Review Article

Update on diagnosis and management of refractory corneal infections

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Infectious keratitis is a medical emergency resulting in significant visual morbidity. Indiscriminate use of antimicrobials leading to the emergence of resistant or refractory microorganisms has further worsened the prognosis. Coexisting ocular surface diseases, delay in diagnosis due to inadequate microbiological sample, a slow-growing/virulent organism, or systemic immunosuppressive state all contribute to the refractory response of the ulcer. With improved understanding of these varied ocular and systemic factors contributing to the refractory nature of the microbes, role of biofilm formation and recent research on improving the bioavailability of drugs along with the development of alternative therapies have helped provide the required multidimensional approach to effectively diagnose and manage cases of refractory corneal ulcers and prevent corneal perforations or further dissemination of disease. In this review, we explore the current literature and future directions of the diagnosis and treatment of refractory keratitis.

Key words: Adjunctive therapy, corneal ulcers, refractory infectious keratitis

Infectious keratitis is a global cause of concern for visual disability and corneal blindness.[1] A refractory corneal ulcer can be defined as an ulcer with an inadequate healing response to conventional therapy. Scant evidence exists in the literature on a clear clinical definition or specified time duration of refractory keratitis. Under normal conditions, once the infective component is neutralized, corneal ulcers heal due to the proliferative ability of the corneal epithelium. However, various systemic, ocular, and organism characteristics predispose to the development of non-healing or refractory corneal ulcers.[2,3] Prompt etiological diagnosis and appropriate antimicrobial therapy constitute the mainstay of treating infectious corneal ulcers; however, an inadequate response results in progressive worsening requiring surgical intervention, leading to a poor outcome in refractory keratitis.[2]

This review explores the current literature for various factors contributing to infective keratitis refractory, diagnosing, and managing them along with probing future directions. In this article, we have considered ulcers with inadequate healing response to conventional treatment, ulcers worsening on treatment, and refractory or virulent organisms or infiltrates in specific post-surgical interventions such as laser refractive surgery (LRS), keratoplasty, or post collagen cross-linking based on their location and the altered local tissue response as refractory keratitis.

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Contributory factors

Factors, both ocular and systemic, can contribute to a refractory ulcer [Table 1]. Identifying them will not only help in early diagnosis and timely management but also help prevent ulcers from becoming refractory.

Systemic factors

Systemic risk factors such as diabetes, use of oral steroids/immunosuppressives, and underlying autoimmune conditions weaken the ocular immune system, increasing the severity of the infection and resulting in inadequate or delayed response to treatment. Lim *et al.*[4] noted them to be significant risk factors for polymicrobial keratitis as compared to monomicrobial infections. Diabetes is an independent risk factor for fungal infection, which correlates with the severity of the infection and worsens the prognosis.[5] Similarly, the use of systemic immunosuppressive medications is known to exacerbate the severity and delay fungal clearance.[6] O' Neill *et al.*[7] reported diabetes, systemic immunosuppression, and use of systemic steroids/oral immunosuppressives to be independent risk factors for microbial keratitis-associated endophthalmitis, thus making it necessary to manage the immunosuppressed state effectively and, if possible, to discontinue the immunosuppressives for a while after consulting with the treating physician/rheumatologist.

Table 1: Ocular and systemic factors contributing to a refractory ulcer

Ocular Factors	Systemic Factors
Ulcer profile	Uncontrolled diabetes mellitus
Inaccurate Diagnosis	On oral Immunosuppression
Coexisting ocular diseases	Immunosuppressed state
Superadded infection	Malnutrition
Antimicrobial resistance	
Refractory organism	
Specific situations (Post PK/LK/LRS/CXL)	

PK - penetrating keratoplasty, LK - lamellar keratoplasty, LRS - laser refractive surgery, CXL - collagen cross-linking)

Ocular factors

Ulcer profile

Signs of a healing ulcer include decreased symptoms, reduced ulcer size, infiltrates and hypopyon, epithelialization, and finally, scarring. However, cases refractory to conventional medical therapy show worsening of most of the abovementioned features in addition to progressive corneal stromal melt/thinning.[2,3]

Recurrence of infection, indolent ulcers, neurotrophic ulcers, ulcers larger than 6 mm in size, deep stromal or full-thickness infiltrates, and impending perforation are clinical profiles of refractory keratitis apart from those caused by multidrug-resistant or virulent organisms such as *Pythium insidiosum* or *Pseudomonas aeruginosa* or a polymicrobial infection. Indolent slow-growing infiltrates tend to resist susceptibility to potent antimicrobials due to biofilm formation.[8] Deep stromal infiltrates and endothelial plaques in chronic mycotic ulcers have poor penetration and accessibility of therapeutic agents to the depth of posterior stroma and endothelium, with the overlying epithelium having healed. This leads to recalcitrant fungal infections,[8] necessitating the need to adopt a targeted therapeutic approach for drug delivery.[8,9]

Diagnosis

An ulcer more than 2 mm in size or involving the visual axis must be investigated microbiologically.[8] An inaccurate diagnosis or empirical therapy with multiple medications causes surface toxicity and alters the clinical picture in addition to leading to an inadequate response to treatment, thus emphasizing the need for microbiological tests.

Basic diagnostic techniques

The mainstay in the diagnosis of corneal ulcers is an examination of corneal smears obtained by corneal scraping and culture of corneal samples.[9-11] Gram stain accurately detects causative organisms 60%–75% of the time for bacterial cases[12] and 35%–50% for fungal.[13] Potassium hydroxide (KOH) wet mount has a sensitivity of 76.3% for diagnosing fungal keratitis.[13] Calcofluor white stain is helpful in fungal, *Acanthamoeba*, and *Microsporidial keratitis*.[13,14] Blood and chocolate agar are the most commonly used culture media for bacteria. Sabouraud's dextrose agar or potato dextrose agar is best for isolating fungi, and non-nutrient agar enriched with *Escherichia Coli* is employed to culture *Acanthamoeba*.[13-15]

Clinically refractory fungal keratitis can also be reviewed for *Pythium insidiosum*. The hyphae of P. insidiosum stain positive for calcofluor-KOH, acridine orange hydrochloride, and lactophenol blue,[16] and it grows well in blood, Sabouraud's dextrose, and chocolate agar;[16] however, polymerase chain reaction (PCR) is considered as the diagnostic test.[16]

Viral keratitis is primarily a clinical diagnosis.[8] PCR is noted to be highly sensitive, especially in diagnosing various viral pathogens such as herpes simplex virus, adenovirus, and cytomegalovirus, along with multiple other organisms such as bacteria, fungus, *Acanthamoeba*, and microsporoidosis.[17]

In a retrospective study of 23897 cases of presumed keratitis over 10 years at Aravind Eye Hospital in India, 38% of corneal scrapings tested negative, both on culture and smear.[18] Culture-negative keratitis remains a significant problem for clinicians in the management of refractory keratitis. If the ulcer is refractive to empirical therapy and cultures are negative, repeat cultures of the ulcer and referral to a cornea specialist may be warranted. While doing repeat scraping, it is recommended that antimicrobial therapy be stopped at least 24–48 h prior.[19] When the repeat culture of a progressive, non-responding corneal ulcer is negative, histological examination of the corneal biopsy specimen is indicated. Superficial keratectomy or corneal biopsy specimen can be obtained by a trephine or free lamellar dissection with a sharp blade for immunohistochemical and light-microscopic examination. This approach is beneficial for the detection of fungi and *acanthamoeba* in deep ulcers.[20] Despite repeating these basic investigations not infrequently the organism remains unidentified and there arises a need to look for alternate/advanced options.

