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An overview of fungal keratitis

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Abstract

Purpose of review: Fungal keratitis is associated with significant morbidity and is of major concern in the developing world where filamentous fungi such as *Fusarium* species predominate. Standardised diagnostic modalities and treatment strategies are not well established, and thus management is constantly evolving. This review is aimed at providing clinicians with an updated overview of fungal keratitis.

Recent findings: Molecular identification methods such as polymerase chain reaction offer superior diagnostic and prognostic utility and, unlike conventional sensitivity testing, may predict in vivo antifungal sensitivity. Water moulds such as *Pythium insidiosum* result in a keratitis morphologically similar to typical filamentous fungi, but exhibit marked treatment resistance. There is currently insufficient evidence to suggest that corneal crosslinking is a useful therapeutic modality. Solid lipid nanoparticle formulations of natamycin are being developed to improve ocular penetration. Fungal keratitis may recur after keratoplasty in up to 15%, with three-quarters of these cases requiring further surgery. The site of recurrence heavily influences prognosis.

Summary: Fungal keratitis is a globally significant and relatively understudied entity. It poses formidable challenges to the clinician, both diagnostically and therapeutically. Advances in molecular identification have enabled improved understanding of this disease, and management protocols are starting to emerge from various high incidence centres.

Keywords: *Fusarium*, *Candida*, fungal keratitis, corneal ulcer, keratoplasty, management

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Introduction

Fungi are eukaryotic saprophytic microorganisms with chitinous cell walls. These are broadly classified as yeasts or filamentous fungi (moulds). Yeasts are oval single-celled microbes that multiply via budding. A well-known example is *Candida* species. Moulds are multicellular microbes that develop filaments termed hyphae. Examples include *Aspergillus*, *Penicillium* as well as *Fusarium* species.[1]

Fungal keratitis (FK) is of major concern in the developing world, and in the tropics where it is responsible for as much as half of infective keratitis cases.[2] The prevailing infection tends to be filamentous, of which *Fusarium* species is most prevalent,[3] followed by *Aspergillus* and *Curvularia*.[4] FK is not common in temperate climes or developed areas, but when it occurs, yeasts are more prevalent than moulds (most commonly *Candida* species).[1] FK carries a poorer prognosis than bacterial keratitis, and evidence-based protocols are in short supply.[2]

Methods

A PubMed database search was conducted to find recently published, original English articles concerned with the topic of FK. Articles relevant to an up-to-date review were included. Furthermore, important publications referenced by the authors of the aforementioned articles were also reviewed and included where appropriate. All research included is in accordance with the Declaration of Helsinki.

Mycology

Filamentous fungi organise into a mycelium, which is composed of interconnecting hyphae. When these fungi exhibit individual nucleated cells with dividing cell walls, they are sub-classified as **septate**. Well-known septate fungi include *Aspergillus* and *Fusarium* species. When moulds lack cell wall divisions between their many nuclei, they are termed **non-septate**. Well-known non-septate fungi include *Rhizopus* and *Mucor* species.

Water moulds are morphologically similar to typical filamentous fungi, but closely related to brown algae. *Pythium insidiosum* is such a fungus and is newly identified as the cause of treatment-resistant keratitis in Southeast Asia.

Yeasts are single-celled organisms that do not form hyphae. *Candida* is described as a **dimorphic** fungus in that it may occur in two distinct configurations. These are the typical single-celled yeast form, and a multicellular form characterised by pseudohyphae. These pseudohyphae enable corneal invasion by producing proteases and phospholipases.[3]

Epidemiology

FK occurs over a wide geographic range. This is due to differences in climate,

employment type, income, and access to medication. Over 70 species have been identified globally as causes by the Indo-Hungarian Fungal Keratitis Working Group.[4]

Five to 10% of keratitis is caused by fungi in the USA, but in 2006 there was a major US outbreak of FK due to *Fusarium* contaminating the contact lens cleaning product 'Renu with MoistureLoc'. The Pacific Rim was also involved.[3]

Pathogenesis

Fungi require a violated corneal epithelium to cause keratitis. Once in situ, hyphae or pseudohyphae form which are recognised by dendritic cells and macrophages. These cells release chemokines, interleukin-1b, and tumour necrosis factor alpha which in turn attract neutrophils to the area. Significant inflammation accompanies the infection which worsens the clinical picture.[4] Filamentous fungi may progress rapidly to a thinned or perforated necrotic cornea. Even without frank perforation, the Descemet membrane may be breached by the infection to cause endophthalmitis.[1]

