Current Fungal Infection Reports (2018) 12:66–70
https://doi.org/10.1007/s12281-018-0315-z

ADVANCES IN DIAGNOSIS OF INVASIVE FUNGAL INFECTIONS (S CHEN, SECTION EDITOR)

Fungal Eye Infections: New Hosts, Novel Emerging Pathogens but No New Treatments?

Christina C. Chang [1] · Sharon C-A Chen [2,3]

Published online: 22 March 2018

Abstract

Purpose of Review We sought to explore the current incidence and associated risk factors associated with fungal eye infections. We also reviewed new diagnostic strategies and recent clinical studies exploring the use of topical and oral antifungal agents.

Recent Findings Incidence and associated risks continue to vary with geographic region, and access to timely healthcare. Nosocomial fungal endophthalmitis can result from minor surgical procedures to the eye. Molecular methods offer increasing diagnostic utility. Clinical treatment studies have mainly focussed on the treatment of fungal keratitis and have been conducted in South Asia. Topical natamycin remains superior to topical reconstituted voriconazole and remains the preferred therapy including for *Fusarium* eye infections. Neither adjunctive oral ketoconazole nor oral voriconazole has been shown to have added clear benefit to topical treatment.

Summary Larger international studies with more heterogenous populations are required for future clinical studies which should include patients with contact lens fungal keratitis and those with fungal endophthalmitis. Basic science studies exploring the immunology of fungal eye infections and drug levels to understand the differences in clinical outcomes are encouraged.

Keywords Fungal keratitis · Fungal endophthalmitis · Invasive fungal infection · Contact lens infection · Topical natamycin · Voriconazole · Mycotic ulcer

Introduction

Fungal eye infections predominantly present as *keratitis*, involving the cornea or "anterior eye", or more uncommonly *endophthalmitis*, involving the vitreous or aqueous humour or both, or the retina. The term "endophthalmitis" is typically used to refer to bacterial or fungal causes of this entity, while "uveitis" is generally reserved for intraocular infections due to viruses or parasites.

Endophthalmitis may be classed as exogenous or endogenous endophthalmitis. In general, and especially for fungal endophthalmitis, endophthalmitis is more frequently due to exogenous causes where organisms are introduced into the eye through an external source while in endogenous endophthalmitis, fungal organisms seed into the eye haematogenously, such as during candidemia or fungal endocarditis. Thus, *Candida* spp. is the most commonly isolated organism in endogenous fungal endophthalmitis, while moulds such as *Fusarium* spp. and *Aspergillus* spp. predominate in exogenous fungal endophthalmitis. Here, we will focus on discussing fungal keratitis and both exogenous and endogenous fungal endophthalmitis. We discuss data pertaining to new host risk groups for fungal eye infections, recent advances in diagnostic tools and the expanding repertoire, though of uncertain role if any, of antifungal agents in its management.

This article is part of the Topical Collection on *Advances in Diagnosis of Invasive Fungal Infections*

✉ Sharon C-A Chen
Sharon.chen@health.nsw.gov.au

[1] Department of Infectious Diseases, Alfred Hospital, Monash University, Melbourne, Australia

[2] Marie Bashir Institute for Emerging Infectious Diseases and Biosecurity, University of Sydney, Sydney, Australia

[3] Centre for Infectious Diseases and Microbiology Laboratory Services, ICPMR, New South Wales Health Pathology, Westmead Hospital, 3rd Level, ICPMR Building, Darcy Road, Westmead, NSW 2145, Australia

Fungal Keratitis

Fungal causes of cornea inflammation are most often associated with ocular trauma and have a higher rate of corneal perforation than bacterial keratitis. The incidence of fungal keratitis is higher in resource-limited settings where occupational and safety measures are less entrenched, further aggravated by delayed presentations to health services. Patients often present with a painful eye and often relate a history of trauma associated with vegetative matter contaminated with soil. In resource-rich settings, contact lens fungal keratitis has become prominent. Filamentous fungi such as *Fusarium*, *Aspergillus*, and *Scedosporium* are often found as the causative agent, though a broad range of pathogens including rare fungi may be implicated.