Advanced diagnostic techniques

Internal transcribed spacer (ITS) gene sequencing establishes a rapid and prompt diagnosis of fungal keratitis in refractory cases[21] and has been described in Pythium along with other non-sporulating molds.[22-33]

Molecular identification also helps diagnose rare fungal species such as *Beauveriabassiana*, which was found to be highly resistant to antifungal therapy, along with *Colletotrichumgloeosporioides* and *Trametesbetulina*[22,34,35]

Apart from these, *in vivo* confocal microscopy (IVCM), a non-invasive method, is increasingly being used due to its rapidity and high sensitivity in detecting larger and deep-seated organisms inaccessible by routine scraping, such as filamentous fungus, *Acanthamoeba*, and *Nocardia*[36-40] [Fig. 1]. Anterior segment optical coherence tomography (AS-OCT) has been used to provide an objective measure of the size of the corneal infiltrate/scar dimensions or to monitor the progress of corneal thinning during treatment.[41,42]

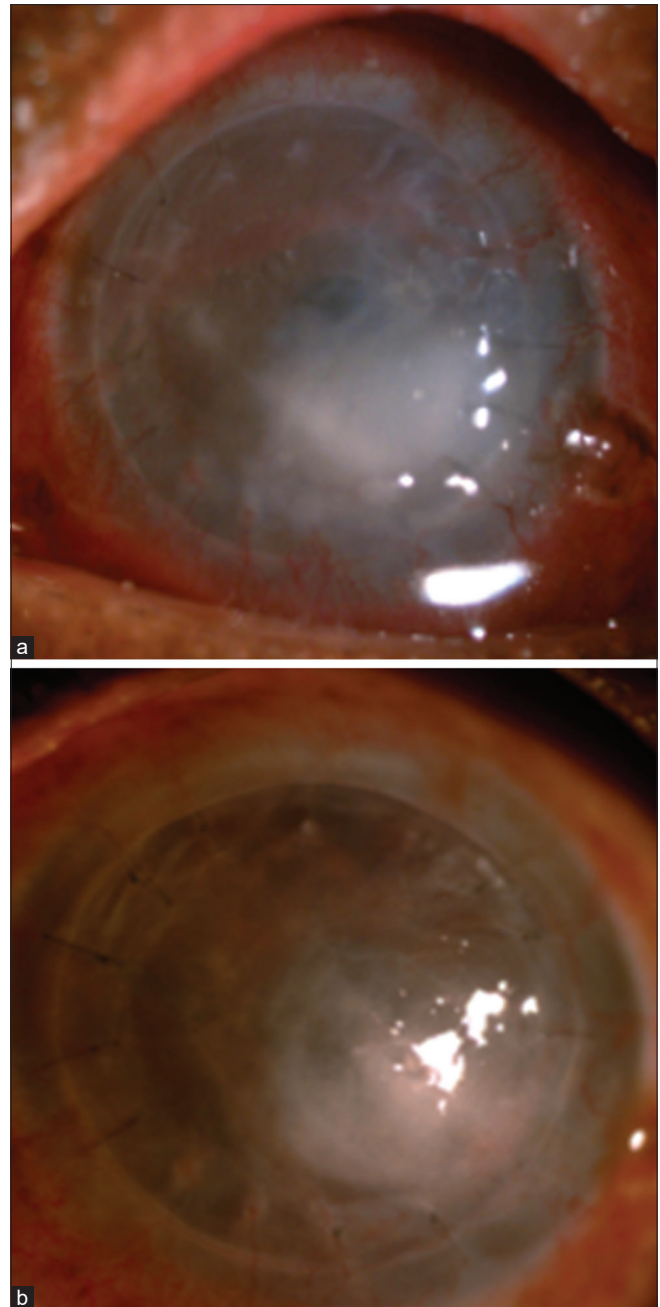Next-generation sequencing (NGS) has emerged as a novel method that may improve the diagnostic accuracy of infectious keratitis, particularly for organisms that are difficult to culture by conventional methods such as atypical or anaerobic bacteria.[43] However, it is not clear whether these approaches can be used to effectively determine the etiology of infection or antibiotic sensitivity data.[44]
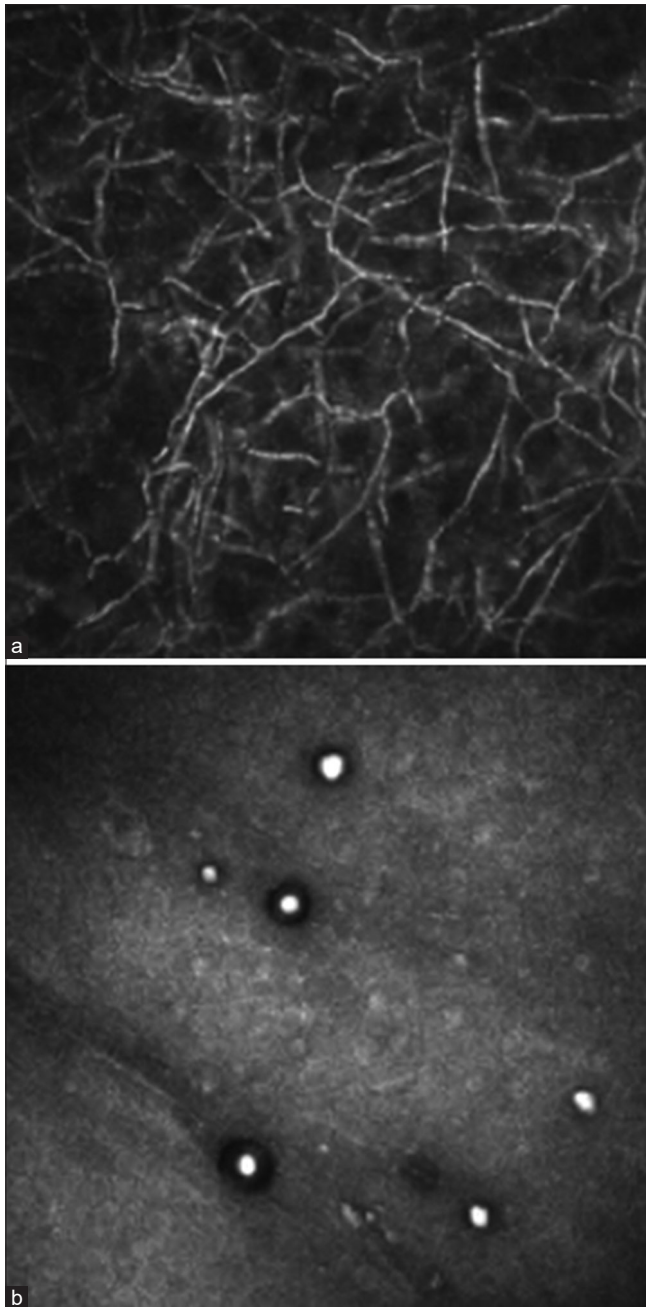
Figure 1: Laser confocal microscopy images showing (a) hyperreflective beaded string-like branching structures suggestive of fungus/pythium; (b) Acanthamoeba cysts showing highly reflective nucleus surrounded by a low refractile ring-like wall

Figure 2: Clinical picture depicting (a) suture-related infiltrate in an optical graft; (b) resolved infiltrate leaving behind a scarred graft

