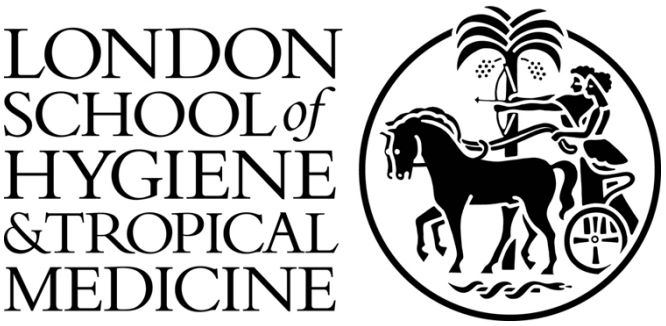

Managing Fungal Keratitis in Nepal: A Randomised Controlled Trial Comparing Chlorhexidine 0.2% to Natamycin 5%

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Thesis submitted in accordance with the requirements for the degree of Doctor of Philosophy of the University of London

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Declaration

I, Jeremy Hoffman, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

24/01/2023

Glossary

AHD	Automatic hyphae detection
AHW	Auxiliary health workers
AI	Artificial intelligence
AK	Acanthamoeba keratitis
AMG	Amniotic membrane graft
ANM	Auxiliary nurse midwifes
aOR	Adjusted odds ratio
BK	Bacterial keratitis
BSCVA	Best spectacle corrected visual acuity
CF	Counting fingers vision
CFW	Calcofluor white
CHX	Chlorhexidine
CI	Confidence interval
CL	Contact lens
CPE	Cytopathic effects
CXL	Corneal collagen cross-linking
DDA	Department of Drug Administration
DM	Diabetes mellitus
DSMB	Data Safety Monitoring Board
ECC	Eye care centre
ED	Epithelial defect
EPI	Expanded programme of immunisation
EQ-5D	EuroQol-5 dimensions
EREC-P	Eastern Region Eye Care Programme
FCHV	Female community health volunteers
FDA	Food and Drug Administration
FFI	Filamentous fungal infection
FK	Fungal keratitis
GDP	Gross domestic product
GPS	Global Positioning System
HIV	Human immunodeficiency virus
HLA	Human Leukocyte Antigen
HP	Health post
HRQoL	Health-related quality of life

ICAB	Intracameral amphotericin B
ICK	Infectious crystalline keratopathy
IQR	Interquartile range
ITT	Intention-to-treat
IVCM	*In vivo* confocal microscopy
KOH	Potassium hydroxide
LAMP	Loop-mediated isothermal amplification
LK	Lamellar keratoplasty
LMIC	Low- and middle-income country
logMAR	Logarithm of the Minimum Angle of Resolution
LPCB	Lactophenol cotton blue
LSHTM	London School of Hygiene & Tropical Medicine
MALDI-ToF MS	Matrix-assisted laser desorption / ionization time of flight mass-spectrometry
MBP	Mannose-binding protein
MIC	Minimum inhibitory concentration
MK	Microbial keratitis
MOH	Ministry of Health
MUTT	Mycotic Ulcer Treatment Trial
NATA	Natamycin
NGO	Non-governmental organisation
NHRC	Nepal Health Research Council
NLDO	Nasolacrimal duct obstruction
NNJS	Nepal Netra Jyoti Sangh
NPR	Nepali Rupees
NPV	Negative predictive value
NSS	Nothing significant seen
NTD	Neglected tropical disease
OR	Odds ratio
OSD	Ocular surface disease
PCR	Polymerase chain reaction
PHC	Primary health clinics
PHC/OC	Primary health care outreach centre
PHCW	Primary healthcare worker
PHMB	Polyhexamethylene-biguanide

PK	Penetrating keratoplasty
PL	Perception of light vision
PPV	Positive predictive value
PVA	Presenting visual acuity
QoL	Quality of life
RAAB	Rapid Assessment of Avoidable Blindness
RCT	Randomised controlled trial
RPA	Recombinase polymerase amplification
SCEH	Sagarmatha Choudhary Eye Hospital
SCUT	Steroids for Corneal Ulcers Trial
SD	Standard deviation
SNP	Single nucleotide genetic polymorphisms
SSA	Sub-Saharan Africa
TEM	Traditional eye medicine
TLR	Toll-like receptor
TPK	Therapeutic penetrating keratoplasty
TST	Topical, Systemic and Targeted Therapy
UFF	Unspecified filamentous fungi
UML	Unsupervised machine learning
VDC	Village development committee
VIF	Variance inflation factor
VRQoL	Vision-related quality of life
WHO	World Health Organization
WHO/PBD-VF20	World Health Organization Prevention of Blindness and Deafness 20-item Visual Functioning Questionnaire
WHOQOL-BREF	World Health Organization Quality of Life: Brief Version

Abstract

Background: Filamentous fungal infections of the cornea, filamentous fungal keratitis (FK), are challenging to treat. Current topical antifungals are not always effective and are often unavailable. Topical natamycin 5% is usually first-line treatment, however, even when treated intensively, infections may progress to corneal perforation; alternative antifungal medications are needed. Previous pilot studies suggest that topical chlorhexidine 0.2% compares favourably with topical natamycin. Full-scale randomised controlled trials (RCTs) of topical chlorhexidine 0.2% are warranted to answer this question definitively. In addition, outcomes for patients with FK are poor as they often present late, clinical diagnosis is challenging, and investigations are limited. Developing the evidence-base to guide practice and ultimately improve outcomes is therefore required.

Methods: All consenting patients with microbial keratitis (MK) attending a tertiary ophthalmic referral hospital in Eastern Nepal were assessed for the presence of FK by *in vivo* confocal microscopy (IVCM) and/or smear microscopy. Demographic, clinical, journey, and microbiological data were collected from all these patients. These data were analysed in a cohort study to investigate reasons associated with delayed presentation as well as two nested case-controlled studies, investigating indicative clinical features and evaluating investigations. Patients with confirmed FK were enrolled in the RCT and randomly allocated to receive topical chlorhexidine 0.2% or topical natamycin 5%. Primary analysis (intention-to-treat) was by linear regression, using baseline logarithm of the minimum angle of resolution (logMAR) best spectacle-corrected visual acuity (BSCVA) and treatment arm as prespecified covariates. The primary outcome measure was BSCVA at 3 months. Secondary outcome measures included perforation or therapeutic penetrating keratoplasty by 90 days.

Results: Between 3 June 2019 and 9 November 2020 we enrolled 643 patients with MK. Of these, 354 were eligible for the RCT and randomly assigned: 178 to chlorhexidine and 176 to natamycin. Excluding mixed infections, primary outcome data were available for 141 and 143 of the chlorhexidine and natamycin groups, respectively. We did not find evidence to suggest chlorhexidine was noninferior to natamycin and in fact found strong evidence to suggest that natamycin-treated participants had significantly better 3-month BSCVA than chlorhexidine-treated participants, after adjusting for baseline BSCVA (regression coefficient, −0.30; 95% confidence interval [CI], −0.42 to −0.18; $P < 0.001$). There was no difference in re-culture positivity between arms at day 7. The majority of chlorhexidine-treated patients healed (151/175, 86.3%), although this was less than natamycin-treated cases (163/173, 94.2%; $P = 0.018$). Furthermore, there were more perforations and emergency corneal grafts in the chlorhexidine arm (24/175, 13.7%) than in the natamycin arm (10/173, 5.8%; $P = 0.018$, mixed infections included).

A fungal cause was identified in 482/642 (75.1%) of cases, which increased to 532/642 (82.9%) when including mixed infections. Unusually, dematiaceous fungi accounted for half of the culture-positive cases (50.6%). Serrated infiltrate margins, patent nasolacrimal duct, raised corneal slough, and organic trauma were independently associated with fungal keratitis ($p < 0.01$). Smear microscopy had the highest sensitivity (90.7% [87.9-93.1%]), followed by IVCM (89.8% [86.9-92.3%]) and culture (75.7% [71.8-79.3%]). Of the three smear microscopy stains, KOH had the highest sensitivity (85.3% [81.9-88.4%]), followed by Gram stain (83.2% [79.7-86.4%]) and calcofluor white (79.1% [75.4-82.5%]).

In the cohort study, the majority of patients (96%) self-referred. Over half (328/643) of all cases presented after at least seven days. The total cost of care increased with increasing numbers of facilities visited ($P<0.001$). Those living furthest away were least likely to present directly ($P<0.001$). Factors independently associated with delayed presentation included distance >50km from the eye hospital (aOR 5.760 [95% CI 1.829-18.14, p=0.003]), previous antifungal use (aOR 4.706 [95% CI 3.139-5.360]), and two or more previous journeys (aOR 1.442 [95% CI 1.111-3.255]).

Conclusion: Treatment with natamycin is associated with significantly better visual acuity, with fewer perforations, compared to treatment with chlorhexidine. However, the proportion of healed chlorhexidine-treated cases is comparable to that of voriconazole reported in earlier trials. Natamycin remains the preferred first-line monotherapy treatment for filamentous fungal keratitis. Chlorhexidine 0.2% may be considered in situations where natamycin is unavailable. Smear microscopy and IVCM were the most sensitive tools for identifying FK in our cohort, whilst certain clinical signs can help direct the clinician to find a presumptive infectious cause, allowing appropriate treatment to be started without delay. Distance to the eye hospital is a significant barrier to prompt, direct presentation.

Format of the Thesis

The thesis for this PhD utilises the “research papers” format, recently introduced by the London School of Hygiene and Tropical Medicine. It therefore includes several papers which are either published, accepted or in submittable format for publication in peer-reviewed journals. The chapters listed in italics in the Contents are in this research/review paper format, and each chapter includes publication details in a cover sheet, including acknowledgement of the contributions of other people. The other chapters of the thesis are composed of “linking material” which includes information/data not covered in the research papers and helps to make the thesis a coherent body.

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Dedication

I dedicate this thesis to the countless patients across the world who are suffering with, or have suffered from, fungal keratitis; I hope that this work goes a little way in the quest to improve the outcome for them and future patients.