Geography plays a major role. In a study of nearly 24,000 corneal patients in India, more than a third of infectious corneal ulcers were of fungal aetiology with *Fusarium* and *Aspergillus* spp. as the predominant organisms [1]. A staggering majority are due to avoidable trauma. In a paediatric population of 234 cases of infectious keratitis, 53% were associated with trauma including from thorn, stick and vegetative material or stone, sand and dust with nearly 60% of cultured fungi identified as *Fusarium* spp. [2]. Similarly, nearly half or the participants in a large fungal keratitis study of 326 persons were agricultural workers where nearly two thirds experienced ocular trauma from vegetative material as their main risk factor.

Obtaining a timely tissue diagnosis is important and the importance of fungal culture and identification cannot be over-emphasised. Eye swabs are usually inadequate as penetration into deeper corneal layers is common. Hence, corneal scraping using a surgical blade or platinum spatula is recommended. A Gram stain is the usual first step followed by a wet preparation by potassium hydroxide (KOH), ink-KOH, lactophenol cotton blue, Giemsa or calcofluor white. For isolation of fungi and bacteria, blood agar and chocolate agar may be used for bacterial culture while Sabouraud dextrose agar (SDA) is the culture medium of choice for fungi.

Endopthalmitis

Endophthalmitis is a medical emergency and can lead to irreversible blindness in a matter of hours [3]. It is however uncommon. The incidence varies by category—the rate of endophthalmitis after cataract surgery is approximately 0.1% while the rate after penetrating eye trauma is 1 to 18% (reviewed in [3]). Globally, post-operative cases account for 40–80% and case posttrauma for 2–15% of all endophthalmitis cases seen at centres in Brazil, England, Israel, Iran, India, Australia and South Korea (reviewed in [3]). In a 10-year review of the culture-proven endophthalmitis cases seen at Bascom Palmer Eye Institute, Miami, fungal causes accounted for 71 of 448 cases (15.8%), of which more than one third of was due to *Candida albicans* [4].

Exogenous Endophthalmitis

Most cases of endophthalmitis are due to exogenous causes and may be classified primarily by risk factor such as post-cataract after cataract surgery, post-traumatic after penetrating eye injury, keratitis-related associated with corneal ulceration and bleb-related usually in association with glaucoma, and more recently post-injection after intravitreal injection (reviewed in [3]).

Post-injection After Intravitreal Injection

Following on from the devastating outbreak of *Exserohilum rostratum* fungal meningitis from contaminated methylprednisolone vials involving more than 700 cases of which more than 150 were culture-positive [5, 6], a concerning outbreak of bacterial endophthalmitis due to *Granulicatella adiacens* and *Abiotrophia* spp. was reported attributed to repackaged single-use vials of bevacizumab, an antineoplastic agent commonly used off-label to treat retinal disorders [7].

Ordinarily, the overall incidence of "all-comer" causes of endophthalmitis following anti-VEGF (vascular endothelial growth factor) agents is very low, confirmed in two recent large retrospective reviews. In a multisite study around the USA of 503,890 injections, the rate of endophthalmitis was 0.039, 0.035 and 0.035% for bevacizumab, ranibizumab and aflibercept, respectively [8]. Of 173 patients with endophthalmitis, one was culture-positive for *Candida parapsiloisis* and nearly 60% were culture-negative. In another review of 54,101 injections of bevacizumab, 5614 injections of ranibizumab and 3468 injections of aflibercept, the incidence of suspected causes of endophthalmitis was < 0.006% and culture-positive cases were reported in only 0.017, 0.02 and 0.03% for bevacizumab, ranibizumab and aflibercept, respectively. While bacterial infections were reported in 11 of the cases and no fungal endophthalmitis cases were specifically reported, the remaining 15 (57.7%) were culture-negative [9]. Contemporary experience with intravitreal bevacizumab in the Middle East reported similarly low incidence rates [10, 11].

In contrast, an outbreak of fungal exophthalmitis caused by *Bipolaris hawaiiensis* was reported in eight patients all whom developed floaters and were diagnosed with endophthalmitis 41 to 97 days after receiving the intravitreal injection of compounded combined bevacizumab and triamcinolone, which was prepared by the same compounding pharmacy [12]. These events have led to stricter regulations of compounding pharmacies and an increasing awareness of possible fungal contamination.