The severity of the infection depends on the virulence of a specific fungal pathogen. Factors associated with increased virulence include filament formation, biofilm formation, proteinase production, and phospholipase production.[5]

The most prominent risk factor for FK is corneal injury,[3] especially involving vegetable matter or agrarian implements. Additional risk factors include: an unhealthy ocular surface, chronic topical steroid administration, contact lens wear, immunosuppressive conditions (HIV, diabetes, chemotherapy, etc.),[1] persistent epithelial erosions, surgery such as keratoplasty or refractive surgery.[3]

Clinical presentation

Most cases are not diagnosed immediately, but rather have been previously treated as bacterial keratitis. It is difficult to distinguish these entities from one another clinically, but suspicion may be raised earlier if typical fungal features are present, or if anti-bacterial agents are ineffective.[1]

The patient may have shown a partial response to empirical antibacterial therapy. While this may be due to mixed bacterial and fungal infection, it may also represent a direct antifungal effect of fluoroquinolones (such as moxifloxacin and gatifloxacin), aminoglycosides (such as gentamycin and tobramycin), or the preservative benzalkonium chloride. In vitro studies have demonstrated the antifungal effects of these agents, and several case series have reported successful resolution of FK with topical moxifloxacin 0.3% monotherapy while awaiting laboratory results. This is further supported by fluoroquinolone monotherapy cure rates of 16 to 36% during the MoistureLoc-related *Fusarium* outbreak of 2006. This phenomenon appears to be limited to a small subset of FK cases however, and most cases will not respond significantly to antibacterial monotherapy.[6]

Because chronic topical steroid use is an important risk factor for FK, a high index of suspicion is appropriate where there is a history of keratoplasty, inflammatory disorders (such as ocular cicatricial pemphigoid), or allergic conjunctivitis.[5]

Symptoms of FK are typically subacute. Patients report discomfort, photophobia, redness and decreased vision. A discharge may be noted, ranging from mucopurulent to watery. Signs include epithelial defect, although this may be small or absent with an underlying yellow/white/grey infiltrate.[1] An elevated corneal epithelium is often seen.[3] Yeasts tend towards dense stromal abscesses and are usually more superficial,[1] although may rarely cause deep invasion.[3] Moulds may display fluffy/feathery borders, ringed infiltrate and satellite lesions (*Figure 1*).[1] The finding of satellite lesions is less common than classically believed. Moulds tend to deep invasion, and the epithelium is often intact. Hypopyon and an exudate on the endothelial surface are common. Moulds may invade the anterior chamber, the sclera, the iris and the vitreous. This is heralded by worsening inflammation, and the prognosis worsens drastically at this point. Secondary angle closure glaucoma may result due to pupil block.[3]

Investigations

Investigations include corneal scraping which is best obtained prior to starting antifungal medications. Avoid calcium-based swabs as they may limit polymerase chain reaction activity. Any contact lenses and their containers should be sent for culture.[1] Laboratories usually rely primarily on mycelium and spore morphology to recognise the organism. It is not uncommon for microscopy to show fungi followed by a negative culture. For this reason, the exact organisms may not be identified, which has negative implications for treatment success. As a result, molecular identification is now the preferred diagnostic modality, and multi-locus DNA sequencing has been done for 65 different *Fusarium* keratitis samples by the Indo-Hungarian Fungal Keratitis Working Group.[4]

The available diagnostic modalities are listed below:

1. **Stains:** Gram/Giemsa (50% sensitivity,[1] only *Candida* will stain with Gram stain[3]), potassium hydroxide wet mount (very sensitive), calcofluor-white, methenamine silver.[1]
2. **Culture:** Mediums include Sabouraud dextrose agar, blood agar,[1] brain-heart infusion agar,[3] chocolate agar,[7] and fungal broth.[1]
3. **Sensitivity testing:** Antifungal sensitivity in vitro does not guarantee efficacy in vivo.[1] As a result, in vitro sensitivity