I also dedicate this work to my late grandfather, Dr Adrian J. Salter, an alumnus of the London School of Hygiene & Tropical Medicine, and lead-researcher into antimicrobials and antivirals during the late 20th century at the Wellcome Research Laboratories. He left us well before his time but has nevertheless been an inspiration for me throughout this journey. I know he would have read this work from cover-to-cover and I hope this would have made him proud.

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Finally, to the Wellcome Trust for funding this work, without this support we would have achieved nothing.

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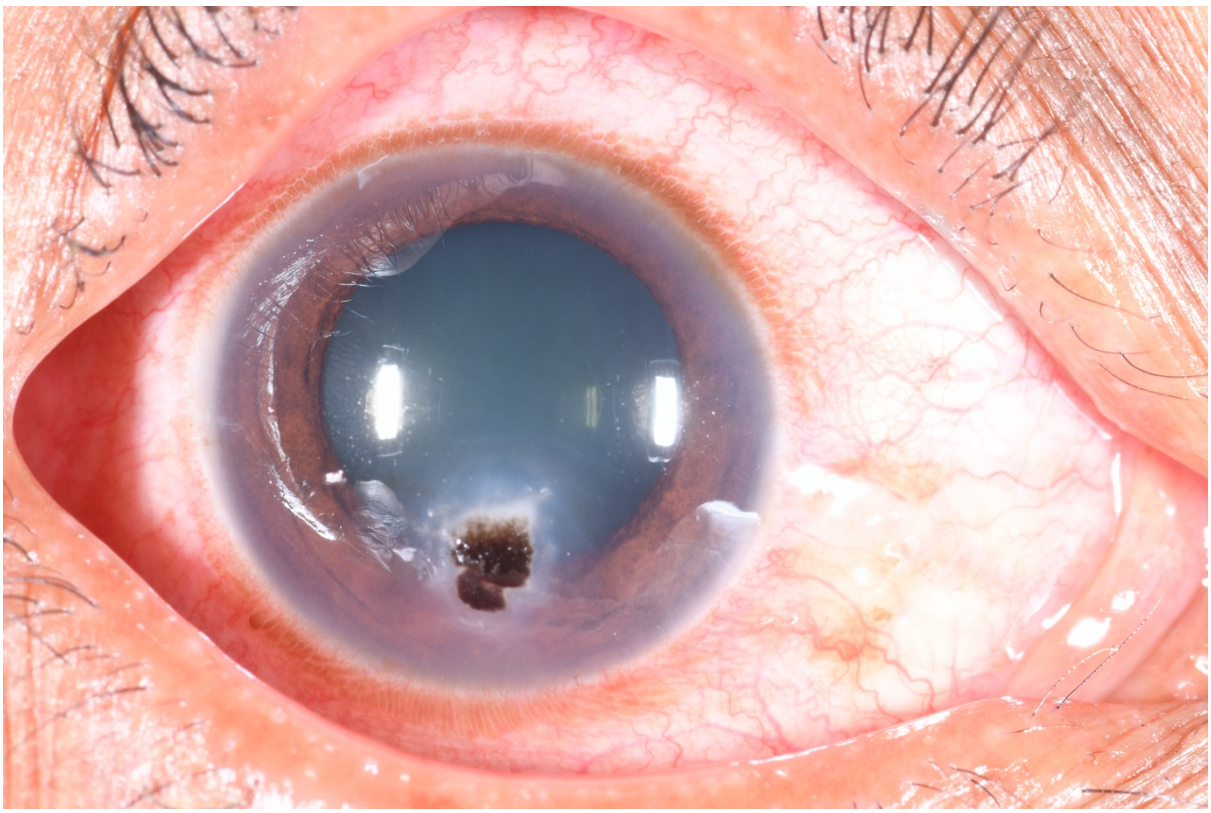
Chapter 1: Background [1]



The right eye of a 40-year-old Nepali farmer with filamentous fungal keratitis (*Curvularia* spp.), presenting at one week from symptom onset

[1] In this section, the epidemiology, clinical features, and diagnostic investigations for fungal keratitis within the broader context of microbial keratitis are presented. These subtopics are largely covered by a published review article in the *Journal of Fungi*, which is included here in full.[1] Additional background information, relating to other causes of microbial keratitis and *in vivo* confocal microscopy that is not fully covered within that review article, are given in subsequent sections below.

Microbial keratitis

Corneal infection, or microbial keratitis (MK), is an ocular emergency that often leads to permanent sight-loss in the affected eye. MK is caused by several different pathogenic groups of microorganisms. Causative microorganisms include bacteria, protozoa (particularly *Acanthamoeba* spp.), non-filamentous fungi (yeasts and microsporidia), and filamentous fungi.[1] Viruses can also cause infectious keratitis, although broadly MK indicates non-viral causes.[2] The predominant clinical features of pain, conjunctival injection and corneal ulceration (epithelial defects associated with stromal infiltrates) are typical for MK regardless of the underlying causative organisms, making clinical diagnosis challenging, although some clinical features may be suggestive of certain organisms.[3-6]

MK is associated with significant adverse clinical outcomes including monocular blindness (defined as presenting distance vision less than 3/60 in the affected eye)[7] as a result of dense corneal scarring, and eye-loss due to corneal perforation and endophthalmitis.[8-10] As a result, it is the leading cause of monocular blindness in tropical low- and middle-income countries (LMICs) after cataract, with an approximate annual incidence of 2 million cases in Africa and Asia.[9] Bilateral corneal scarring (excluding that caused by trachoma or vitamin A deficiency) is believed to be responsible for blindness (defined as presenting distance vision of less than 3/60 in the better eye)[7] in 1.3 million people globally, or 3.2% of the global burden.[10] The negative impact of MK goes beyond clinical morbidity associated with pain and blindness: MK is known to reduce quality of life,[11-13] cause direct economic costs,[14] place a significant burden on healthcare infrastructure,[15, 16] and likely cause a similar burden on wider society, given that MK most frequently affects individuals during their most productive years of life.[17]

Defining the magnitude of MK is challenging as most data are reported as “corneal blindness”, a heterogenous range of conditions including corneal trauma, infection, inflammation and inherited disorders. A recent review from Ung and co-workers was only able to identify 11 suitable published studies on MK, three of which included data only from contact lens (CL) wearers (**Table**).[2] Of note, there were no published studies from Africa included. There is considerable global variation in the incidence of MK: the highest incidence is found in Asia (apart from a study from Hong Kong),[18-21] whilst the lowest incidence is found in Europe and North America. [22-29]

Table 1: Incidence of microbial keratitis from population-based studies (adapted and updated from Ung et al. (2019)[2]

Source	Location	Years studied	Overall incidence (incidence in contact lens wearers) per 100,000
South and Southeast Asia			
WHO (2004)[18]	Bhutan	NS	339
WHO (2004)[18]	Burma	NS	710
Lam et al (2002)[19]	Hong Kong	1997-1998	6.3 (33.9)
Upadhyay et al (2001) [20]	Nepal (Bhaktapur)	1992-1993	799
Gonzales et al. (1996) [21]	India (Tamil Nadu)	1993	113
North America, Australasia and Europe			
Jeng et al. (2010)[22]	USA (Northern California)	1998-1999	27.6[a]
Erie et al. (1993)[23]	USA (Minnesota)	1950-1988	2.5-11[b]
Poggio et al (1999)[24]	USA (New England)	1987	(20-209)[c]
Ibrahim et al (2012)[25]	UK (Portsmouth)	1997-2003	52.1
Ibrahim et al (2012)[25]	UK (Portsmouth)	2006	40.3
Seal et al (1999)[26]	UK (West Scotland)	1995	3.6 (18.1)
Ting et al (2021)[27]	UK (Nottingham)	2007-2019	34.7
Cheng et al (1999)[28]	The Netherlands	1996	(11-209)[c]
Stapleton et al (2008)[29]	Australia	2003-2004	(42)

WHO, World Health Organization; NS, not specified. [a] per 100,000-person-years, [b] 2.5/1000,000 years in the 1950s and 11/100,000 years in the 1980s; [c] range of incidence amongst contact lens wearers depending on type of lens (lower incidence in hard/ rigid gas permeable lenses, highest incidence in extended-wear soft)

Causative organisms and pathogenesis of microbial keratitis

As discussed in more detail in the review below, there is clear geographic variation in causative organisms for MK.[1] In summary, filamentous fungal organisms are more frequently responsible for MK in tropical LMICs compared to bacterial organisms, whereas in developed, temperate countries this situation is reversed.[1, 30, 31] Environmental factors (including climate and potentially rainfall) and poverty (in particular countries where there is a high degree of manual subsistence farming resulting in ocular trauma) are most likely responsible for this variation.[1] Contact lens use is the main risk factor for developing microbial, and in particular bacterial, keratitis in temperate, high-income countries. However, there have been reports of a recent increase in fungal keratitis cases amongst contact lens wearers in these locations.[31,

[32] Although rare overall, *Acanthamoeba* keratitis (AK) is also frequently associated with contact lens use, and the incidence of AK is also believed to be increasing in certain countries, such as the UK.[33] In this section, we briefly describe the relevant microbiology (particularly relating to virulence) of the most commonly implicated microorganisms and their pathogenesis.

Bacterial keratitis

Regardless of location, the most common bacteria responsible for MK are *Streptococcus pneumoniae, Staphylococcus aureus,* and *Pseudomonas aeruginosa.*[34] In terms of pathogenicity, they all share a common mechanism: adhesion, bacterial invasion, and cytotoxicity, and in some cases exotoxin production.

Staphylococcus aureus

Staphylococcus aureus is a Gram-positive, round-shaped facultative anaerobe that is often part of the normal skin flora of individuals, with 35% of the general public and up to 66% of healthcare professionals colonised with this organism.[35, 36] Keratitis can typically only develop in the absence of an intact corneal epithelium. Host immunity is largely afforded by various components of the tear film, including but not limited to cytokines, secretory IgA, surfactant, proteases and phospholipases.[37] However, any defect in the corneal epithelium or reduction in this innate protection as a result of dry eye disease, corneal trauma, surgery, or contact lens wear, can allow the organism to adhere to the cornea, and ultimately enter the corneal stroma.