Endogenous Fungal Endophthalmitis

The current opioid crises globally and particularly in the USA have led to increasing reports of endogenous fungal endophthalmitis in injecting drug users with 10 patients reported in New England, in a 2-year period compared to three in the preceding similar period [13]. Of the 10 patients, all had unilateral involvement and presented variably with floaters (80%), reduced vision (60%) and eye pain (50%) and photophobia (30%); 90% of patients were systematically well and blood culture was positive for fungus (*Candida tropicalis*) in only one [13]. Of the nine patients who acceded to hospitalisation, all received empirical systemic antifungals followed by a diagnostic vitreous tap and intravitreal injection within 3 days of presentation. As such, only 2 vitreous biopsies were positive for *C. albicans* and one for *Candida dublinensis* [13].

In contrast, in a retrospective review of 9 patients with microbiologically confirmed ocular candidiasis in Japan over 9 years, 4 (44.4%) patients had a fever and 66.7% had a positive blood culture. Guidelines continue to advocate for clinicians to assess for ocular involvement in those with candidemia [14, 15] but a lower threshold is necessary in injecting drug users as transitory fungemia is sufficient to cause ocular tissue involvement. This was noted elegantly in a paper published in 1973 where a patient, despite only having 36 h of candidemia and clinical resolution of fever with systemic antifungals, developed endophthalmitis 7 days later [16]. It is likely that visual symptoms are under-reported, under-appreciated and under-investigated. In the current climate of increasing recreational drug use, long-term vascular catheterisation and long-term intravascular devices, eliciting a history of visual disturbance, examination for visual acuity and retinal involvement should be encouraged in all such patients with formal ophthalmological assessment.

Recent Advances in Diagnostics

The small volume of vitreous fluid and minute sizes of corneal biopsy are major barriers to successful laboratory diagnostics. Prioritisation of laboratory tests is paramount. Non-culture techniques including large multiplex polymerase chase reaction assays have become more prominent, in the same way smaller multiplexes (often in-house PCRs) detecting the *Herpesviridae* are routinely used in many laboratories. Others have attempted to apply large multiplex PCR against a multitude of bacteria, mycobacteria, viruses and fungi.

Nonetheless, culture-based methods remain the mainstay to microbiological diagnosis. There have been recent attempts at culturing vitreous fluid in blood culture bottles to enhance sensitivity. A large study of 247 patients with clinically suspected endophthalmitis over an 8-year period in Germany compared direct intra-operative inoculation of 1–3 ml of vitreous fluid into BACTEC Peds Plus/F bottle (for the detection of aerobic bacteria) and into the BACTEC anaerobic bottle (BACTEC 9240 system, Becton Dickinson, Heidelberg, Germany) to 10 μL plated onto solid media including chocolate agar, blood agar, MacConkey agar, chromogenic yeast medium and two solid anaerobic media and 100 μL into liquid broth including aerobic brain heart infusion broth and anaerobic Schaedler Broth for the detection of microorganisms [17]. Of the 86 culture-positive samples, microorganisms were more frequently grown from blood culture bottles ($n = 77$, 55%) compared to broth solution ($n = 63$, 45%, $p = 0.007$) and solid media ($n = 46$, 33%, $p < 0.0001$) [17]. Nine specimens were positive for *C. albicans* and two for *Aspergillus fumigatus*. All 11 grown fungi were detected by blood culture bottles whereas broth solution recovered 64% and solid media 46% of grown fungi, suggesting the value of inoculating vitreous samples into blood culture bottles may be of even larger value in suspected fungal infections [17].

Reflecting on the various laboratory protocols in three teaching institutions in MA, USA, applied on 5736 vitreous samples, authors recommended that appropriate vitreous sample processing involves categorising the sample into themes, such as lymphoma or malignancy, infectious and therapeutic [18]. In those suspicious for infection, an undiluted refrigerated specimen (0.5–1.0 ml) should be sent for PCR analysis of Herpes simplex virus, varicella zoster virus, toxoplasmosis, *Mycobacterium tuberculosis* complex, cytomegalovirus and blood taken for *Toxocara canis* antibody testing, depending on clinical suspicion.