Emerging New Pathogenic Microbes

Several new pathogenic fungi causing keratitis, with varying or suboptimal susceptibility to antifungal therapy, are emerging [Table 2].[23-35,45-52] Knowledge of the sensitivity profile of antifungals by antifungal susceptibility test (AFST) against various species helps in initiating appropriate treatment and improving the outcome. A recent study from South America reported *A. fumigatus* isolate from post-traumatic keratitis in a 27-year-old male worker carrying the substitution G54E at Cyp51Ap associated with itraconazole resistance, highlighting

the possibility of mutation-induced resistance to common antifungal therapy.[53]

Coexisting Ocular Diseases

The ocular surface is directly exposed to the environment, where it interacts with a myriad of pathogens. The gel-forming mucins and tight intercellular junctions of the epithelium prevent the entry of organisms, and tears help flush the noxious substances out of the eye, help maintain healthy epithelium, and limit the growth of pathogens with the help of proteins such as lysozyme, immunoglobulins, and lactoferrin.[54] If any of the abovementioned factors or mechanisms are overwhelmed

Table 2: Details of emerging new fungal corneal infections reported in recent literature

Study	Risk	Microorganism	AFST
[23]Tan SJ *et al*. Contact lens associated keratitis due to Tintelnotiadestructans. Med Mycol CaseRep. 2019; 27: 8-10. [45]Kaufmann *et al*. Tintelnotiadestructans Keratitis: A Clinicopathological Report and Review of the Literature. Cornea. 2021; 40: 380-382. [46]Behrens-Baumann WJ *et al*. Keratomycosis due to Tintelnotiadestructans refractory to common therapy treated successfully with systemic and local terbinafine in combination with polyhexamethylene biguanide. IntOphthalmol. 2019; 39: 1379-1385.	CL wear Immuno-compromised CL wear	*Tintelnotiadestructans*	amphotericin B, ciclopirox, natamycin, posaconazole, voriconazole, and terbinafine
[24]VanamHP *et al*. First report of Lasiodiplodiapseudotheobromae keratitis susceptible to voriconazole in an Indian mango grower. Access Microbiol. 2019; 1: e000055.	Trauma	*Lasiodiplodiapseudotheobromae* (dematiaceous fungi)	voriconazole and amphotericin B
[25]Homa M *et al*. Characterization of Aspergillus tamarii Strains from Human Keratomycoses: Molecular Identification, Antifungal Susceptibility Patterns and Cyclopiazonic Acid Producing Abilities. Front Microbiol. 2019; 10: 2249.	Trauma	*Aspergillus tamarii*	Azoles
[26]Shigeyasu C *et al*. Keratomycosis caused by Aspergillus viridinutans: an Aspergillus fumigatus-resembling mold presenting distinct clinical and antifungal susceptibility patterns. Med Mycol. 2012; 50: 525-8.	CL wear	*Aspergillus viridinutans*	Micafungin
[27]Ozkurt Y *et al*. Pseudallescheria boydii keratitis. Case Reports J PediatrOphthalmol Strabismus. 2006; 43: 114-5.	Trauma	*Pseudallescheria boydii*	voriconazole and posaconazole
[47]Chew R *et al*. Purpureocilliumlilacinum keratitis: a case series and review of the literature. Review Can J Ophthalmol. 2016; 51: 382-385. [48]Todokoro D *et al*. Topical voriconazole therapy of Purpureocilliumlilacinum keratitis that occurred in disposable soft contact lens wearers. IntOphthalmol. 2014; 34: 1159-63.	Immuno-compromised CL wear	*Purpureocilliumlilacinum*	voriconazole
[28]Lu X *et al*. Rare Fungal Keratitis Caused by Coprinellus Radians. Case Reports Mycopathologia. 2020; 185: 389-394.	Trauma	*Coprinellus radians*	amphotericin B, posaconazole, itraconazole and voriconazole
[29]Rosa PD *et al*. Antifungal Susceptibility, Morphological and Molecular Characterization of Lasiodiplodiatheobromae Isolated from a Patient with Keratitis. Case Reports Mycopathologia. 2018; 183: 565-571.	Immuno-compromised	*Lasiodiplodiatheobromae*	amphotericin B and voriconazole
[30]Kiss N *et al*. New Species of the Genus Curvularia: C. tamilnaduensis and C. coimbatorensis from Fungal Keratitis Cases in South India. Pathogens. 2019; 9: 9.	Trauma	*Curvularia. tamilnaduensis, Curvariacoimbatorensis*	natamycin and amphotericin B
[49]Guarro J *et al*. Mycotic keratitis due to Curvularia senegalensis and in vitro antifungal susceptibilities of Curvularia spp. J ClinMicrobiol. 1999; 37: 4170-3.	Immuno-compromised	*Curvularia senegalensis*	amphotericin B, miconazole, itraconazole and ketoconazole
[50]Sreepurna AT *et al*. Multidrug-resistant Fusarium in keratitis: a clinico-mycological study of keratitis infections in Chennai, India. Mycoses. 2017; 60: 230-233.	Immuno-compromised Trauma	*Fusarium keratoplasticum, Fusarium falciforme, Fusarium sporotrichioides*	natamycin and amphoterecin B
[31]Al-Hatmi AMS *et al*. Keratitis by Fusarium temperatum, a novel opportunist. BMC Infect Dis. 2014; 14: 588.	Immuno-compromised Trauma	*Fusarium temperatum (Fusarium fujikuroi species complex)*	micafungin, posaconazole and amphotericin B
[32]Sun S *et al*. Identification and Characterization of Fusarium proliferatum, a New Species of Fungi that Cause Fungal Keratitis. Sci Rep. 2018; 8: 4859.	Immuno-compromised CL wear	*Fusarium proliferatum*	natamycin and voriconazole

Contd...

Table 2: Contd...

Study	Risk	Microorganism	AFST
[33]Monden Y *et al*. First case of fungal keratitis caused by Pestalotiopsisclavispora. ClinOphthalmol. 2013;7:2261-4	Multiple ocular surgeries, herpetic infection, bullous keratopathy	*Pestalotiopsisclavispora*	Micafungin
[51]Gajjar DU *et al*. Severe pigmented keratitis caused by Cladorrhinumbulbillosum. Indian J Med Microbiol. 2011; 29: 434-7.	Immuno-compromised	*Cladorrhinumbulbillosum*	natamycin, amphotericin B, fluconazole and itraconazole
[34]Wang L *et al*. Fungal keratitis caused by a rare pathogen, Colletotrichumgloeosporioides, in an east coast city of China. Case Reports J Mycol Med. 2020; 30: 100922.	Trauma Topical steroids	*Colletotrichumgloeosporioides* (filamentous fungi)	amphotericin B, voriconazole, itraconazole, posaconazole, micafungin and capsofungin
[35]Hardin JS *et al*. Fungal Keratitis Secondary to Trametesbetulina: A Case Report and Review of Literature. Mycopathologia. 2017; 182: 755-759.	Trauma	*Trametesbetulina* (filamentous fungi)	voriconazole
[52]Aggarwal S *et al*. Exophialaphaeomuriformis Fungal Keratitis: Case Report and In Vivo Confocal Microscopy Findings. Case Reports Eye Contact Lens. 2017; 43: e4-e6.	Post PKP	*Exophialaphaeomuriformis* (pigmented yeast)	voriconazole