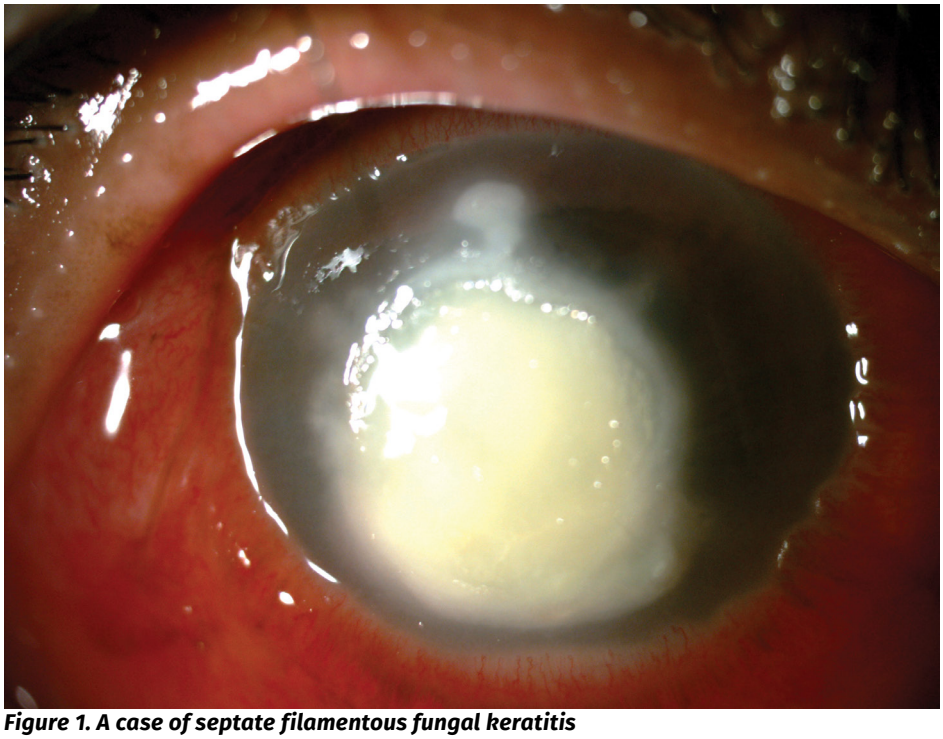


Figure 1. A case of septate filamentous fungal keratitis

testing of antifungal agents is not routinely employed. This outlook is starting to change, however, as more standardised data is becoming available for specific organisms such as *Candida* and *Aspergillus*.[4]

4. **Polymerase chain reaction:** Sensitivity approaches 90%, and it is likely the single most useful investigation.[1] Exact molecular identification may predict antifungal sensitivity in vivo.[4]
5. **Corneal biopsy:** This is indicated where fungal infection is strongly suspected but no improvement is seen after four days without a positive culture. A 2 to 3 mm square of deep corneal stromal tissue is excised, and the sample is divided for histology and culture.[1]
6. **Anterior chamber aspiration:** This may be positive if endothelial deposits are present.
7. **Confocal microscopy:** This may be employed as an adjunct where available,[1] especially to detect the hyphae of septate moulds.[3]

General therapeutic measures

Admission is indicated, as is debridement of the ulcer base or epithelium overlying the stromal abscess.[1] This debridement increases penetration of topical agents, and is especially useful for superficial infection.[3] Intraocular pressure must be observed and managed where necessary.[1] Perforation develops in as many as 50% of patients, and must be monitored for. Prajna *et al*. (2017) found that hypopyon at presentation, a deep infiltrate involving the inner 33% of the stroma, and larger infiltrate size significantly increased perforation risk.[8] Doxycycline 100 mg bd PO is useful where there is corneal thinning as it inhibits collagenases.[1]

Topical therapy

Relatively few antifungal medications are available, in contrast with the wide range of fungal pathogens. In resource-limited settings, chlorhexidine gluconate has shown efficacy, albeit less so than natamycin. Silver sulphadiazine 0.5%/1% has also been shown to be efficacious when resources are limited.[9]

Antifungals are administered topically every hour for at least the first two days, then tapered according to response.[1] Twenty-one to 50 days of treatment may be required.[7] In vitro studies have shown a synergistic effect when dual topical antifungal therapy is used. While there are case reports to support this notion, further study is needed to elucidate the role of dual therapy.[5] A topical antibiotic is prudent for prophylaxis and treatment of superimposed bacterial infection.[1] Moxifloxacin or gentamycin may be preferred for this purpose as they may enhance the effect of antifungal medication.[6] Cycloplegic agents improve comfort and prevent posterior synechiae.[1]