There has been a recent attempt to explore the ability of next-generation sequencing in identifying pathogens retrospectively from stored formalin-embedded specimens derived from 14 penetrating keratoplasties, an enucleation due to perforation of a corneal ulcer and a small limbal biopsy [19]. The Illumina NextSeq instrument produced 75 base-pair reads per DNA fragment which were analysed with two different metagenomics classification databases Krakens and Centrifuge where the former contains a wide range of human, mouse, viral and bacterial genomes but few eukaryopathic pathogens, while the latter comprised of finished and partial genomes of nearly a million species across 75 thousand genera including many eukaryotes [19]. Five of these 14 cases had previously identified fungi by culture, including *Aspergillus flavus*, *C. albicans*/*C. dublinensis*, *Curvularia clavata* and *Fusarium solani* and an unidentified yeast. The unidentified yeast was identified as *C. parapsillosis*, the *Fusarium* was identified only on the Centrifuge platform, the *A. flavus* was mis-identified as *A fumigatus* and *A oryzae* and both methods detected *C albicans* [10]. The lack of

breadth and depth in the sequence library particularly in eukaryotes remains a major limiting factor.

Recent Clinical Trials in Fungal Keratitis

There have been some new insights albeit with some disappointing results in recent clinical studies of treatment of fungal corneal ulcers. Natamycin eye drops was introduced in the 1960s and has long been the only federal drug authority (FDA)-approved topical treatment for fungal keratitis. With the advent of newer azoles in the last 15 years, clinicians have developed a liking for topical reconstituted voriconazole and many have attempted adjunctive oral antifungal agents including oral voriconazole.

Unfortunately, results of recent clinical studies have been disappointing. The Mycotic Ulcer Topical Treatment Trial I (MUTT I) was a National Eye Institute-supported, randomised, double-blind, multi-centre clinical trial comparing outcomes in patients with fungal corneal ulcers receiving topical natamycin, 5% (Natacyn; preserved with benzalkonium chloride, 0.01%) and topical voriconazole, 1% (Vfend IV; reconstituted in sterile water for injection with benzalkonium chloride, 0.01%) which enrolled 326 patients in South India [20]. One drop was applied to the affected eye every 1 h while awake for 1 week, then every 2 h while awake until 3 weeks from enrolment with further continuation of the masked medication at the discretion of the physician. The most commonly isolated organisms were *Fusarium* (128 patients [40%]), followed by *Aspergillus,* species (54 patients [17%]) [20].

The primary outcome was best spectacle-corrected visual acuity (BSCVA) at 3 months and patients randomised to receive voriconazole fared 1.8 times worse than those receiving natamycin (regression coefficient = − 0.18 logMAR; 95% CI, − 0.30 to − 0.05; $p = 0.006$) [20]. Further, more patients in the voriconazole arm (48%) tested culture-positive at 6 days than individuals randomised to natamycin (15%), ($p < 0.001$) [20]. Patients with ulcers randomised to natamycin were less likely to undergo perforation or transplantation (odds ratio = 0.42; 95% CI, 0.22 to 0.80; $p = 0.009$) [20]. Interestingly, the difference in efficacy noted in this trial was primarily attributable to cases caused by *Fusarium* spp. *Fusarium*-infected patients randomised to receive natamycin had outcomes 4.1 times better than those for patients randomised to voriconazole (regression coefficient = − 0.41 logMAR; 95% CI, − 0.61 to − 0.20; $p < 0.001$) [20].

A smaller RCT of 118 patients with fungal keratitis compared a commercially available 1% voriconazole eye drop (Aurolab, Madurai, India) to 5% natamycin eye drops also showed no difference between the groups in the number of patients who either did not improve or marginally worsened on day 7, thought the authors reported marginal advantage at the last, (albeit undefined) follow-up visit [21]. However, this study was not powered to demonstrate statistical difference.