either because of the underlying disease or an adverse effect of treatment, an organism can gain entry.[55] Green *et al*.[56] identified ocular surface disease (OSD) as a predisposing factor for microbial keratitis. These patients presented with more severe infections, higher incidence of polymicrobial or mixed infections, and took longer to heal. Among the OSDs, blepharitis followed by dry eye, SJS, and OCP were the most prevalent; coagulase-negative *Staphylococcus aureus* was the most common species with non-healing epithelial defect, resulting in corneal perforation being the most common complication in a five-year study on microbial keratitis with OSD in Australia.[57] Lacrimal duct obstruction or chronic dacryocystitis by delaying the tear clearance alters the ocular flora, thus making the cornea more susceptible to infections. Staphylococcus species is reported to be the most common; however, fungal infections have also been reported.[58] Several measures can be employed to prevent further deterioration of the surface. These include the use of preservative-free drops, tarsorrhaphy, and punctal occlusion in cases of neurotrophic or severe dry eyes to control factors causing underlying inflammation. In addition, a heightened awareness regarding the possibility of altered microbial flora and anticipating delayed epithelization are essential. A low threshold is adopted for applying cyanoacrylate glue to prevent perforation as stromal melt tends to progress quickly in these compromised eyes. Judicious use of the abovementioned measures aids in faster resolution of non-healing corneal ulcers.[59-63]

Superadded infections

Occasionally, an ulcer with a good healing response could worsen. This indicates either compromised compliance or a superadded/secondary infection. Patient compliance needs to be reaffirmed, and a repeat corneal scraping helps rule out a secondary infection. The presence of an epithelial defect, history of steroid use, and previous recurrent episodes of keratouveitis were identified as risk factors for secondary bacterial and fungal infection in herpes simplex keratitis.[64]

Specific situations

a) Post-penetrating keratoplasty

Infective keratitis following optical keratoplasty is one of the important causes of graft failure and poor visual outcome. The predisposing risk factors are grouped into three categories: donor-related (infected donor tissue), host-related (ocular surface disorders, use of topical steroids or contact lens, recurrence of previous infection, or underlying systemic disease), and graft-related (suture related, persistent epithelial defect, or wound leak/dehiscence).[65] Most studies report a higher incidence of infection within the first year of surgery, thus warranting a close follow-up, particularly in those with underlying risk factors stated above.[66] To prevent this, proper surveillance of the donor tissue, and in particular, consideration of intraoperative suturing techniques and wound integrity are essential. Improving the ocular surface health by punctal occlusion, tarsorrhaphy, lid corrective surgeries, and epilation are additional procedures that are planned as required.[67] In addition, oral acyclovir 400 mg twice a day is recommended as a prophylactic dose in patients undergoing a graft for healed viral keratitis; however, the exact duration for which it needs to be continued is unclear.[68] Use of prophylactic antibiotics in the absence of a persisting defect is not recommended as it has limited or no role.[69] Medical management alone with topical/systemic antimicrobials is found to control when the infiltrate is <4 mm in 66% of cases,[66] whereas larger ones require a regraft if the organism is not very sensitive. Despite the resolution of infection, the visual prognosis is poor because of a high incidence of graft failure [Fig. 2].[65-67,70]

b) Post-lamellar keratoplasty

The graft host interface remains a potential space for infection to occur following lamellar keratoplasty, and though rare, results in significant visual morbidity. As the site of infection is deep within the stroma, it restricts access to the infiltrate for microbiological testing. In addition, it impacts penetration of topical drugs, thus delaying the diagnosis and response to treatment besides the use

of topical steroids in the postoperative period being a risk factor.[71] *Candida* species has been the most common organism reported to cause interface infection, followed by *Klebsiella*.[71] Infected donor tissue was the most common risk factor identified.[71-73] Tissue warming during the tissue processing for lamellar keratoplasty promoted Candida growth in donor rims. However, the addition of antifungal agents to storage media raised concerns about endothelial toxicity.[74,75] A single or multiple whitish infiltrate/s seen in the interface should raise suspicion of an interface infection warranting close observation. This is especially important because these infections rarely produce significant symptoms. As the infection is deep-seated restricting access to the microbiological sample, confocal microscopy offers additional value; however, the role of anterior segment OCT is limited.[76,77] Based on the donor corneal rim culture and clinical appearance, empirical treatment is initiated with topical and systemic antimicrobials. Washing the interface with antimicrobial agents or deep intrastromal/intracameral injections with antifungal shave has been attempted with limited success, with most cases requiring a therapeutic penetrating keratoplasty.[71,78] Removal of the donor lenticule with the aim of reducing the microbial load too has achieved limited success. This has, on the contrary, led to recurrence of infection in the interface, and of dissemination of infection in the anterior and posterior chamber, causing endophthalmitis in posterior lamellar keratoplasty.[79,80] High degree of clinical suspicion, close watch, especially in eyes with positive donor rim culture reports, along with antimicrobial injections in the interface, should be attempted to avoid further interventions.

c) Post-refractive surgery

The incidence of post-laser refractive surgery (LRS) infection is 0.0001%–1.5%, and it is higher after photorefractive keratectomy (PRK) than laser *in situ* keratomileusis (LASIK) or small-incision lenticule extraction (SMILE), probably due to a large epithelial defect following PRK.[81] Risk factors include preexisting dry eyes, blepharitis, Meibomian gland dysfunction, intraoperative contamination of instruments or surgical field, and the use of bandage contact lens postoperatively.[81,82] Based on the onset, keratitis is defined as early (within 1 week of surgery) and is usually caused by *Staphylococci/Streptococci* or late (beyond 1 week of surgery) wherein slow-growing organisms such as fungus, mycobacteria, *Nocardia*, or *Acanthamoeba* should be suspected. Herpetic keratitis too can present following laser refractive procedure as a primary infection or due to reactivation.[82,83] In flap procedures, the infiltrate usually occurs in the interface or is limited only to the lamellar flap, flap margin, or stroma.[83,84] For microbiological assessment in procedures with an interface, the flap needs to be lifted. The undersurface of the flap or the interface is scraped, followed by a thorough wash with fortified antibiotics. Most cases respond to medical management, but in non-responding cases, repeated interface irrigation, flap amputation, PACK CXL, tissue adhesives, and surgical intervention might be needed.[82-84] Improving the health of the ocular surface preoperatively and a close watch in the postoperative period with timely intervention taking into account the possible microorganism based on their presentation can help improve outcomes [Table 3].