Moulds are managed with natamycin 5% (first line[3]) or econazole 1%.[1] Other options are voriconazole 1%/2%, amphotericin B 0.15% (shown to be especially efficacious for *Aspergillus* species),[3] or miconazole 1%.[1] Despite being fungicidal,[10] natamycin 5% does not penetrate the entirety of the stroma, and so it was postulated that voriconazole 1%/2% may be a more efficacious drug. Indeed, it was shown to have excellent penetration and a wide spectrum in vitro.[2] The Mycotic Ulcer Treatment Trial (MUTT) disproved its superiority in vivo for filamentous fungi however, and natamycin emerged as superior in both safety and efficacy for the treatment of moulds. The results were so marked that the trial had to be stopped ahead of schedule, and all patients switched to natamycin. Natamycin monotherapy resulted in better visual outcomes at three months, and was less likely to result in perforation or therapeutic keratoplasty than was voriconazole monotherapy. This effect was much more marked for *Fusarium* keratitis; non-*Fusarium* cases fared similarly. The regimen employed was hourly until the cornea epithelialised, then four times daily for no less than three weeks.[7]

Natamycin remains the only FDA-approved medication for FK. Its hydrophobic nature is thought to limit ocular penetration, and so solid lipid nanoparticle formulations are being developed, which have shown promising preliminary safety and efficacy enhancement.[10]

Yeasts such as *Candida* are managed with amphotericin B 0.15–0.30% which is the most efficacious agent,[3] but needs to be compounded.[2] Other options include econazole 1%, natamycin 5%, clotrimazole 1%, voriconazole 1%/2% or fluconazole 2%.[1]

Toxic ocular surface effects may result with topical therapy such as hyperaemia, chemosis, punctate epithelial erosions and corneal epithelial erosions.[9]

Regional therapy

Regional antifungal therapy options available include subconjunctival injection, intrastromal injection and intracameral injection. The aim is to improve ocular penetration of the antifungal agent. Subconjunctival injections, such as fluconazole for severe keratitis, are described but cause significant pain and may result in necrotic conjunctival ulcers.[9] Intrastromal voriconazole has been used in patients not responding to topical natamycin and voriconazole with contradictory results. No definitive benefit has been proven over topical voriconazole at this stage. Intrastromal natamycin has been shown to be no better than topical treatment. Intracameral injections have been described in the literature, but no clear efficacy has been shown as outcomes differ.[4] Amphotericin B may be administered intracamerally, usually after anterior chamber washout.[2] Intracameral injection may be helpful in cases where stromal infiltrate is unchanged but endothelial deposits are increasing.[1] At present intrastromal and intracameral injection are considered an unproven adjunct to conventional treatment, and require further study.[2]

Systemic therapy

Systemic antifungal agents are indicated for severe infections[1] and extension into the anterior chamber.[3] These are especially helpful when the infection is close to the limbus.[1] Options include voriconazole (400 mg twice daily for one day, then 200 mg twice daily),[1] posaconazole (800 mg daily),[3] itraconazole (200 mg per day, then tapered to 100 mg per day), as well as fluconazole 200 mg twice daily.[1] Older agents such as ketoconazole, fluconazole and itraconazole are being replaced by the newer agents such as voriconazole and posaconazole as these have superior ocular penetration and spectrum of cover.[3]

MUTT II randomised patients with severe filamentous FK to oral voriconazole or placebo. A 400 mg bd loading dose for one day, then 20 days of maintenance dose (200 mg bd) was used if patients weighed more than 49 kg. The loading and maintenance doses for patients weighing 40–49 kg were 300 mg and 150 mg respectively. Patients weighing less than 40 kg received a 200 mg loading dose and a 100 mg maintenance dose. All patients received topical dual therapy with natamycin and voriconazole. The oral voriconazole group achieved no statistically significant benefit over the placebo group. The rate of perforation, therapeutic penetrating keratoplasty (PKP), and corneal re-epithelialisation were not different. Moreover, spectacle visual acuity and scar size were not different at three

months. Oral voriconazole is expensive (approximately $4 180 per patient), and significantly increases side-effect incidence such as transaminase elevation, visual disturbance and gastrointestinal upset. The study concluded against the use of oral voriconazole for severe mould-related FK.[11] A subgroup analysis of the data from MUTT II was performed looking specifically at cases of *Fusarium* keratitis. This analysis showed lower risk of perforation and therapeutic PKP, as well as smaller corneal scars. Re-epithelisation rates and spectacle visual acuity were not different. Based on this, there may be a role for oral voriconazole in severe *Fusarium*-related FK.[12]