Staphylococcus aureus possess several virulence factors. These include the bacterial binding proteins (fibronectin binding protein and collagen binding protein) as well as inflammation and tissue damage mediators, including teichoic acid, alpha-toxin, gamma-toxin, Panton-Valentine leucocidin, super-antigen like protein, and *S. aureus* elastase.[37] Through genetic variation, different strains of *S. aureus* possess various combinations of virulence factors, making certain strains more (or less) virulent in terms of being able to cause MK. Once infection is established, tissue damage occurs through both direct bacterial virulence factors as well as indirectly through “bystander” damage resulting from the immune response and pro-inflammatory molecules and host-produced proteases;[37] this pathogenesis is common for most causes of MK.

Streptococcus pneumoniae

Streptococcus pneumoniae is a Gram-positive, facultatively anaerobic alpha haemolytic pathogen.[38] As well as keratitis, it can cause systemic disease including pneumonia, meningitis, otitis media and sinusitis.[39] The host defences it needs to evade are the same as those described for *S. aureus,* and it is typically also unable to establish infection in the presence of an intact corneal epithelium. *S. pneumoniae* keratitis is therefore frequently caused by improper contact lens wear, trauma or previous ocular surgery.[38]

There are several virulence factors implicated in *Streptococcus pneumoniae* keratitis.[38] Although acting as a virulence factor for other diseases, the polysaccharide capsule is not believed to be necessary for pathogenesis in keratitis.[40] Conversely, the inflammation encountered during pneumococcal keratitis is largely due to the host reaction to pneumolysin, one of the key virulence factors for this organism.[41, 42]

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a versatile, encapsulate, Gram-negative rod that is able to thrive in a wide range of environmental conditions but also acts as an opportunistic pathogen, resulting in various infections.[43] *Pseudomonas aeruginosa* keratitis is frequently associated with contact lens wear, with many studies implicating it in the majority of MK cases associated with contact lens use.[34, 44, 45]

Visual outcomes for patients with bacterial keratitis caused by *P. aeruginosa* is known to be significantly worse than infection caused by other bacteria.[46] This is a combination of the multitude of virulence factors and the resulting significant host immune response to acute infection. The full array of virulence factors are beyond the scope of this thesis, however a number of key virulence factors include proteases, elastases, flagella, adhesins, phospholipases, type IV pili, exotoxins and alginate.[45] Unlike the other two bacteria discussed above, a certain strain of *P. aeruginosa* are able to invade intact corneal epithelium. This is possible as this invasive strain possess the *exoS* gene which codes for production of the exotoxin S. This strain is associated with poor prognosis and increased resistance to first-line antibiotics (fluoroquinolones).[47] Another important strain contains the *exoU* gene, this isolate secretes exotoxin U that is acutely cytotoxic;[48] this strain is associated with contact lens wear.[47]

Fungal keratitis

Fusarium spp. and *Aspergillus* spp. are the most common causes of filamentous fungal keratitis.[1, 31, 49] Dematiaceous fungi such as *Curvularia* spp. are also occasionally implicated.[50-53] Yeasts, in particular *Candida* spp., are commonly reported in more temperate climates.[32, 54-56] The pathogenesis of fungal keratitis first starts with the adhesion of fungal conidia to the host cornea, followed by invasion, morphogenesis, and toxin production.

Fusarium spp.

Fusarium spp. are found in the soil, where they act as saprophytes to break down dead plants and are also commonly implicated as plant pathogens, particularly of cereal crops and bananas.[30] There are many species within the genus that have been implicated in fungal keratitis, however *Fusarium solani* and *Fusarium oxysporum* have been most frequently implicated.[57] Fusaria are unable to establish keratitis through an intact corneal epithelium, and therefore corneal epithelial defects are required for infection to develop. This is typically following vegetative trauma, which allows direct inoculation of fungal conidia to the corneal stroma.[58]

There are several virulence factors that have been identified for *Fusarium* spp. PacC is part of an intracellular signalling system that is activated at neutral to alkaline pH through enzymatic proteolysis.[59, 60] This then results in the upregulation and expression of several fungal genes including proteases and phosphatases, facilitating opportunistic fungal invasion into traumatised host cornea.[60, 61] These extracellular proteases produced by *Fusarium* spp. are able to digest the corneal stromal collagen, allowing deeper invasion into the host tissue.[62] Like other filamentous fungi, including *Aspergillus* spp., *Fusarium* spp. produces biofilms, which are able to protect the fungus from the host immune response as well as antifungal medications.[63] This work also indicated that *F. solani* species complex forms more biofilms than *F. oxysporum* species complex.[63] Fusaria are also known to produce mycotoxins including fusaric acid, moniliformin, and/or fumonisin B1. [64] These toxins are cytotoxic to corneal tissue.[64]

Aspergillus spp.

Aspergilli conidia are found ubiquitously in the air throughout the world, with no apparent seasonal or climatic variation in spore concentration.[65] The most common aspergilli isolated in fungal keratitis are *A. fumigatus* and *A. flavus*.[66-68] Similar to fusarium keratitis, aspergilli are unable to penetrate the corneal epithelium, meaning that corneal trauma is also a significant risk factor for developing aspergillus keratitis.[69, 70]

As with all other causes of microbial keratitis, disease ensues because of the interaction between the direct pathogenic effects of the organism and the host's immune response. The aspergilli possess several virulence factors that are integral in this interplay. Although likely common across much of the genus, virulence factors have most often been studied in *A. fumigatus* and *A. flavus* models. These are discussed briefly here.

The conidia are coated in surface active proteins called hydrophobins that are attached to the fungal cell wall, that assist in evading host detection (specifically by c-type lectins).[71] Models have shown that when these hydrophobins are absent, certain molecules on the fungal cell wall are exposed (beta 1,3-glucan and alpha-mannan) which activate the innate immune system by binding to c-type lectins.[72] Avoiding this early detection allows the spores to germinate into hyphae and ultimately enhances fungal survival. Aspergillus conidia are also able to suppress the host proinflammatory response by modulation of Toll-like receptor (TLR)2 and TLR4 signalling through the effects of certain cell wall components: beta-glucan, alpha-glucan, and galactomannan.[73]

Like the fusaria, the aspergilli are able to produce biofilms, which act as a physical barrier against the host's immune system and antifungal drugs.[74] Furthermore, aspergilli can produce toxins that are cytotoxic to corneal cells, including aflatoxin B1 and proteases, implicated in corneal melting and reduced host immune response.[62, 75]

Dematiaceous fungi

After the fusaria and aspergilli, the dematiaceous fungi are the next leading cause of FK. This is a broad group of moulds, characterised by their ability to produce melanin, and believed to lend a significant pathogenic advantage to these fungi.[76] Similar to *Fusarium* spp., dematiaceous fungi are ubiquitous plant pathogens that infrequently cause disease in humans, and similar to other causes of FK, a corneal epithelial defect is required in order for disease to become established. The virulence factors of these fungi for causing keratitis are not well established, although it is likely that many of the virulence factors described above for the aspergilli and fusaria are similar. In addition to these, the production of melanin is believed to be a key virulence factor by allowing fungi to evade various components of the host immune response. For example, melanin can reduce the toxicity of microbicidal peptides, oxygen free radicals and inhibit phagocytosis, as well as increasing the mechanical strength of the fungal cell wall, allowing penetration of host tissue.[76, 77] Melanins have also been shown to reduce the efficacy of commonly used antifungals.[78]

Candida spp.

Keratitis caused by yeast-like fungi, in particular *Candida spp.*, was previously the predominant cause of FK in countries such as the UK and USA, although recent trends suggest this may no longer be the case in certain locations. Typically, FK caused by *Candida* spp. occurs in patients with a history of preceding topical corticosteroid use, post-surgery, systemic disease and immunosuppression, or reduced ocular immunity. *C. albicans* and *C. parapsilosis* are the most commonly isolated species.[79, 80]

Candida spp. possess several virulence factors involved in colonisation and adhesion, invasion, and host immune-escape, although most described relate to non-ocular infection.[79, 81, 82] Biofilm formation is a potential virulence factor for *Candida* keratitis, specifically in conferring resistance to antifungal medications,[83] although work from India did not find any significant reduction in antifungal susceptibility for biofilm-producing strains.[79]

Acanthamoeba keratitis

AK is an uncommon but very serious, painful and debilitating cause of microbial keratitis, resulting from infection by parasites from the *Acanthamoeba* genus.[33, 84-87] Contact lens wear is the leading risk factor for developing AK,[33, 86] although it can also occur in non-contact lens wearers.[88, 89] The incidence of AK is increasing in countries including the UK and USA.[33, 90, 91] Animal models suggest that corneal surface injury is a pre-requisite for developing AK.[92]

The first step that is critical in the establishment of AK is the adhesion of the parasitic organism to the host surface. A major virulence factor for *Acanthamoeba* is a mannose-binding protein (MBP) that mediates the adhesion of amoeba to the corneal surface, and is also implicated in subsequent events that can lead to significant cytopathic effects (CPE).[84]

Acanthamoeba MBP is a transmembrane protein with a similar structure to a typical cell surface receptor.[93] MBP mediates adhesion to host corneal cells, after which the amoeba produce contact-dependent metalloproteinase plus numerous contact-independent serine proteases.[84, 94] Together, these enzymes result in potent CPE by killing direct cytotoxicity, digestion of the epithelial basement membrane and corneal stroma, and penetration into the deeper layers of the cornea.[84, 94] Fortunately, human tears contain both secretory anti-MBP IgA that helps to protect against adhesion of parasites to host cells,[84, 95] as well as innate factors that can inhibit cytotoxic proteinases.[84]

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Thesis Title	Managing Fungal Keratitis in Nepal: A Randomised Controlled Trial Comparing Chlorhexidine 0.2% to Natamycin 5%		
Primary Supervisor	PROF. MATTHEW J. BURTON		

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Review

Mycotic Keratitis—A Global Threat from the Filamentous Fungi

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Abstract: Mycotic or fungal keratitis (FK) is a sight-threatening disease, caused by infection of the cornea by filamentous fungi or yeasts. In tropical, low and middle-income countries, it accounts for the majority of cases of microbial keratitis (MK). Filamentous fungi, in particular *Fusarium* spp., the aspergilli and dematiaceous fungi, are responsible for the greatest burden of disease. The predominant risk factor for filamentous fungal keratitis is trauma, typically with organic, plant-based material. In developed countries, contact lens wear and related products are frequently implicated as risk factors, and have been linked to global outbreaks of *Fusarium* keratitis in the recent past. In 2020, the incidence of FK was estimated to be over 1 million cases per year, and there is significant geographical variation; accounting for less than 1% of cases of MK in some European countries to over 80% in parts of south and south-east Asia. The proportion of MK cases is inversely correlated to distance from the equator and there is emerging evidence that the incidence of FK may be increasing. Diagnosing FK is challenging; accurate diagnosis relies on reliable microscopy and culture, aided by adjunctive tools such as in vivo confocal microscopy or PCR. Unfortunately, these facilities are infrequently available in areas most in need. Current topical antifungals are not very effective; infections can progress despite prompt treatment. Antifungal drops are often unavailable. When available, natamycin is usually first-line treatment. However, infections may progress to perforation in ~25% of cases. Future work needs to be directed at addressing these challenges and unmet needs. This review discusses the epidemiology, clinical features, diagnosis, management and aetiology of FK.

