to the Department of Ophthalmology, University of Miami Miller School of Medicine. The funding organizations had no roles in the design or conduct of the research.
- b. **Financial Disclosures:** No financial disclosures.
- c. **Other Acknowledgements:** None.

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Figure 1. Time to Presentation. Box-plot demonstrating the months between the predisposing event to initial presentation with endophthalmitis. The graph excludes 2 patients whose surgical dates were unknown.

Figure 2. Endophthalmitis resolution without intravitreal antifungal. An 81 year old male presented with pain and blurriness in the right eye 2 months after cataract surgery. The initial vision was 20/200 in the right eye. The patient was found on examination to have white plaques on the capsular bag that was visible on slit lamp microscopy (**Top Left**) and retro-illumination (**Top Right**). The patient underwent a PPV/intravitreal vancomycin/intravitreal ceftazidime in the right eye, and the vision improved to 20/50. Three months later, however, the patient developed a recurrent hypopyon and vitritis (**Bottom Left**). He underwent a PPV/IOL removal/intravitreal voriconazole/intravitreal triamcinolone/vitreous cultures; he was also on oral voriconazole. The endophthalmitis resolved, and at the last examination 21 months later, the vision had improved to 20/30 in the right eye (**Bottom Right**).

Table 1. Demographics and Clinical Course. Summary of the patient demographics, clinical course, and final visual acuities in patients with persistently culture-positive fungal endophthalmitis.

	Fungal Endophthalmitis
Mean Age	68.8 ± 18 years
Mean Follow-Up	42.0 ± 72 months
Mean Number of Antibiotics	1.1
Mean Number of Antifungals	2.7
Mean Number of Treatments	4.4
Mean time to 1 st treatment	2.0 ± 1.6 months
Mean Initial Visual Acuity (logMAR, Snellen equivalent)	1.76 ± 0.9 ≈ 20/1200
Mean Final Visual Acuity (logMAR, Snellen equivalent)	1.84 ± 1.2 ≈ 20/1400

Table 2. Fungal Minimum Inhibitory Concentrations. Comparison of the Minimum Inhibitory Concentrations ($\mu\text{g/ml}$) for different antifungal therapies in patients with persistent culture-positive endophthalmitis.*

Fungal Isolate (Number of isolates= 9)	Amphotericin B ($\mu\text{g/ml}$)	Voriconazole ($\mu\text{g/ml}$)	Fluconazole ($\mu\text{g/ml}$)
Acremonium (1)	1	0.5	0.5
Aspergillus (1)	8	0.25	64
Candida (1)	0.06	0.02	0.25
Curvularia (1)	0.5	0.06	4
Dematicus (1)	0.2	-	-
Fusarium (2)	1.0	2.1	64
Paecilomyces (1)	16	0.25	64
Phialophora (1)	0.5	-	-

* Included are 3 eyes whose specimens were previously re-cultured to determine the antifungal sensitivities.¹²

Table 3. Mean Visual Acuities. Comparison of mean initial and final best corrected visual acuities based on clinical setting of patients with persistent culture-positive fungal endophthalmitis.

Clinical Setting (Number of patients)	Initial Visual Acuity		Final Visual Acuity	
	logMAR	Snellen Equivalent	logMAR	Snellen Equivalent
Cataract Surgery (9)	1.4 ± 0.9	20/470	1.4 ± 1.1	20/500
Glaucoma Surgery (4)	1.6 ± 0.9	20/730	2.3 ± 1.2	Hand Motion
Trauma (2)	2.3 ± 0.6	Hand Motion	2.9 ± 0.2	Light Perception
Corneal Surgery (1)	1.3 ± 0	20/400	1.3 ± 0	20/400

Figure 1

**Months between Inciting Event
and Endophthalmitis**

Figure 2