Photoactivated chromophore for keratitis crosslinking (PACK-CXL)

In vitro studies of PACK-CXL monotherapy have not demonstrated fungal inactivation.[2] In vivo studies have evaluated PACK-CXL as a possible adjunct to medical therapy for resistant cases of FK. These studies tend to be case series and their results do not cohere. As such there is currently insufficient evidence to suggest that PACK-CXL is a useful modality for the treatment of FK, especially in severe cases where deep corneal stroma is involved.[13]

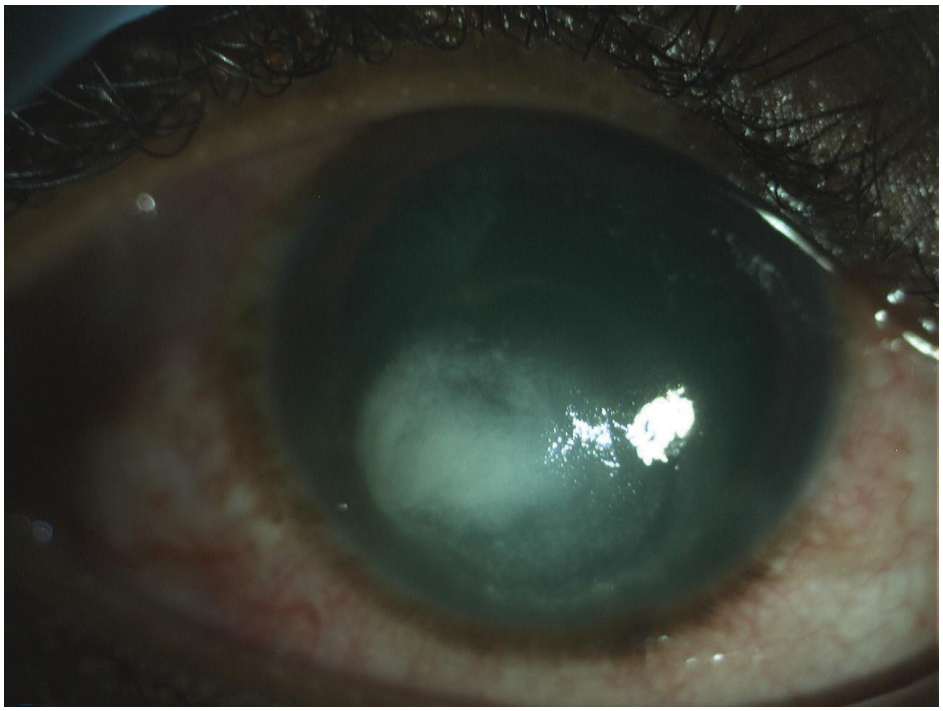
Rose bengal photodynamic antimicrobial therapy (PDAT)

Rose bengal PDAT is a novel treatment for infectious keratitis that has shown some preliminary success in the treatment of FK.[5] Rose bengal is a well-known ophthalmic dye used to detect corneal and conjunctival pathology. During PDAT, green light is used to activate this dye. This results in the generation of reactive oxygen species that exert effects on nearby organic structures. These effects include antimicrobial activity, and a degree of corneal crosslinking. In vitro studies have shown fungicidal activity against *Fusarium, Aspergillus* and *Candida* species. In vivo treatment has been shown to be safe for keratocytes and deeper ocular tissues. A recent case series (2019) by Naranjo *et al.* reports the successful use of rose bengal PDT as a last resort to avoid therapeutic PKP in cases of *Fusarium* and *Curvularia* keratitis.[14] Randomised control trials are needed to further evaluate the role of this modality.[5]

Surgical management

Medical treatment of FK is often not sufficient.[15] Superficial keratectomy (SK) may be employed where it is possible remove the majority of the infection.[1] This is possible due to the relatively minimal inflammation in the adjacent stromal bed which allows good visualisation of the mycelium. SK may also be employed for diagnostic purposes where culture has yielded nothing despite a high index of suspicion for FK. SK is employed in cases of moderate keratitis, where infiltration depth is more than one-third but less than two-thirds of the stroma, and infiltrate is 3–6 mm in diameter. Phototherapeutic keratectomy may also be used in these cases. Early keratectomy may shorten disease duration. Further studies are needed to define the effects of SK on perforation rates.[15]