Keywords: microbial keratitis; fungal keratitis; microbiology; mycotic keratitis; epidemiology; *Fusarium*; *Aspergillus*; dematiaceous fungi; blindness

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1. Introduction

Mycotic or fungal keratitis (FK) is a severe and potentially blinding infection of the cornea (Figure 1) and is considered an ophthalmic emergency [1,2]. It is one of the leading causes of microbial keratitis (MK) or corneal ulcer. The latest conservative estimates predict that there are close to 1.5 million new infections every year [3], which correlate with estimates published more than 20 years ago [4,5]. The burden of FK is greatest in tropical and subtropical countries, accounting for between 20 and 60% of MK cases presenting in tropical regions [6], likely a result of climate (higher temperatures and relative humidity) and frequent agriculture-related ocular trauma [7].

Fungal keratitis is caused by yeasts and filamentous fungi but the pattern of infection varies globally with respect to aetiology and predisposing risk factors relating to geographical location and occupational exposure. Infections due to *Candida* spp. and other yeasts are typically associated with steroid use, ocular surface disorders, previous ocular surgery, contact lens wear and underlying illness resulting in immuno-incompetency [8], mostly

occurring in temperate climes. However, the main burden of disease globally is attributable to the filamentous fungi and these infections predominantly affect the poorest patients in warm, humid, tropical climatic regions [7]. There have also been reports of an increase in *Fusarium*-related keratitis in contact lens wearers in temperate, industrialised regions [9–11]. Interestingly, even within developed countries fungal keratitis is a disease of poverty: infections are associated with contact lens wearers from deprived or low socioeconomic backgrounds [3,12].

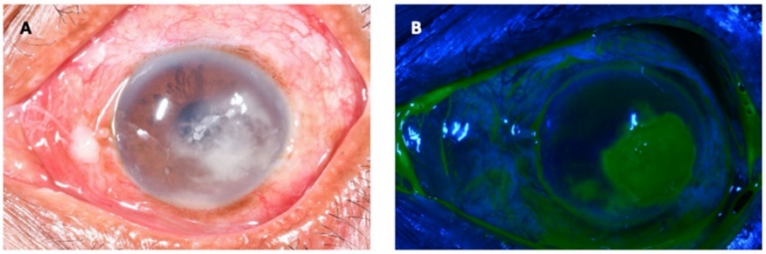


Figure 1. Fungal keratitis in a patient presenting to an ophthalmic hospital in Nepal. The causative organism was confirmed to be *Fusarium* sp. on culture. (**A**): The conjunctiva is hyperaemic, causing the eye to be red. There is a white corneal infiltrate with feathery serrated margins and satellite lesions present. There is also a small hypopyon. (**B**): The same eye as viewed with a cobalt blue filter after instillation of topical fluorescein. The area staining in green represents a defect in the corneal epithelium.

Over one hundred different species of filamentous fungi isolated from infected corneas have been reported in the literature [13]. The most common genera isolated from filamentous fungal keratitis cases are *Fusarium* spp. and the aspergilli [14], followed by the dematiaceous fungi-a heterogenous group of fungi characterised by melanin-production and pigmentation-*Curvularia* spp. being the most commonly reported genus from this group [15–17].

Patients typically present with a red, painful eye, together with reduced vision. Clinical examination will demonstrate conjunctival hyperaemia, making the eye appear red, in conjunction with a corneal infiltrate-an area of corneal opacity, often white or cream in colour (Figure 1A). There will also usually be loss of the corneal epithelium overlying the infiltrate, which stains with topical fluorescein eye drops and fluoresces green under blue light (Figure 1B). These clinical signs can be observed without a slit-lamp, a simple torch with or without loupes will suffice, aided by a blue filter and fluorescein testing strips. More detailed examination using a slit-lamp biomicroscope yields more subtle signs that can help distinguish the different causative agents of MK to some extent; fungal keratitis is more likely if there are serrated margins, raised slough (dead epithelial tissue), and/or colour other than yellow [2].

Unfortunately, presentation to an appropriate eye care provider is usually delayed, with patients often taking a convoluted journey to reach an ophthalmic clinician [18]. Compounding this is the fact that patients often self-medicate with traditional eye medicine which commonly contains non-sterile plant matter, or inappropriate conventional medication (such as topical corticosteroids), exacerbating disease [1,19,20]. Primary health-care workers have little training in recognising, treating or referring MK [21]. This delay leads to advanced infections, which have poor outcomes [1,18]. Accurate diagnosis remains challenging as it is frequently not possible to clinically distinguish bacterial and fungal MK. Microbiology services are usually unavailable. Due to resource limitations in the populations most at risk of these infections microscopy and culture remain the mainstay for diagnosis-Gram, Potassium Hydroxide (KOH), calcofluor white (CFW). Microscopy is the gold standard with visualisation of fungal hyphae in corneal tissue specimens.

Added to this is the fact that FK is particularly challenging to treat. Current topical antifungals are not consistently effective and infections can progress despite prompt

treatment, Figure 2 [1,22–26], with up to 30% of patients receiving current 'gold-standard' therapy progressing to corneal perforation and/or eye-loss, mediated by human and pathogen derived proteases [1,22,24]. Antifungal drops are rarely available in sub-Saharan Africa and often scarce elsewhere where the burden is greatest [1].

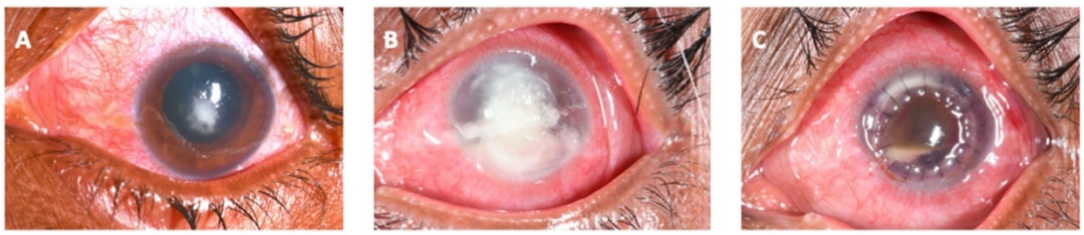


Figure 2. The progression of a patient with fungal keratitis caused by *Aspergillus* sp. This patient presented early in the course of the disease with a relatively small corneal ulcer, with serrated feathery margins to the corneal infiltrate (**A**). Despite intense, appropriate, prompt treatment with topical natamycin 5%, the corneal infiltrate increased in size, ultimately perforating, and was temporarily treated with corneal gluing and bandage contact lens insertion (**B**). The patient ultimately underwent a therapeutic penetrating keratoplasty (**C**).

Treatment can be administered topically (first-line, with intensive hourly dosing for at least the first 48 h), orally or by intravenous, subconjunctival, corneal stromal or intracameral injection. The treatment of yeast infections is often different to filamentous fungi, with the former being more common in temperate climates and the latter in hot and humid locations [27,28]. Surgical therapy, typically with therapeutic penetrating keratoplasty (PK, corneal transplantation replacing the diseased cornea with donated corneal tissue), is generally reserved for cases of corneal perforation or progressive infection refractory to medical therapy. PK can also be performed for visual rehabilitation after the acute infection has resolved.

In this review, given the wide range of organisms implicated in fungal keratitis, we have classified the causative organisms into three main groups: hyaline fungi, dematiaceous fungi and yeasts; the focus of this review are the filamentous fungi. The epidemiology, clinical features, diagnosis and management will also be discussed for fungal keratitis as a distinct entity.

2. Epidemiology of Fungal Keratitis

2.1. Incidence

Until recently, the annual global incidence of fungal keratitis had never been estimated. In 2020, Brown et al. estimated the incidence of fungal keratitis to be 1,051,787 cases per annum, within a range of between 736,251 and 1,367,323 cases per annum [3]. The incidence may in fact be higher at 1,480,916 cases per annum (range 1,036,641–1,925,191) if it is assumed that all unconfirmed culture negative cases of microbial keratitis were in fact fungal in aetiology. The morbidity associated with FK is also important to note: approximately 10–25% of eyes with FK will perforate or need surgical removal, whilst at least 60% of patients, even if treated, are left monocularly blind, equating to approximately 800,000 people per year [1,22,24].

2.2. Geographical Distribution

The incidence of FK varies across regions, with the highest incidence in Asia and Africa and the lowest incidence in Europe [3]. Similarly, the proportion of fungal keratitis as a subset of microbial keratitis varies between geographical regions, within the range of 1% of MK cases in Spain to 60% in Vietnam (Table 1) [29,30]. There have been four large reviews that have considered the proportion of MK caused by filamentous fungi by geographical region [3,7,31,32]. The first study from 2002 plotted the proportion of FK as a subset of MK against latitude, and found the proportion of FK cases increases with

decreasing latitude, i.e., increasing the closer one is to the equator [7]. The second review, from 2011, correlated the proportion of fungal cases of MK in a country with the country's gross domestic product (GDP) [31]. This found the highest proportion of fungal infections within Asia, specifically in India and Nepal. The study found the lower the GDP per capita of a country, the higher the proportion of fungal MK. The most recent study looked at both GDP per capita and latitude as potential determinants of the proportion of fungal cases of all those with MK [3]. The findings here correlated to the previous two reviews, suggesting that both proximity to the equator and low GDP per capita are associated with a higher proportion of fungal MK cases [3,7,31]. However, it is important to note there was some considerable unexplained variability [3].