Exploring the role of oral antifungal agents, adjunctive oral ketoconazole 200 mg twice a day was compared to placebo in a study of 115 culture-positive fungal keratitis treated with topical natamycin. This showed no difference in clinical cure rates between the two groups nor when corrected by ulcer size [22]. Disappointingly, MUTT II another double-blind, randomised RCT followed and compared oral voriconazole to placebo as an adjunct to topical eye drops, in 240 participants with severe fungal corneal ulcers, enrolled in India and Nepal [23]. There was no difference in the rate of corneal perforation or the need for therapeutic penetrating keratoplasty for oral voriconazole vs placebo (hazard ratio 0.82; 95% CI, 0.57–1.18; $p = 0.29$). Indeed, patients in the voriconazole arm experienced significantly more adverse events compared to the placebo arm.

In the updated Cochrane review of medical interventions for fungal keratitis where 12 clinical trials were reviewed, at least seven were deemed at high risk of bias in one or more domains with overall inconclusive results as most comparisons only one small trial available [24]. The exception was the comparison of topical natamycin and topical voriconazole for which three trials were available. This combined analysis revealed that people randomised to natamycin had better spectacle-corrected visual acuity at 2 to 3 months compared to people randomised to voriconazole but the estimate was uncertain and the 95% confidence intervals included 0 (no difference) (mean difference − 0.12 logMAR, 95% CI − 0.31 to 0.06, 434 participants; 3 studies, low-quality evidence) and a decreased risk of corneal perforation or therapeutic penetrating keratoplasty, or both (RR 0.61; 95% CI 0.40 to 0.94, 434 participants, high-quality evidence) [24].

Conclusions

Fungal keratitis is common especially in resource-poor settings, has well-defined risk factors in this context including from occupational or environmental exposure. Endophthalmitis conversely is uncommon, may follow minor trauma or be a complication of haematogenous infection where *Candida* spp. are the commonest cause. Molecular methods offer increasing diagnostic utility. Clinical treatment studies have mainly focussed on the treatment of fungal keratitis, and topical natamycin remains superior to topical reconstituted voriconazole and remains the preferred therapy including for *Fusarium* eye infections. Neither adjunctive oral ketoconazole nor oral voriconazole has been shown to have added clear benefit to topical treatment. Larger international studies with more heterogenous populations are required for future clinical studies which should include patients with fungal endophthalmitis. Basic science studies exploring the host

response to fungal eye infections to understand the differences in clinical outcomes are encouraged.

Compliance with Ethical Standards

Conflict of Interest Christina C. Chang declares no conflict of interest. Sharon C-A Chen has received grant funding from MSD (AUS).