Up to 50% of severe FK cases eventually perforate.[8] Therapeutic keratoplasty is indicated when medical therapy fails or perforation occurs, and options include either penetrating or lamellar keratoplasty.[1] These grafts have a higher failure rate than conventional keratoplasty, but may facilitate control of the infection.[8] Lamellar keratoplasty may be performed if Descemet membrane has not been breached, otherwise PKP is performed (*Figure 2*). Post-operative



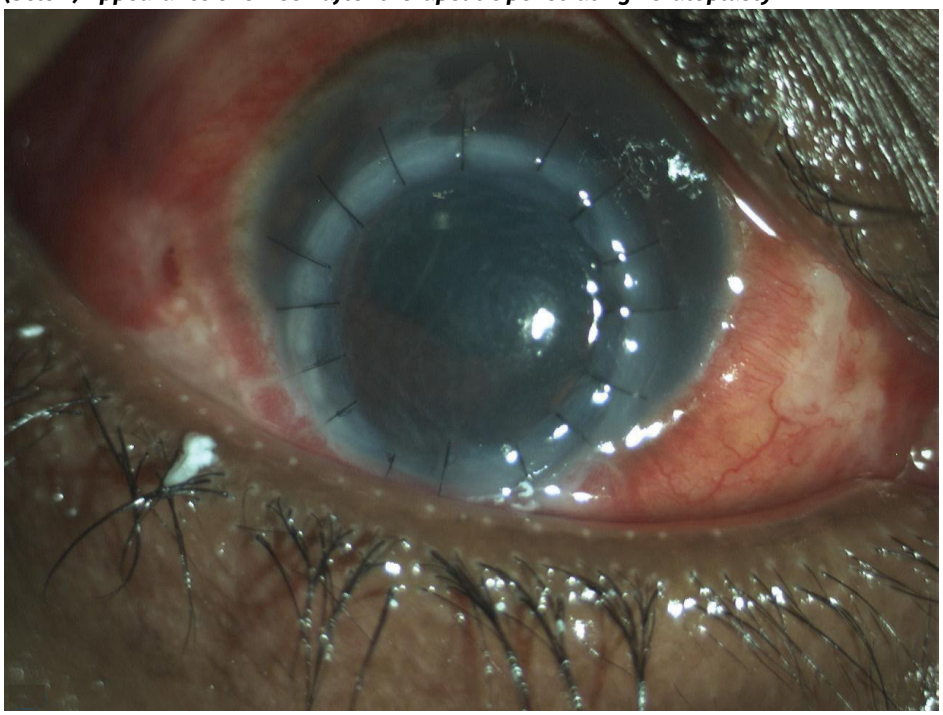


Figure 2. A case of* Fusarium *keratitis
(above) Clinical appearance before therapeutic penetrating keratoplasty
(below) Appearance one week after therapeutic penetrating keratoplasty

topical antifungals, antibiotics and nonsteroidal anti-inflammatory drugs are employed. Topical steroid is added at two weeks if no recurrence is present. Oral antifungals are used pre- and post-operatively.[16]

FK may recur after keratoplasty in as many as 10–15% of cases, and treatment of such cases is not currently protocol driven. Where lamellar keratoplasty is performed, the recurrence is in the stroma of the host, while in PKP cases 15% of recurrence may be intraocular. The overall incidence of recurrence is not different between PKP and lamellar keratoplasty, however. Prognosis depends heavily upon the site of recurrence. In descending order of prognosis, the recurrence sites are: anterior chamber; host corneal stroma; vitreous; atypical. Medical management of recurrence includes dual topical therapy such as natamycin with voriconazole, as well as subconjunctival fluconazole or voriconazole, and may successfully treat a quarter of cases. Three-quarters of recurrences may need surgical treatment, and this is site dependent. Anterior chamber recurrence responds excellently to early washout and intracameral voriconazole. Stromal recurrence may require injections, focal excision, PKP or corneoscleral patch graft. Posterior segment-based recurrence necessitates intravitreal antifungals, lens extraction and pars planar vitrectomy.[16] Evisceration or enucleation may become necessary if all else fails.[3]

Conclusion

FK is a globally significant and relatively understudied entity. It poses formidable challenges to the clinician, both diagnostically and therapeutically. Recent advances in molecular identification have enabled improved understanding of this disease, and treatment protocols are starting to emerge from various high incidence centres. The management of FK is still very much evolving and as such there remains much to be learned from future studies.

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