These epidemiological reviews have been updated in Table 1 and Figure 3, which plots the proportion of FK as a subset of MK against distance from the equator [3,32]. There remains a clear inverse correlation with the highest proportion of FK as a subset of MK close to the equator, with the proportion decreasing with increasing distance from the equator. There is, however, considerable variability, and a number of important outliers: Singapore for example is 143 km from the equator but FK accounts for 0.7% of MK cases suggesting that FK is not only climate dependent but also probably linked to rural and occupational risk factors.

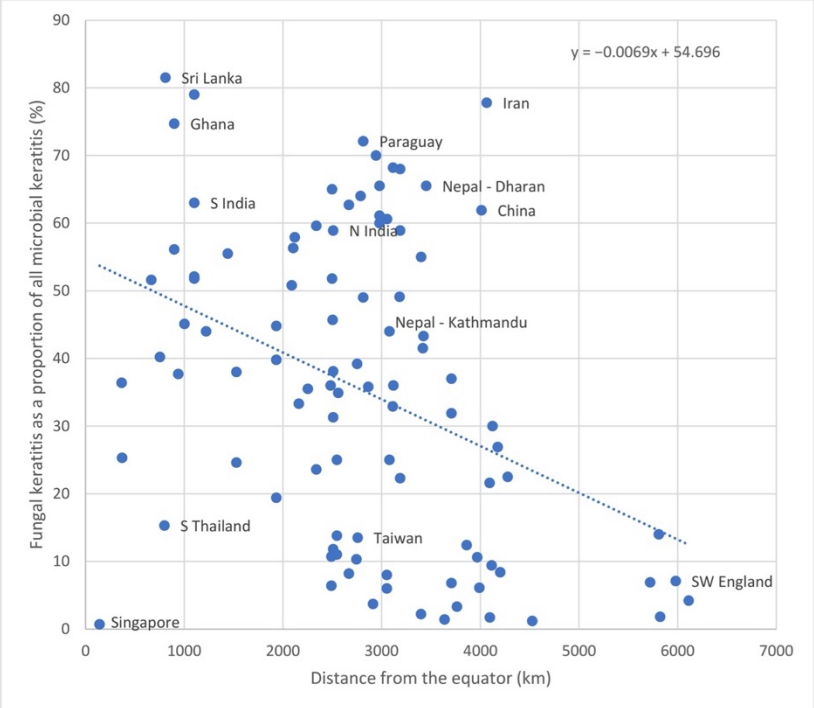


Figure 3. Fungal keratitis as a proportion of all culture positive microbial keratitis cases, by distance from the equator, with select locations shown, with calculated line of best fit given (dotted line, y = − 0.0069x + 54.696).

In general, filamentous fungal keratitis is a relatively rare cause of MK in temperate regions, where it is often associated with contact lens usage. Table 1 details the proportion of FK (as a subset of MK) in temperate regions such as Europe and temperate North America, which is in the range of 1.2–14.0% [33,34]. This contrasts to tropical regions, such as sub-Saharan Africa and South Asia, where the proportions are considerably higher, with rates reported as between 37.7% and 81.5%, Table 1 [35,36].

Table 1. Global epidemiology of fungal keratitis (FK), most frequently isolated fungal organisms and summary results of select papers on risk factors for developing FK, grouped as per their geographical region (as defined by the UN).

Country	Year	% FK #	% Culture Negative Cases ~	Age (Mean)	% Male	% Trauma	% Steroid	% TEM	% CL	% OSD	% HIV	% DM	Distance from Equator (km)	N (All MK Cases)	Organism 1 (%)	Organism 2 (%)	Reference
AFRICA																	
Egypt (Zagazig)	2012	55	-	-	-	63.6	6.1	-	12.1	-	-	18.2	3401	60	*Penicillium* spp. (24.2)	*Aspergillus* spp. (21.2)	[37]
Egypt (Mansoura)	2013–2015	65.5	55.5	-	66.1	51.4	5.3	-	2.4	4.5	-	15.1	3452	247	*Aspergillus* (41.0)	-	[38]
Egypt (Tanta)	2011–2013	43.3	15.5	49.2	65.9	58.4	32.7	-	-	-	-	-	3424	834	*Aspergillus flavus* (29.1)	*Aspergillus niger* (16.1)	[39]
Ethiopia	2014–2015	45.1	-	-	58.0	78.3	5.8	-	-	-	-	7.2	1003	153	*Fusarium* spp. (27.6)	*Aspergillus* spp. (25.0)	[40]
Ghana	1995	56.1	42.7	36.3	69.3	-	-	-	-	-	-	-	900	199	*Fusarium* spp. (52.3)	*Aspergillus* spp. (15.3)	[41]
Ghana	1999-2001	74.7	60.0	-	-	-	-	-	-	-	-	-	900	290	*Fusarium* spp. (42.2)	*Aspergillus* spp. (17.4)	[7]
Libya	2008–2010	32.9	^	-	60.7	78.5	3.6	-	21.4	-	-	17.8	3114	85	*Aspergillus* spp. (50.0)	*Fusarium* spp. (39.3)	[42]
Sierra Leone	2005–2006	37.7	5.5	-	-	-	-	-	-	-	-	-	941	73	UFF (69.2)	*Aspergillus* spp. (15.4)	[35]
South Africa	1982-1983	3.7	-	-	-	0	0	-	0	-	33.3	50.0	2913	164	*Curvularia* spp. (33.3)	-	[43]
South Africa	2013–2015	2.2	-	-	-	-	-	-	-	-	-	-	3399	46	-	-	[44]
Tanzania	2008–2010	51.6	45.6	-	-	-	-	-	-	-	-	-	667	170	UFF (87.5)	*Candida* spp. (12.5)	[1]
Tanzania	2013	40.2	8.9	-	-	-	-	-	-	-	-	-	755	202	*Candida* spp. (60.8)	UFF (39.6)	[45]
Tunisia	1995-2012	12.4	7	47.2	63.3	61.6	18.3	3.3	3.3	10.0	-	5	3862	483	*Fusarium* spp. (49.0)	*Aspergillus* spp. (22.0)	[46]
Tunisia	2010–2015	30.0	40	48.9	56.6	43.3	3.3	-	3.3	-	-	10	4124	30	*Fusarium* spp. (50.0)	*Aspergillus* spp. (33.3)	[47]
Tunisia	1996–2004	21.6	58	-	-	50.0	-	-	-	25	-	-	4094	100	*Fusarium* spp. (87.5)	*Acremonium* spp. (12.5)	[48]

Table 1. *Cont.*

Country	Year	% FK #	% Culture Negative Cases ~	Age (Mean)	% Male	% Trauma	% Steroid	% TEM	% CL	% OSD	% HIV	% DM	Distance from Equator (km)	N (All MK Cases)	Organism 1 (%)	Organism 2 (%)	Reference
ASIA																	
Bangladesh	2008	39.2	5	-	-	49	-	-	-	-	-	-	2752	120	-	-	[49]
Bangladesh	1994	36	18.3	-	-	-	-	-	-	-	-	-	2483	142	*Aspergillus* spp. (40.0)	*Fusarium* spp. (21.0)	[50]
China	2009–2013	45.7	53.9	-	-	-	-	-	-	-	-	-	2504	2973	*Fusarium* spp. (29.3)	*Aspergillus* spp. (24.1)	[51]
China	1999-2004	61.9	-	-	-	-	-	-	-	-	-	-	4012	1054	*Fusarium* spp. (73.3)	*Aspergillus* spp. (12.1)	[52]
China (Hong Kong)	1997-1998	6.4	65.0	-	-	20	20	-	-	-	-	-	2491	223	*Fusarium* spp. (60.0)	*Penicillium* spp. (20.0)	[53]
China (Hong Kong)	2004–2013	10.7	67.7	-	-	-	-	-	-	-	-	-	2491	260	*Fusarium* spp. (33.3)	*Candida* spp. (25.0)	[54]
China (Taiwan)	1992-2001	13.5	51.0	-	-	-	-	-	-	-	-	-	2758	453	*Fusarium* spp. (29.4)	*Candida* spp. (29.4)	[55]
China (Taiwan)	2012–2014	8.2	69	-	-	-	-	-	-	-	-	-	2669	233	-	-	[56]
India (West Bengal)	2007–2011	58.9	27.0	-	61.7	88.7	16.3	-	-	6.0	-	11.8	2510	928	*Aspergillus* spp. (37.8)	*Fusarium* spp. (20.3)	[57]
India (West Bengal)	2008	38.1	32.7	53	65.0	48.0	16.0	16.0	-	-	-	-	2510	289	*Aspergillus* spp. (55.4)	*Fusarium* spp. (10.8)	[58]
India (Odisha)	2006–2009	35.5	18.6	-	70.0	40.2	-	-	-	-	-	2.2	2253	997	*Aspergillus* spp. (27.9)	*Fusarium* spp. (23.2)	[59]
India (Assam)	2007–2009	60.6	-	-	68.8	76.40	-	-	-	1.5	-	2.5	3056	310	*Fusarium* spp. (25.0)	*Aspergillus* spp. (19.0)	[60]
India (Delhi)	2010–2015	68	65	-	76	89.4	-	-	-	5.3	-	1.3	3188	400	*Aspergillus* spp. (30.8)	*Fusarium* spp. (27.6)	[61]
India (Chandi-garh)	2005–2011	Ψ	49	-	-	66.5	2	-	-	11.7	-	-	3418	765	*Aspergillus* spp. (47.6)	Dematiaceous fungi (21.9)	[62]
India (Rajasthan)	2005–2012	68.2	45	-	71.7	62.8	-	-	3.9	-	1.1	8.9	3116	480	*Aspergillus* spp. (63.3)	*Alternaria* spp. (8.3)	[63]