d) Post collagen cross-linking

Infective keratitis post collagen cross-linking (C3R) is rare and most commonly involves staphylococcus species. Large epithelial defect, damage to stromal keratocytes by UV light, use of topical steroids and bandage contact lens postoperatively, and an altered ocular surface in patients with vernal or atopic keratoconjunctivitis or blepharitis might be the predisposing factors and need to be considered.[85-87] Reactivation of the
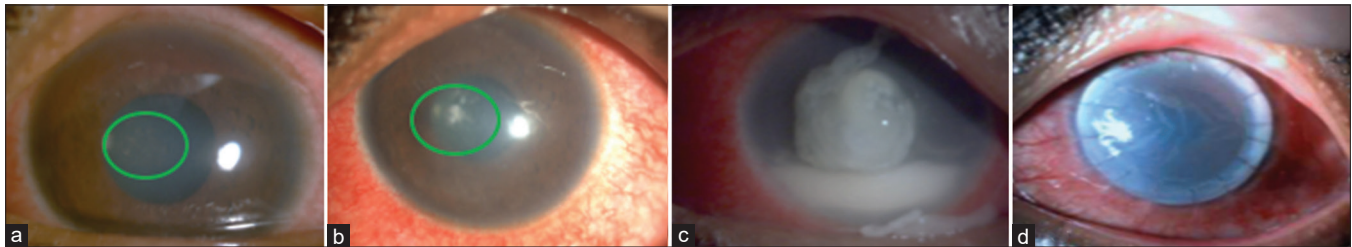
Figure 3: Clinical picture showing (a) early endothelial exudates noted one week following collagen crosslinking for keratoconus; (b) increase in endothelial exudates despite being on topical antibiotic therapy; (c) worsening of infiltrate, causing corneal melt and cultures growing Staphylococcus aureus; (d) no recurrence noted one week following therapeutic penetrating keratoplasty
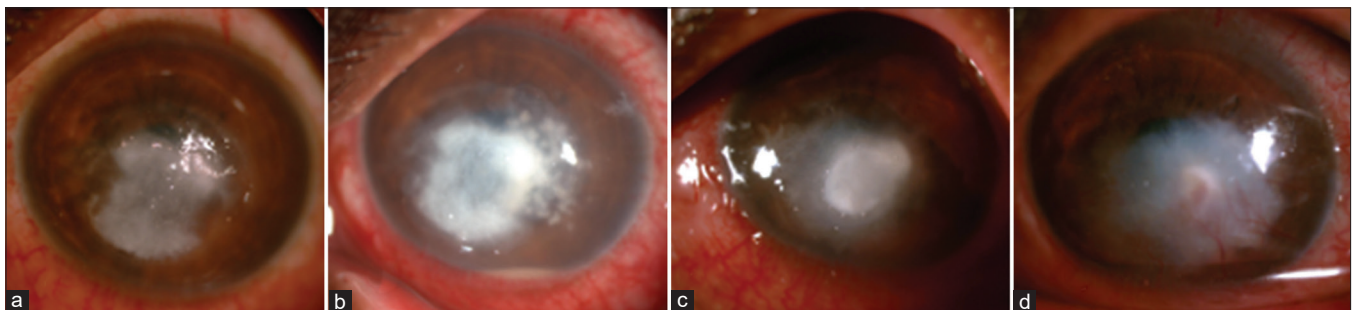
Figure 4: Clinical picture depicting (a) deep stromal fungal infiltrate; (b) worsening on maximum topical antifungal therapy; (c) infiltrate responding well following two intrastromal voriconazole injections along with topical antifungals; (d) completely resolved following five intrastromal injections along with topical antifungals

herpes simplex virus by UV light has been hypothesized by Kymonis *et al.*[88] to be responsible for causing herpetic epithelial keratitis and uveitis inpatients following C3R. Though most cases respond well to topical antibiotics, it must be borne in mind that in the immediate postoperative period, in the absence of keratocytes, corneal melt in the presence of an infection can proceed very rapidly, at times necessitating a therapeutic penetrating keratoplasty in these eyes [Fig. 3]. A close watch for resistant microorganisms and altered sensitivity patterns should be monitored in case of worsening of infections.

Management of refractory ulcers

Antimicrobial therapy forms the mainstay of treatment. However, with the emergence of antimicrobial resistance (AMR), virulent and new pathogens, attention is focused on the development of novel antimicrobial compounds with better penetration and adjunct therapeutic modalities to prevent the need for surgical intervention and augment the treatment response.

Antimicrobials

For bacterial keratitis, single-drug therapy using fluoroquinolone has been traditionally the mainstay of management.[88] Combined fortified topical antibiotics should be considered for large and/or visually significant corneal ulcers, especially if a hypopyon is present and for eyes unresponsive to initial treatment.[89] In various studies, including some randomized controlled trials, both moxifloxacin and gatifloxacin performed at least

as well as standard fortified cefazolin/tobramycin combination therapy.[88-92] However, Methicillin-resistant *S. aureus* isolates are generally resistant to fluoroquinolones but susceptible to vancomycin.[93,94] Vancomycin-resistant *S. aureus* is very rare but sensitivity to topical linezolid has been demonstrated in such cases.[95] Keratitis from multidrug-resistant *Pseudomonas aeruginosa* has also been reported, with high morbidity further highlighting the need for antibacterial sensitivity.[93,94] Topical colistin 0.19%, imipenem, or polymyxin B 10000–20000 IU/ml may be considered in such cases.[95] Systemic antibiotics may be considered in severe cases where the infectious process has extended to adjacent tissues (e.g., the sclera) or when there is impending or frank perforation of the cornea.[96] Systemic therapy is also necessary in cases of gonococcal keratitis.[97] Gram-positive rods (non-tuberculous mycobacteria) can be treated with amikacin, clarithromycin, or azithromycin therapy, whereas gram-positive rods (*Nocardia*) are susceptible to sulfacetamide, amikacin, or trimethoprim/sulfamethoxazole therapy [Table 4].[96]

The use of adjuvant corticosteroids has long been debated in the treatment of bacterial keratitis.[98-100] Steroids for Corneal Ulcers Trial (SCUT) compared adjunctive topical corticosteroids to placebo in treating bacterial corneal ulcers. Despite the comprehensive data showing no difference in outcomes such as 3-month visual acuity, scar size, or perforation rate, subgroup analyses suggested that corticosteroids are beneficial in specific subgroups.[101]

Table 3: Infective keratitis associated with kerato-refractive surgical procedures

Refractive surgery	Site of infection	Organism (most common)	Treatment Recommended
PRK	Base/edge of epithelial defect	Staphylococci/Streptococci	Topical antibiotics based on antimicrobial sensitivity
LASIK	Flap/interface	Early -Staphylococci/Streptococci Late- Candida/Nocardia/Mycobacteria	Topical antibiotics based on antimicrobial sensitivity Topical antibiotics/amputation of flap/interface wash
SMILE	Interface	Staphylococci	Interface wash with antibiotics/PACK-CXL

PRK - Photorefractive keratectomy, LASIK - Laser in-situ keratomileusis, SMILE - Small-incision lenticule extraction

Table 4: Antimicrobial therapy recommended against various microorganisms causing infective keratitis

Microorganism	Recommended antimicrobial agents
Gram-positive cocci[63,64,71]	Cefazolin, Vancomycin, Fluoroquinolones, Bacitracin
Gram-negative bacilli[63,64,71]	Tobramycin, Gentamicin, Ceftazidime, Fluoroquinolones
Gram-negative cocci[63,64,71]	Ceftriaxone, Ceftazidime, Fluoroquinolones
Gram-positive bacilli (Non-tuberculous mycobacteria)[71]	Amikacin, Clarithromycin, Azithromycin, Fluoroquinolones
Gram-positive bacilli (*Nocardia*)[71]	Sulfacetamide, Amikacin, Trimethoprim, Sulfamethoxazole
Methicillin-resistant *S. aureus* (MRSA)[68,69]	Vancomycin
Vancomycin-resistant *S. aureus* (VRSA)[70]	Linezolid
Pseudomonas aeruginosa[70]	Polymyxin B, Colistin
Filamentous fungi[4,77-85]	Natamycin, Ketoconazole
Yeasts (e.g., *Candida* spp.)[4,77-85]	Amphotericin B, Natamycin, Ketoconazole, Flucytosine
Newer/resistant fungal strains[5-25]	Voriconazole, Posaconazole, Micafungin, Capsofungin, Itraconazole, Fluconazole, Ciclopirox, Terbinafine
Herpes Simplex Virus[86-90]	Trifluridine, Acyclovir, Ganciclovir, Valacyclovir
Varicella Zoster Virus[87,90]	Acyclovir, Ganciclovir, Valacyclovir
Acanthamoeba spp.[91]	Chlorhexidine, Polyhexamethylene biguanide, Propamidine
Pythium insidiosum[92,123]	Linezolid, Azithromycin, Topical ethanol
Microsporidium spp.[94]	Propamidine, Fumagillin, Fluroquinolones, Albendazole, Itraconazole