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Lalitha P, Prajna NV, Manoharan G, Srinivasan M, Mascarenhas J, Das M, et al. Trends in bacterial and fungal keratitis in South India, 2002-2012. Br J Ophthalmol. 2015;99(2):192–4.
2. Aruljyothi L, Radhakrishnan N, Prajna VN, Lalitha P. Clinical and microbiological study of paediatric infectious keratitis in South India: a 3-year study (2011-2013). Br J Ophthalmol. 2016;100(12):1719–23.
3. Durand ML. Bacterial and fungal endophthalmitis. Clin Microbiol Rev. 2017;30(3):597–613.
4. Schimel AM, Miller D, Flynn HW Jr. Endophthalmitis isolates and antibiotic susceptibilities: a 10-year review of culture-proven cases. Am J Ophthalmol. 2013;156(1):50–2. e1
5. Kainer MA, Reagan DR, Nguyen DB, Wiese AD, Wise ME, Ward J, et al. Fungal infections associated with contaminated methylprednisolone in Tennessee. N Engl J Med. 2012;367(23):2194–203.
6. Kauffman CA, Pappas PG, Patterson TF. Fungal infections associated with contaminated methylprednisolone injections. N Engl J Med. 2013;368(26):2495–500.
7. Edison LS, Dishman HO, Tobin-D'Angelo MJ, Allen CR, Guh AY, Drenzek CL. Endophthalmitis outbreak associated with repackaged bevacizumab. Emerg Infect Dis. 2015;21(1):171–3.
8. Rayess N, Rahimy E, Storey P, Shah CP, Wolfe JD, Chen E, et al. Postinjection endophthalmitis rates and characteristics following intravitreal bevacizumab, ranibizumab, and aflibercept. Am J Ophthalmol. 2016;165:88–93.
9. Forooghian F, Albiani DA, Kirker AW, Merkur AB. Comparison of endophthalmitis rates following intravitreal injection of compounded bevacizumab, ranibizumab, and aflibercept. Can J Ophthalmol. 2017;52(6):616–9.
10. Al-Rashaed S, et al. Incidence of endophthalmitis after intravitreal anti-vascular endothelial growth factor: experience in Saudi Arabia. Middle East Afr J Ophthalmol. 2016;23(1):60–3.
11. Wani VB, al-Kandari J, Sabti K, Aljassar F, Qali H, Kumar N, et al. Incidence of endophthalmitis after intravitreal bevacizumab using aliquots prepared on-site in 2 operating rooms in Kuwait. Middle East Afr J Ophthalmol. 2016;23(1):64–70.
12. Sheyman AT, Cohen BZ, Friedman AH, Ackert JM. An outbreak of fungal endophthalmitis after intravitreal injection of compounded combined bevacizumab and triamcinolone. JAMA Ophthalmol. 2013;131(7):864–9.
13. Tirpack AR, Duker JS, Baumal CR. An outbreak of endogenous fungal endophthalmitis among intravenous drug abusers in New England. JAMA Ophthalmol. 2017;135(6):534–40.
14. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;62(4):e1–50.
15. Chen SC, Sorrell TC, Chang CC, Paige EK, Bryant PA, Slavin MA. Consensus guidelines for the treatment of yeast infections in the haematology, oncology and intensive care setting, 2014. Intern Med J. 2014;44(12b):1315–32.
16. Weinstein AJ, Johnson EH, Moellering RC Jr. Candida endophthalmitis: a complication of candidemia. Arch Intern Med. 1973;132(5):749–52.
17. Kehrmann J, Chapot V, Buer J, Rating P, Bornfeld N, Steinmann J. Diagnostic performance of blood culture bottles for vitreous culture compared to conventional microbiological cultures in patients with suspected endophthalmitis. Eur J Clin Microbiol Infect Dis. 2018;
18. Mehta M, Rasheed RA, Duker J, Reichel E, Feinberg E, Husain D, et al. Vitreous evaluation: a diagnostic challenge. Ophthalmology. 2015;122(3):531–7.
19. Li Z, Breitwieser FP, Lu J, Jun AS, Asnaghi L, Salzberg SL, et al. Identifying corneal infections in formalin-fixed specimens using next generation sequencing. Invest Ophthalmol Vis Sci. 2018;59(1):280–8.
20. Prajna NV, Krishnan T, Mascarenhas J, Rajaraman R, Prajna L, Srinivasan M, et al. The mycotic ulcer treatment trial: a randomized trial comparing natamycin vs voriconazole. JAMA Ophthalmol. 2013;131(4):422–9.
21. Sharma S, Das S, Virdi A, Fernandes M, Sahu SK, Kumar Koday N, et al. Re-appraisal of topical 1% voriconazole and 5% natamycin in the treatment of fungal keratitis in a randomised trial. Br J Ophthalmol. 2015;99(9):1190–5.
22. Rajaraman R, Bhat P, Vaidee V, Maskibail S, Raghavan A, Sivasubramaniam S, et al. Topical 5% natamycin with oral ketoconazole in filamentous fungal keratitis: a randomized controlled trial. Asia Pac J Ophthalmol (Phila). 2015;4(3):146–50.
23. Prajna NV, Krishnan T, Rajaraman R, Patel S, Srinivasan M, Das M, et al. Effect of oral voriconazole on fungal keratitis in the Mycotic Ulcer Treatment Trial II (MUTT II): a randomized clinical trial. JAMA Ophthalmol. 2016;134(12):1365–72.
24. FlorCruz NV, Evans JR, Medical interventions for fungal keratitis. Cochrane Database Syst Rev, 2015(4): p. CD004241.