Table 1. *Cont.*

Country	Year	% FK #	% Culture Negative Cases ~	Age (Mean)	% Male	% Trauma	% Steroid	% TEM	% CL	% OSD	% HIV	% DM	Distance from Equator (km)	N (All MK Cases)	Organism 1 (%)	Organism 2 (%)	Reference
India (Chandigarh)	1999-2003	41.5	46.9	-	80.5	43.8	7.8	-	-	-	-	-	3418	64	*Aspergillus* spp. (41.2)	*Fusarium* spp. (23.5)	[64]
India (Delhi)	2007–2011	58.9	27	-	61.7	88.7	16.3	-	-	19	-	11.8	3188	928	*Aspergillus* spp. (37.8)	Dematiaceous fungi (23.8)	[65]
India (Delhi)	2000–2004	22.3	-	-	77.9	32.4	16.2	2.7	0	-	-	-	3188	346	*Aspergillus* spp. (55.9)	Dematiaceous fungi (7.8)	[66]
India (Madurai)	2012–2013	79	14.5	50	64	70	9	19				8	1103	252	*Fusarium* spp. (39.0)	*Aspergillus* spp. (18.0)	[67]
India (Madurai)	1999–2002	52.1	29.4	-	65	92.1	1.2	-	0	6.7	-	15.7	1103	3183	*Fusarium* spp. (41.9)	Dematiaceous fungi (26.9)	[68]
India (Madurai)	1994	51.8	31.6	-	61.3	-	-	-	-	-	-	-	1103	434	*Fusarium* spp. (47.1)	*Aspergillus* spp. (16.1)	[4]
India (Hyderabad)	1991–2000	39.8	^	40.4	71.2	54.4	5.9	-	-	11.7	-	6.4	1933	3399	*Fusarium* spp. (37.2)	*Aspergillus* spp. (30.7)	[69]
India (Hyderabad)	1991–2001	44.8	39.6	30.9	-	81.9	2.4	-	0.3	18.2	-	-	1933	5897	*Fusarium* spp. (35.6)	*Aspergillus* spp. (26.8)	[70]
India (Madurai)	2006–2009	63	42	-	-	-	-	-	-	-	-	-	1103	6967	*Fusarium* spp. (42.3)	-	[71]
India (Bangalore)	2012–2014	55.5	62.5	-	-	-	-	-	-	-	-		1442	312	*Fusarium* spp. (31.0)	*Aspergillus* spp. (11.0)	[72]
India (Tamil Nadu)	1999–2001	44	31	-	-	-	-	-	-	-	-	-	1223	800	*Aspergillus* spp. (39.9)	*Fusarium* spp. (21.5)	[7]
India (Maharashtra)	2004–2009	57.9	37	-	-	-	-	-	-	-	-	-	2120	852	*Fusarium* spp. (35.0)	*Aspergillus* spp. (18.0)	[12]
India (Gujarat)	2006–2008	65	60		54	73	-	-	-	-	-	-	2498	100	*Aspergillus* spp. (70.0)	*Fusarium* spp. (12.0)	[73]
India (Gujarat)	2003–2005	51.8	45	-	-	-	-	-	-	-	-	-	2498	200	*Fusarium* spp. (29.8)	*Aspergillus* spp. (21.1)	[74]
India (Gujarat)	2007–2008	34.9	40.7	-	-	-	-	-	-	-	-	-	2561	150	*Aspergillus* spp. (35.4)	*Fusarium* spp. (22.5)	[75]
India (West Bengal)	2001–2003	62.7	32	-	-	-	-	-	-	-	-	-	2669	1198	*Aspergillus* spp. (59.9)	*Fusarium* spp. (21.2)	[76]

Table 1. *Cont.*

Country	Year	% FK #	% Culture Negative Cases ~	Age (Mean)	% Male	% Trauma	% Steroid	% TEM	% CL	% OSD	% HIV	% DM	Distance from Equator (km)	N (All MK Cases)	Organism 1 (%)	Organism 2 (%)	Reference
India (Delhi)	2005	49.1	43.2	-	-	-	-	-	-	-	-	-	3183	1000	*Aspergillus* spp. (41.6)	*Fusarium* spp. (19.8)	[77]
India (Hyderabad)	2002	19.4	33.5	-	-	-	-	-	-	-	-	-	1933	170	*Fusarium* spp. (72.7)	-	[78]
Iran (Tehran)	2011–2013	Ψ	94.4		79.3	-	-	-	-	-	-	-	3969	2180	*Fusarium* spp. (49.6)	*Aspergillus* spp. (26.4)	[79]
Iran (Sari)	2004–2005	77.8	59.1	61.5	71.4	28.6	0	-	0	14.3	-	14.3	4065	22	*Fusarium* spp. (50.0)	*Aspergillus* spp. (50.0)	[80]
Iraq	2002–2005	31.9	41.4	-	-	90	-	-	0	6.8	-	-	3707	396	*Aspergillus* spp. (56.8)	*Fusarium* spp. (27.0)	[81]
Iraq	2013–2014	6.8	30.5	-	-	-	-	-	-	-	-	-	3707	105	*Aspergillus* spp. (60.0)	*Alternaria* spp. (40.0)	[82]
Iraq	2017–2018	37	^	73	61	-	-	-	-	-	-	41	3707	234	*Aspergillus* spp. (70.0)	*Penicillium* spp. (13.0)	[83]
Japan	1999–2003	6.1	41.5	-	-	-	-	-	-	-	-	-	3991	122	*Candida* spp. (83.3)	-	[84]
Japan	2003	10.6	56.7	-	-	-	-	-	-	-	-	-	3969	261	-	-	[85]
Korea (RO)	2003–2008	26.9	37.3	-	-	-	-	-	-	-	-	-	4177	83	*Candida* spp. (57.0)	*Aspergillus* spp. (28.6)	[86]
Malaysia	2007–2011	25.3	12.8	-	61.7	48.9	17.0	-	4.3	10.6	-	10.6	371	186	*Fusarium* spp. (46.0)	*Aspergillus* spp. (9.8)	[87]
Malaysia	2017	36.4	59.9	-	-	-	-	-	-	-	-	-	367	137	*Fusarium* spp. (60.0)	-	[88]
Nepal (Dharan)	2004–2008	61.1	20.8	-	-	-	-	-	-				2980	351	*Aspergillus* spp. (33.3)	*Fusarium* spp. (12.7)	[89]
Nepal (Dharan)	1998–1999	65.5	32.6	-	-	-	-	-	-	-	-	-	2980	86	*Aspergillus* spp. (60.5)	*Fusarium* spp. (13.2)	[90]
Nepal (Nepalgunj)	2011–2012	36	^	-	59.3	58	12	-	-	6	-	-	3120	1880	*Fusarium* spp. (31.9)	*Curvularia* spp. (17.7)	[91]
Nepal (Dharan)	2007–2008	60	54.5	-	-	-	-	-	-	-	-	-	2980	44	*Aspergillus* spp. (66.6)	-	[92]
Nepal (Kathmandu)	2014	44	55.4	-	-	-	-	-	-	-	-	-	3080	101	*Fusarium* spp. (24.0)	*Aspergillus* spp. (20.0)	[93]

Table 1. *Cont.*

Country	Year	% FK #	% Culture Negative Cases ~	Age (Mean)	% Male	% Trauma	% Steroid	% TEM	% CL	% OSD	% HIV	% DM	Distance from Equator (km)	N (All MK Cases)	Organism 1 (%)	Organism 2 (%)	Reference
Nepal (Kathmandu)	1981	25	50	-	-	-	-	-	-	-	-	-	3080	133	-	-	[94]
Nepal (Biratnagar)	2011	*70*		-	-	-	-	-	-	-	-	-	2944	1644	No culture performed, microscopy only		[95]
Oman	2004–2007	31.3	57.9	-	59.4	25	31.3	15.6	-	18.8	-	9.4	2510	242	*Fusarium* spp. (50.0)	*Aspergillus* spp. (34.4)	[96]
Oman	2000–2006	11.8	56.9	-	-	-	-	-	-	-	-	-	2510	188	-	-	[97]
Pakistan	2010	64	32.3	-	-	-	-	-	-	-	-	-	2788	133	-	-	[98]
Saudi Arabia	1984–2004	10.3	69.4	55	79	20.9	16.9	-	0.8	8.87	-	12	2746	1200	*Aspergillus* spp. (37.0)	*Trichophyton* spp. (20.0)	[99]
Singapore	1991–2005	Ψ	^	40	79.3	55	24	-	7	14	-	-	143	29	*Fusarium* spp. (52.0)	*Aspergillus* spp. (17.0)	[100]
Singapore	2012–2014	0.7	-	-	-	-	-	-	-	-	-	-	143	531	-	-	[56]
Sri Lanka	1976–1981	81.5	59.1	-	-	-	-	-	-	-	-	-	811	66	UFF (63.6)	*Aspergillus* spp. (18.0)	[36]
Thailand (Central)	1988–2000	24.6	52.7	-	-	-	-	-	-	-	-	-	1529	292	*Fusarium* spp. (34.3)	*Aspergillus* spp. (20.0)	[101]
Thailand (South)	1982–2003	15.3	-	46.4	72.3	66	-	-	-	-	-	-	800	556	*Fusarium* spp. (64.5)	*Aspergillus* spp. (10.5)	[102]
Thailand (North)	2003–2006	50.8	74.4	-	-	-	-	-	-	-	-	-	2090	305	*Fusarium* spp. (58.1)	*Aspergillus* spp. (12.9)	[103]
Thailand (Central)	2001–2004	38	^	-	67.7	77.5	-	-	0	9.68	-	-	1529	127	*Fusarium* spp. (26.0)	Dematiaceous fungi (20.0)	[104]
Turkey (Adana)	2014–2015	9.4	-	39.3	50	50	-	-	25	-	-	-	4115	64	*Aspergillus* spp. (66.7)	*Fusarium* spp. (33.3)	[105]
Turkey (West Anatolia)	1990–2005	22.5	63.8	-	-	-	-	-	-	-	-	-	4278	620	*Fusarium* spp. (50.0)	*Aspergillus* spp. (20.0)	[106]
Vietnam (North)	2008	59.6	47.2	-	44.1	83.8	1.4	1.4	-	-	-	-	2338	1153	*Fusarium* spp. (40.7)	*Aspergillus* spp. (25.9)	[30]
Vietnam	1974–1982	23.6	-	-	-	-	-	-	-	-	-	-	2338	1219	-	-	[107]