For fungal keratitis, treatment with topical natamycin 5% is the mainstay of management.[102] Topical amphotericin B 0.15%–0.5% is an alternative primarily for yeasts, but its use requires access to a compounding pharmacy and is limited by toxicity. Voriconazole, a newer generation triazole, has gained popularity in treating fungal keratitis due to its excellent ocular penetration.[103] The first Mycotic Ulcer Treatment Trial (MUTT I) showed a benefit of natamycin over voriconazole for topical treatment of fungal keratitis, particularly for *Fusarium* keratitis,[104] which was also confirmed by a second randomized clinical trial[105] and a recent Cochrane review.[106] The Mycotic Ulcer Treatment Trial II (MUTT II) investigated the effect of adjuvant oral voriconazole versus oral placebo for smear-positive filamentous fungal keratitis and did not report a significant benefit of adding systemic voriconazole.[107] Therefore, currently, topical natamycin remains the most evidence-based treatment for filamentous fungal keratitis, and oral voriconazole can be considered if the organism is *Fusarium*, or if there is the risk of impending/frank perforation or associated scleritis. Other potential adjuvant treatments for endothelial plaques in fungal keratitis include intracameral injection of amphotericin or voriconazole with or without hypopyon drainage[108-110] or intrastromal injection of voriconazole in cases of deep stromal infiltrates[110,111] [Fig. 4]. Terbinafine has been suggested to be efficacious in treating severe cases of fungal keratitis due to the rare fungi, *Tintelnotia destructans*, which is refractory to common antifungal therapy.[25] New strains identified within the same mycotic family might exhibit differences in their susceptibility to antifungal agents.[26,30] Inaccurate etiological diagnosis or ineffective antimicrobial therapy with partially sensitive or resistant therapeutic agents in the setting of empirical antifungal therapy without AFST is responsible for the progression of the ulceration in refractory cases; therefore, AFST is recommended despite the increased economic burden, especially in refractory cases.[112,113] Resistance to amphotericin B has been found to correlate with the proteinase production ability of filamentous fungi; however, multidrug resistance to antifungal treatment is considered rare.[114,115]

Management of viral keratitis includes antiviral medications with or without adjuvant topical corticosteroids. Topical acyclovir is the first-line treatment for HSV epithelial keratitis and oral for stromal and endothelial keratitis.[116] Ganciclovir is a newer synthetic medication with more broad-spectrum antiviral coverage. In addition to treating HSV and VZV keratitis, topical ganciclovir is also effective in treating keratitis caused by CMV.[117] Ganciclovir has been shown to be just as effective as acyclovir and can especially be used in patients resistant or intolerant to acyclovir.[117] The Herpetic Eye Disease Study I (HEDS I) evaluated the effectiveness of corticosteroids in treating HSV stromal keratitis. Time to resolution of infection was significantly shorter in the group receiving topical corticosteroid than those taking placebo.[118] Oral valacyclovir, a newer antiviral, is well-tolerated, and there is some evidence that it may have better ocular penetration. Additionally, the treatment dose for valacyclovir is 1 g three times daily, as opposed to acyclovir which is 400 mg five times daily (800 mg five times daily for VZV), which aids in patient compliance.[119] HEDS II examined the prolonged use of oral acyclovir for prophylaxis of recurrent ocular HSV and reported that ocular HSV recurrence was 45% lower in the acyclovir group at 12 months.[120]

Medical therapy for *Acanthamoeba* keratitis typically begins with topical chlorhexidine 0.02% or a combination of chlorhexidine/polyhexamethylene biguanide 0.02% and propamidine 0.1%. Therapy needs to be continued for 6–12 months.[121] Corticosteroids need to be used with extreme caution only once the infective aspect is well taken care of and are indicated only in cases where the immune component is contributing like uveitis, scleritis, or optic neuritis.

For *Pythium insidiosum*, various studies have evaluated the effect of a combination of topical linezolid with topical and oral azithromycin and have found mixed results. However, most cases are not amenable to medical therapy and early surgical treatment with or without adjuncts may be warranted.[122] Recently, the safety and efficacy profile of topical ethanol in the treatment of Pythium keratitis was reported; however, the exact dose and strength of ethanol that will be most effective needs further work.[123]

The most appropriate treatment for microsporidial stromal keratitis has not yet been established, and therapeutic keratoplasty is recommended in the majority. Treatment with 0.02% polyhexamethylene biguanide does not offer any significant advantage over placebo.[124] Microsporidial infections in HIV-infected individuals may respond to the combination of antibiotics and antiparasitic agents, including topical propamidine, topical fumagillin, topical fluoroquinolones, oral albendazole, and/or oral itraconazole.[125]

Future perspectives

Biofilm promotes adherence of microbes to the surface, interferes with drug penetration, and increases the resistance to antimicrobials; thus, the need for increased understanding of the role of biofilm formation in infections may aid in the development of improved antimicrobial strategies. The biofilm formation that occurs in *Fusarium solani* has been cited for functioning as a survival strategy that provides antifungal resistance.[126] The description of efflux pumps[127] in *Fusarium solani* species complex (FSSC) biofilms and promethazine challenged biofilms showing increased sensitivity to amphotericin B offer prospects to explore this therapeutic strategy for effective management of fusarium infections.

Modifications of the antimicrobials to improve their penetration and efficacy have gained significant importance in recent times. Cyclodextrins are natural cyclic oligosaccharides with a hydrophilic outer surface comprising (α-1,4-)-linked α-D-glucopyranose units and a lipophilic central cavity. Hydroxypropyl β CD, a cyclodextrin used as a carrier for ketoconazole, led to a 20-fold increase in drug bioavailability compared to suspensions.[128,129]

Nanoparticles are the colloidal carriers and can be divided into nanocapsules, wherein the drug is generally enclosed in a polymer shell or nanosphere, wherein the drug is uniformly distributed within the polymer. Chitosan oligosaccharides (CS) are naturally biocompatible mucoadhesive positively charged polymers. Ofloxacin loaded on CS-modified nanolipid carriers were found to have excellent penetration, improved preocular residence time-controlled drug release, and improved corneal bioavailability.[130]

Liposomes are yet another form of nanoformulations comprising lipid vesicles. Investigators have studied liposomes

to deliver idoxuridine, fusidic acid, amphotericin B, and minocycline.[131-133]

Over the past few decades, contact lenses have gained attention to be used as a tool for delivering therapeutics against diseases prevailing in the anterior segment of the eye, including keratitis. Desirable drug-eluting contacts lens devices include biocompatibility, flexibility and toughness, transparency with no visual obstruction, and desirable drug release profiles. Some such approaches with potential application in corneal ulcers are being studied over time and have fetched favorable results in bacterial and fungal ulcers.[134,135]

Vaccination using microbe-derived products to reduce the host inflammatory responses have been tested in animal models and appear promising.[136]

Adjunct measures

Despite maximum antimicrobial therapy, the infective keratitis worsens frequently, resulting in melts, sclera extension, perforations, endophthalmitis, and, in some cases, panophthalmitis. To circumvent such sight-threatening complications in cases of non-responding corneal ulcers, adjunct measures or alternate therapies can be considered in addition to the continuing treatment modality.[137]

a) Photodynamic therapy (PDT)

PDT involves a non-toxic dye (photosensitizer), a low-intensity visible light (red to the near red range), which in the presence of oxygen combines to produce cytotoxic reactive oxygen species. The two basic mechanisms by which PDT induces lethal damage on the microorganisms are by damaging the DNA and cytoplasmic membrane, thus allowing leakage of cellular contents or inactivation of membrane transport systems and enzymes.[138,139] It is also found to increase the stiffness of the corneal tissue, reduce enzymatic digestion by pathogenic microorganisms, and prevent corneal melt.[140] Most PDT studies have attempted collagen cross-linking by using riboflavin and UVA following the Dresden protocol and termed it a photoactivated chromophore for infectious keratitis corneal cross-linking (PACK-CXL).[44] Based on available evidence, PACK-CXL is most effective in resolving bacterial keratitis with limited success in fungal keratitis. However, the data is insufficient to comment on acanthamoeba, viral, and mixed infective keratitis.[141,142]

A fundamental difference has been noted in susceptibility to PDT between different organisms because of the variation in their cell membranes and cellular organelles.[138,139] This made researchers to experiment with different permutations of photosensitizers and light, for example, toluidine blue O with red light for bacterial keratitis, methylene blue with argon laser for Candida, and rose bengal (RB) with a green light for Fusarium, Aspergillus, Candida, Acanthamoeba, and methicillin-resistant *Staphylococcus aureus* (MRSA).[139,143-145] RB-PDT was reported to have better efficacy than riboflavin CXL in inhibiting fungal growth in an *in vitro* study.[146] However, the depth of penetration of photosensitizers in inflamed corneas, exact duration/dose, and the possibility of intraocular complications because of the light remains to be determined.
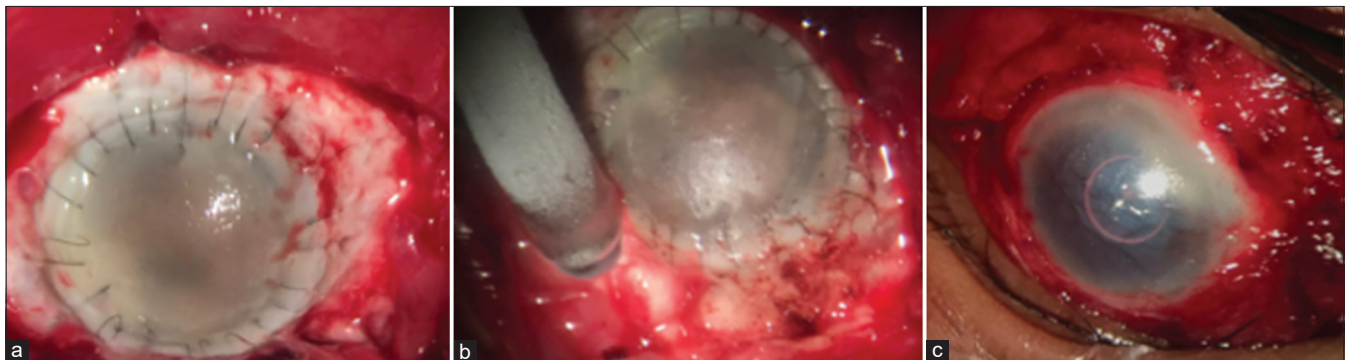
b) Phototherapy

The major disadvantage of PDT is the two-part combination approach (photosensitizer + light), with challenges in introducing the same photosensitizer in different microorganisms along with limitations in tissue penetration of the light. To counter this, only light-based therapy is being investigated as an option. Blue light has been gaining attention due to its intrinsic antimicrobial effect and is supposedly less damaging to mammalian cells than ultraviolet light.[147] The exact mechanism of action is still unclear, but the accepted hypothesis is that it excites endogenous intracellular porphyrins, which produce highly cytotoxic reactive oxygen species, mainly singlet oxygen, similar to PDT.[148-150]

Lasers produce a coherent, monochromatic, and high-energy form of light, causing photocoagulation of the tissue. Argon laser was first used by Fromer *et al.*[151] to treat Pseudomonas keratitis in rabbit corneas. It causes heating and denaturation, leading to cell death. The temperature of the corneal tissue rises over 90° after argon laser, which is believed to contribute to its fungicidal action and increase the epithelial permeability of antimicrobials.[152,153]

Though heartening results, light therapy still needs further studies to determine appropriate timing, dose, and protocol.

c) Cold plasma

Plasma is an ionized gas and consists of ultraviolet light, electromagnetic fields, visible light, ions, heat radiation, and excited species. The effect of all these single components together leads to the disinfecting effect of plasma.[154] Argon and helium are two gases that have been used for plasma generation.[154,155] Reitberger *et al.*[154] devised an argon cold

Figure 5: Clinical picture showing (a) recurrence beyond the graft host junction 10 days following a therapeutic graft for pythium keratitis; (b) intraoperative cryotherapy to the base and edges of the infected area; (c) two days following repeat therapeutic graft
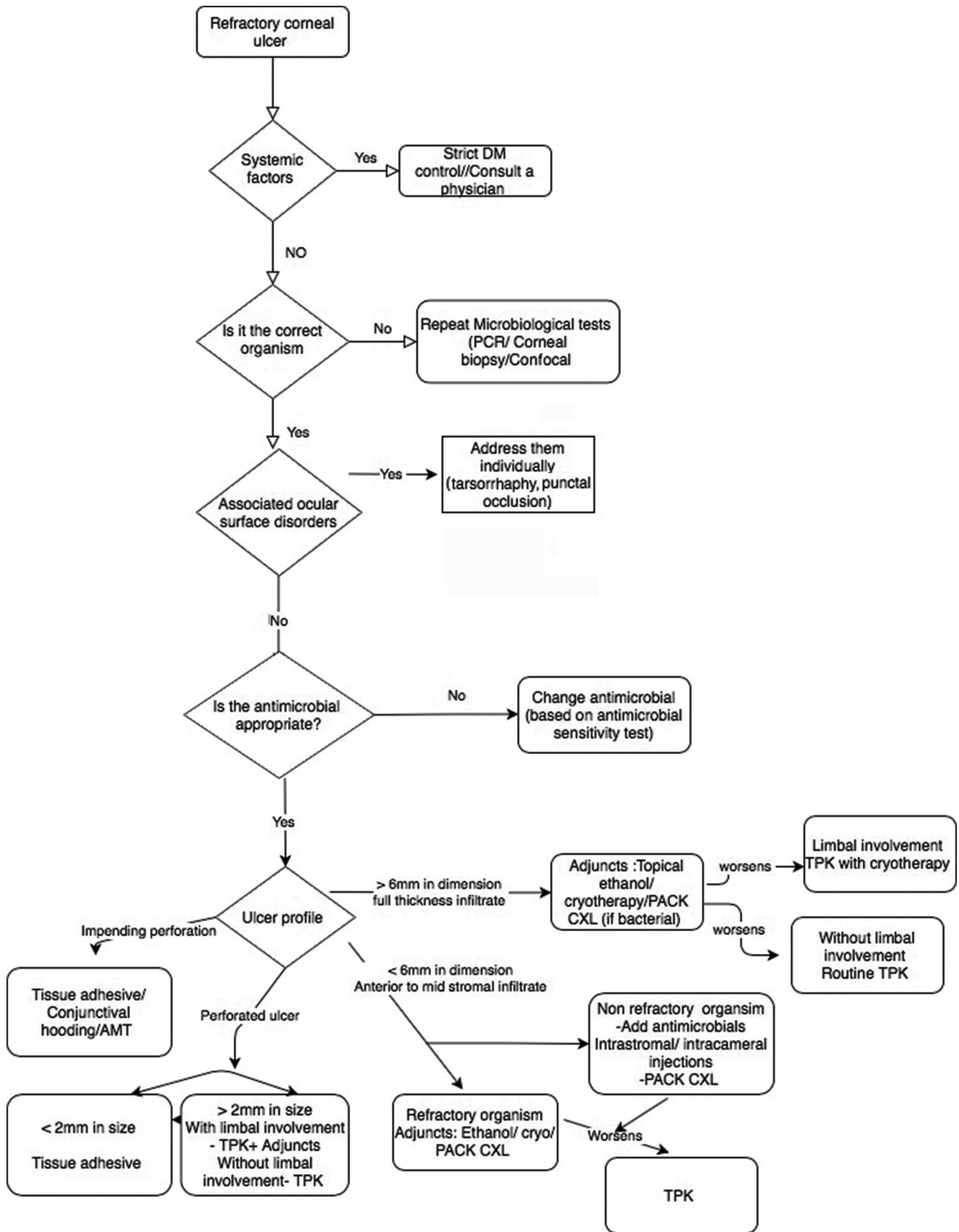
Figure 6: Flowchart depicting a stepwise multidimensional approach for managing refractory corneal ulcers