Table 1. *Cont.*

Country	Year	% FK #	% Culture Negative Cases ~	Age (Mean)	% Male	% Trauma	% Steroid	% TEM	% CL	% OSD	% HIV	% DM	Distance from Equator (km)	N (All MK Cases)	Organism 1 (%)	Organism 2 (%)	Reference
EUROPE																	
Netherlands	2002–2004	1.8	42.0	-	-	-	-	-	-	50	50	-	5823	156	*Candida albicans* (100)	-	[108]
Netherlands	2014–2017	14.0	50	-	-	-	-	-	-	-	-	-	5809	185	-	-	[34]
UK (SW England)	2006–2017	6.9	61.9	-	-	-	-	-	-	-	-	-	5721	2116	UFF (54.2)	*Candida* spp. (45.8)	[109]
UK (London)	2007–2014	-	34.8	47.2	41.4	11.6	32.1	-	57.1	22.3	-	-	5727	112	*Fusarium* spp. (41.8)	*Candida* spp. (38.0)	[28]
UK (NE England)	2008–2017	4.2	55.5	55.3	65	-	-	-	-	-	-	-	6113	407	UFF (50.0)	*Candida* spp. (50.0)	[110]
UK – (NW England)	2004–2015	7.1	67.4	-	-	-	-	-	-	-	-	-	5980	4229	*Candida* spp. (53.2)	*Fusarium* spp. (25.7)	[111]
LATIN AMERICA AND THE CARRIBEAN																	
Brazil (São Paulo)	1975–2007	11	51.4	-	-	-	-	-	-	-	-	-	2547	6804	*Fusarium* spp. (51.9)	*Candida* spp. (17.6)	[112]
Brazil (Uberlandia)	2001–2004	56.3	50.8	-	-	55.6	-	-	0	0	-	-	2104	65	*Fusarium* spp. (61.1)	*Aspergillus* spp. (16.7)	[113]
Brazil (São Paulo)	2000–2004	13.8	63.4	40.7	80.3	-	-	-	-	-	-	-	2547	478	*Fusarium* spp. (66.7)	*Aspergillus* spp. (10.6)	[114]
Brazil (São Paulo)	2003–2010	25	82.4	43	74	49.3	-	-	-	-	-	-	2547	599	*Fusarium* spp. (83.3)	*Aspergillus* spp. (16.7)	[115]
Mexico	2013–2014	33.3	47.1	-	-	-	-	-	-	-	-	-	2161	51	*Fusarium* spp. (44.4)	*Aspergillus* spp. (22.2)	[116]
Paraguay	1988–2001	49	21	-	-	-	-	-	-	-	-	-	2814	660	*Acremonium* spp. (40.0)	*Fusarium* spp. (15.0)	[117]
Paraguay	2009–2011	72.1	10.4	-	71	-	-	-	-	-	-	-	2814	48	*Fusarium* spp. (34.0)	*Aspergillus* spp. (16.1)	[118]

Table 1. *Cont.*

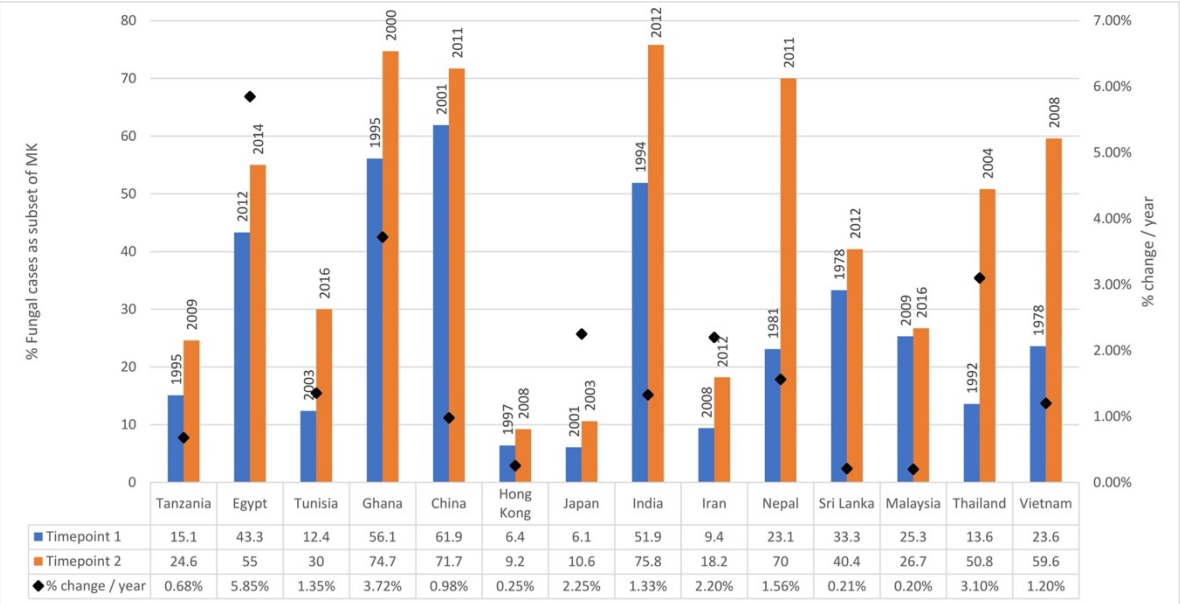
Country	Year	% FK #	% Culture Negative Cases ~	Age (Mean)	% Male	% Trauma	% Steroid	% TEM	% CL	% OSD	% HIV	% DM	Distance from Equator (km)	N (All MK Cases)	Organism 1 (%)	Organism 2 (%)	Reference
NORTH AMERICA																	
USA (N California)	1976–1999	8.4	62	-	-	-	-	-	-	-	-	-	4201	1121	*Candida* spp. (30.5)	-	[119]
USA (Florida)	1968–1977	35.8	44.0	-	-	-	-	-	-	-	-	-	2865	663	*Fusarium* spp. (62.0)	*Candida* spp. (7.5%)	[120]
USA (Florida)	1999–2006	-	29.8	48	75	43	29	-	44	8.3	-	7.1	3298	84	*Fusarium* spp. (41.0)	*Candida* spp. (14.0)	[121]
USA (S California)	1998–2008	1.4	^	56.1	54	14	-	-	24	12.7	1.6	16	3638	4651	UFF (64.0)	*Candida* spp. (32.0)	[122]
USA (New York)	1987–2003	1.2	^	47	35	11	7	-	10	23	25	7	4528	5083	*Candida* spp. (66.0)	*Aspergillus* spp. (12.0)	[33]
OCEANIA																	
Australia (Brisbane)	1999–2004	8	35	-	-	-	-	-	-	-	-	-	3054	231	*Fusarium* spp. most commonly isolated	-	[123]
Australia (Queensland)	1996–2016	-	^	48	65	-	-	-	-	-	-	-	3054	215	*Fusarium* spp. (33.3)	*Aspergillus* spp. (13.0)	[124]
Australia (Sydney)	2009–2017	-	6	60	65	24	54	-	26	34	-	-	3764	51	*Candida* spp. (33.0)	*Fusarium* spp. (28.0)	[125]
Australia (Queensland)	2005–2015	6	^	-	-	-	-	-	-	-	-	-	3054	3182	UFF (75.9)	*Candida* spp. (24.1)	[126]
Australia (Sydney)	2012–2016	3.3	31	63.5	67	25	46	-	28	25	-	8	3764	1052	*Candida* spp. (30.4)	*Fusarium* spp. (21.7)	[127]
New Zealand	2003–2007	1.7	34.4	-	-	-	-	-	-	-	-	-	4097	265	*Fusarium* spp. (66.7)	*Candida* spp. (33.3)	[128]

Confirmed fungal keratitis cases as a percentage of all culture positive microbial keratitis cases, including mixed bacterial-fungal infections. If diagnosis was based on microscopy (culture unavailable), this is a percent of all microbial keratitis cases examined by microscopy, and the results of these are given in italics. ~ Culture negative rate of all cultures taken within the study. - Data not presented. Ψ Studies that only included cases of FK and did not report the number of MK cases. ^ Studies that only included cases that were culture positive and did not report the overall culture negative rate. FK, fungal keratitis; TEM, traditional eye medication; CL, contact lens; OSD, ocular surface disease; HIV, Human Immunodeficiency Virus; DM, diabetes mellitus; MK, microbial keratitis; UFF, unspecified filamentous fungi.

Globally, *Fusarium* spp. and *Aspergillus* spp. are the most commonly isolated fungal causes of FK and are discussed in more detail below. Note, however, that non-filamentous FK is generally more common in temperate climates, where *Candida* spp. is most frequently implicated.

2.3. Changing Incidence over Time

There is evidence that the proportion of MK attributable to fungi is increasing over time, particularly in low and middle-income countries (LMICs) [3]. For example, in Thailand between 1982 and 2003, the mean proportion of FK cases was 13.6% [102]. This increased to 50.8% between 2003 and 2006 [103]. Similar increases have been observed in other parts of Asia, including Nepal with an increase form 23.1% in 1981 to 70% in 2011 [94,95]. Increases have also been observed in Africa, for example in Ghana where the percentage of FK cases increased from 56.1% in 1995 to 74.7% between 1999 and 2001 [7,41]. For countries where there are multiple reports published at different time-points which we reviewed in Brown et al. [3], the relative proportion of FK is plotted against time in Figure 4.



	Tanzania	Egypt	Tunisia	Ghana	China	Hong Kong	Japan	India	Iran	Nepal	Sri Lanka	Malaysia	Thailand	Vietnam
Timepoint 1	15.1	43.3	12.4	56.1	61.9	6.4	6.1	51.9	9.4	23.1	33.3	25.3	13.6	23.6
Timepoint 2	24.6	55	30	74.7	71.7	9.2	10.6	75.8	18.2	70	40.4	26.7	50.8	59.6
◆% change / year	0.68%	5.85%	1.35%	3.72%	0.98%	0.25%	2.25%	1.33%	2.20%	1.56%	0.21%	0.20%	3.10%	1.20%

Figure 4. Percentage of fungal cases as a subset of MK plotted by country at two timepoints. Timepoint 1 represents the earliest year for which values were available, Timepoint 2 represents the latest year for which values are available. The years for the two studies are given as labels. The percentage change per year (calculated from the difference between the two timepoints) is plotted against the secondary y-axis.