plasma pen and reported its successful use in treating corneal infections with both bacterial and fungal organisms, *in vitro* and *in vivo*. However, the response of microorganisms differs from the cold plasma, thus indicating that factors such as the type of plasma, the distance between the jet and the treating surface, duration of treatment, and characteristic features of microorganisms influence the response to treatment.[156]

d) Alcohol

Alcohols have broad-spectrum antimicrobial activity against almost all microorganisms such as bacteria, fungi, viruses, and Acanthamoeba. The antimicrobial property is optimal between 60% and 90%.[157] It acts mainly on the membrane, alters the pH, increases membrane leakage and inhibits growth, reduces sugar uptake, and increases thermal sensitivity.[158] Agarwal *et al*.[123] reported the efficacy and safety of topical absolute ethanol in the treatment of *Pythium insidiosum* keratitis. They noted that the absence of ergosterol in the cell wall of *Pythium* makes it more susceptible to ethanol as compared to fungi in *in vitro* studies. The authors recommend an outpatient procedure for placing a cotton swab soaked in absolute (99.9%) ethanol over the corneal infiltrate for 60 s, in the supine position following application of topical anesthesia and an eye speculum. Repeat applications are based on the improving clinical response. Ethanol is also found to have cytotoxic effects on Acanthamoeba cysts in addition to the trophozoites, and pretreatment with ethanol was found to be safe and effective in controlling Acanthamoeba keratitis in 20 of 24 eyes.[159,160] However, further studies are required to determine the exact dose and duration of treatment.

e) Cryotherapy

Cryotherapy has been used in the treatment of herpes virus and pseudomonas keratitis.[161,162] The possible mechanism of the efficacy include mechanical destruction of microorganisms because of intra and extracellular ice formation, osmotic disequilibrium, disruption of DNA, and other cellular and enzymatic changes.[161] The cooling effect is reported to be best when the diameter of the freezing point is 1 mm larger than the freezing head, with freezing temperatures ranging from −50°C to −60°C for a freezing time of 6–7 s.[163] Cryotherapy causes denaturation and degradation of proteins within fungal cell walls, resulting in their fracture.[164] These cryotherapeutic actions have been found to be cidal for the trophozoites but not for the acanthamoeba cysts.[165] The cornea can tolerate freezing till the endothelium is intact and can regenerate after freeze injury helping regain the normal transparency.[166,167]

Surgical management

Relentless worsening of the infiltrate, limbal involvement, impending or actual perforations, are indications for urgent surgical or specialist measures.

a) Worsening infiltrate

Despite all measures, in eyes with worsening of the infiltrate, extension to and beyond the limbus, a therapeutic graft is recommended to avoid spillover to and involvement of adjacent tissues leading to sclerokeratitis or endophthalmitis. Recurrence has been noted in the graft host interface post lamellar keratoplasty; however, in cases with the stromal participation alone, a deep anterior lamellar keratoplasty can be attempted with a thorough wash of the interface with antimicrobials intraoperatively.[168,169] In full-thickness infiltrates, a penetrating keratoplasty with graft size 0.5–1 mm larger than the clinically

involved area is recommended as there is always a potential risk of subclinical persistence of organisms near the edges of the lesion, and a postoperative histopathological examination helps confirm whether the disease clearance was adequate. A preoperative ultrasound B-scan is advised to rule out the possibility of endophthalmitis.[170] The use of cryotherapy and ethanol as surgical adjuncts in infiltrates involving the limbus has been recommended to address macroscopically invisible involvement, thus reducing the possibility of recurrence. Single freeze-thaw cryotherapy at the trephined edge mark prior to entering the anterior chamber, followed by application of 99.9% ethanol using a sponge placed for 60 s, intraoperatively seemed to reduce the need for a repeat graft and helped salvage the globe in patients with Pythium keratitis [Fig. 5].[171]

b) Impending/small perforations

In cases with progressive corneal stromal melt or descemetocele, tissue adhesives such as cyanoacrylate glue provide the much-required tectonic support and reduce the possibility of perforation.[172] In addition, they also have bacteriostatic action and induce vascularization to promote healing. The use of cyanoacrylate glue is successful in sealing perforations up to 2–3 mm, thus avoiding the need for a therapeutic graft.[172] A conjunctival flap advocated by Gunderson, by bringing in blood vessels to the infected area, facilitates faster healing and provides tectonic support, and can be considered as a treatment option.[173] Halim *et al*. compared the results of amniotic membrane (AMT) and conjunctival flap in eyes with refractory non-viral infectious keratitis with impending/small perforations. They found both to be effective in providing metabolic and mechanical support for corneal healing in accordance with other published reports.[174-176] AMT acts as a biological bandage that promotes epithelization and may have an antimicrobial effect.[177]

c) Large perforations

Perforations larger than 3 mm usually require an urgent full-thickness patch graft or a penetrating keratoplasty as irreversible angle closure and secondary glaucoma or expulsive hemorrhage may occur if the anterior chamber remains flat or the eye remains hypotonous.[170,177] Grafts larger than 9 mm have poorer visual prognosis because of increased risk of stromal vascularization, secondary glaucoma, and recurrence of infection. Thus, in cases of central indolent or progressive infections, an early penetrating keratoplasty can be considered with or without surgical adjuncts as discussed above.[171,177-179]

Conclusion

To conclude, managing refractory corneal ulcers necessitates a stepwise multidimensional approach, involving early and accurate diagnosis, identification of associated factors contributing to its non-responsive behavior (whether local or systemic), and addressing each of them [Fig. 6]. With advances in recent research, newer modalities of treatment have been shown to supplement or act as an alternative therapy for resistant microbial keratitis and improve the overall prognosis.

Conflicts of interest

There are no conflicts of interest.

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