The reason for the increase in LMICs is unclear and has not been formally studied. It could be attributable to the increased use of topical antibiotics as a primary prevention measure following corneal abrasions or as empirical treatment at a primary health level for microbial keratitis, resulting in only severe or resistant bacterial infections presenting to secondary or tertiary care along with all fungal cases. It may also be driven due to greater availability of topical antibiotics available without prescription from pharmacies. Another potential reason for this increase may include climate change: a study from Egypt in 2011 found a strong correlation between the increase in cases of fungal keratitis between 1997 and 2007 and the increase atmospheric temperature and humidity detected during the same period [129]. Other potential reasons include increased availability and use of topical steroids, increased prevalence of diabetes mellitus across the regions or simply due to improved culture and microbiology services in these countries, meaning that under-

reported previous incidence is now being reported more accurately. Increased contact lens wear may also be a contributing factor, although on the whole contact-lens use remains infrequent in poorer countries across Asia and Africa.

In developed countries, there is also evidence of an increasing incidence over time, attributed to the widespread use of contact lenses, including bandage contact lenses, as well as topical steroid use [121,130]. For example, a study from a tertiary referral hospital in Florida showed an increase of over 100% in the number of cases of fungal keratitis between 1999 and 2005; contact lens wear was found to be the most common risk factor in this study [121]. A retrospective multi-centre case series from the US reported a significant increase in the incidence of non-*Fusarium* filamentous fungal keratitis cases between the period 2001–2004 and 2004–2007 ($p < 0.0001$) [130]. The number of *Fusarium* cases increased substantially between 2004 and 2006, when ReNu with MoistureLoc contact lens cleaning solution was on the market, and then returned to the pre-2004 incidence level for the remainder of the study [130]. For the increase described for non-*Fusarium* cases, the authors were unable to give a clear reason why this may have occurred; a contact lens-related product was unlikely to be responsible as the similar trends were seen for both contact lens wearers and non-contact lens wearers [130]. A more recent study from a tertiary eye hospital in the UK also reported a significant increase in the number of cases of filamentous fungal keratitis between 2007 and 2014 ($p = 0.005$), whilst there was no significant change in the incidence of yeast infections ($p = 0.3$) [28]. The same study also compared the incidence between data collected between 1994 and 2006 and data from 2007 to 2014, and found a significant increase in fungal keratitis cases ($p = 0.03$) [28]. All three of these studies report an increasing proportion of filamentous FK compared to yeast FK, with filamentous organisms (and in particular *Fusarium* spp.) now responsible for the majority of FK cases [28,121,130]. More research is required through case–control or national surveillance studies to explore reasons behind this apparent increase in incidence over time in both temperate and tropical locations.

2.4. Risk Factors

There are numerous risk factors for developing fungal keratitis, some attributable to the individual such as age, gender or pre-existing ophthalmic or systemic disease, with others dependent on extrinsic factors including the income status of the patient, occupation, contact-lens use, previous ocular surgery and region. Select risk factors from a number of epidemiological studies on fungal keratitis are presented in Table 1.

2.4.1. Age and Gender

Despite age and gender not being independent risk factors for fungal keratitis, they both affect other risk factors such as trauma, which is more common in younger men who tend to be agricultural labourers [12,131]. It is also important to note that older patients tend to have a more severe disease and worse outcome [108]. Furthermore, older patients are more likely to have predisposing systemic and ocular co-morbidities such as diabetes mellitus and ocular surface disease [108]. Patients between the ages of 20–40 make up the majority of cases [12,28,69,131]. In areas of high incidence of fungal keratitis such as south India, the majority of young patients (aged between 21 and 50) typically have fungal keratitis, compared to the majority of patients over 50 years old who typically have bacterial keratitis [68].

In SSA and India where the burden of FK is greatest, the majority of cases of fungal keratitis are reported in males [12,40,69]. Interestingly, one study from Nepal reports a higher proportion of females compared to males [93], whilst other studies from Nepal report male preponderance [91,95]. The reason for this difference is unclear; it may be due to different socioeconomic factors, health seeking behaviour or differing study methodology. In Europe and North America, there is considerable variation in the reported proportion of men with fungal keratitis [28,121].

2.4.2. Trauma

Preceding ocular trauma is a key predisposing risk factor for the development of fungal keratitis, regardless of geographical region. This is particularly true for trauma with vegetative material and trauma occurring during agricultural practices. Injury to the eye allows for a disruption to the corneal epithelium, permitting fungal pathogens to infiltrate the cornea [24,46,68,132–134]. Furthermore, injury with plant matter can lead to direct inoculation with fungal conidia. For regions where a fungal aetiology is the most common form of microbial keratitis such as South Asia and SSA, the reported rates of trauma range from 24 to 83% [1,76].

2.4.3. Occupation

Given the clear risk that trauma, particularly with organic material, poses to the cornea it is not surprising that occupations that carry a high risk of occupational ocular injury are associated with developing fungal keratitis. In particular, agricultural labourers and subsistence farmers are the most likely to develop fungal keratitis, reported to be between 56–74% of cases from studies in Nepal and India [12,91].

2.4.4. Diabetes Mellitus

Diabetes mellitus (DM) is of increasing public health concern globally, with the incidence increasing at an alarming rate in LMICs [135]. It is well-established that patients with DM are at an elevated risk of developing fungal infections [136], and DM is the most important systemic risk factor for developing fungal keratitis [60]. DM has also been shown to be an independent risk factor for the severity of fungal keratitis [137]. It is thought that hyperglycaemia can alter the ocular surface microenvironment including changes to the commensal organisms and enzyme action, allowing easier fungal adherence, proliferation and corneal penetration [137]. The associated reduced immune response seen in diabetes is also likely to be a significant factor in increasing host susceptibility to fungal infection [138].

2.4.5. HIV

There have been a number of studies from SSA that have suggested an association between HIV infection and fungal keratitis, following a number of case reports of fungal keratitis in AIDS patients at the start of the HIV/AIDS pandemic [139,140]. A prospective study from Tanzania in 1999 found that 81% of the patients with fungal keratitis were HIV positive, compared to only 33% in non-fungal cases ($p < 0.001$) [141]. Another study from Tanzania a few years later found the prevalence of HIV infection amongst MK cases to be double that of the wider population [1], although this did not directly compare the proportion of HIV positive fungal MK cases to bacterial MK cases. A more recent, nested case control study from Uganda where over 60% of MK cases were fungal, found a strong association between HIV infection and MK (OR 83.5, $p = 0.02$) [138,142]. There have been no studies to date looking at this association outside of SSA.

2.4.6. Traditional Eye Medicine

The use of traditional eye medicine (TEM) to treat a wide range of eye problems is commonplace in LMICs [143,144]. Most TEM contain non-sterile preparations comprising plant matter, often herbs or dried leaves, and are therefore a potential route for inoculating the cornea with microorganisms, particularly fungal pathogens [60]. Although there are no studies that have specifically looked at TEM as a risk factor for fungal keratitis, it has been found to be an independent risk factor in developing microbial keratitis in Tanzania and Uganda [20,138,142], where a fungal aetiology make up the majority of MK cases.

2.4.7. Topical Corticosteroids

It is well established that glucocorticoids are associated with an increased risk of invasive fungal infection due to the dysregulation of the patient's immunity [145]. This holds true for prior topical steroid use, which is an independent risk factor for developing

fungal keratitis [146]. Prior topical corticosteroid use is also associated with deeper corneal penetration and a worse clinical outcome [147]. Although topical corticosteroid use is associated with both yeast and filamentous fungal infections, it may be a stronger risk factor for yeast infection [28].

2.4.8. Ocular Surface Disease

Pre-existing ocular surface disease (OSD, a diverse range of disorders that lead to an abnormal ocular surface such as dry eye disease, corneal exposure, blepharitis, persistent epithelial defects or ocular surface inflammatory conditions) compromises the corneal epithelium and therefore allows fungal pathogens to invade the cornea. Furthermore, these conditions are often treated with topical corticosteroids or bandage contact lenses, which further increases the risk of developing fungal keratitis. Although OSD is more often associated with yeast infection [28], it remains a risk factor for filamentous fungal infection: a multi-centre study from the US found 29% of cases of fungal keratitis were associated with OSD, 42.6% of which were filamentous and 53.1% were yeast [148]. Cases of fungal keratitis with pre-existing OSD are less frequently reported in LMICs than in developed countries, other than in areas such as Tanzania, where OSD due to trachoma exists [149].

2.4.9. Contact Lens Usage

In industrialised countries, contact lens use constitutes the main predisposing factor for developing fungal keratitis, with studies showing between 37% and 67% of fungal cases were contact lens wearers [28,130,148]. It is important to consider, however, that it is not simply the contact lens wear itself that carries the risk-it is the type of lens used, the frequency of replacement and how the lenses are cleaned-and with what. For example, the global outbreak of *Fusarium* keratitis between 2005–2006 was caused by a specific contact lens cleaning solution [150]. The current proportion of patients with fungal keratitis in LMICs associated with contact lens usage is low, but this is likely to increase as these countries industrialise leading to an increased number of contact lens wearers and fewer people involved in manual agricultural labour.

2.4.10. Previous Ocular Surgery

A prior history of ocular surgery, including cataract, laser-refractive or corneal transplantation surgery, has been associated with the development of fungal keratitis in both developed and lower-middle income countries [151,152]. Yeasts are often the most commonly implicated pathogen following surgery [8]; for example, in a study from Boston, USA, yeasts accounted for 67% of post-surgical fungal infections. Of note, this group of patients had the worst outcome in terms of final visual acuity. In this study, all surgeries were a form of corneal transplantation [153]. However, it should be noted that prior ocular surgery is more likely to be a stronger risk factor for bacterial, rather than fungal, keratitis [61]; a study from Brazil found 32% of bacterial keratitis cases were associated with previous ocular surgery, compared to just 8% of fungal keratitis cases [154].

Despite intravitreal injections for retinal disease becoming the most commonly performed intraocular procedure globally [155], and corticosteroid periocular injections being used routinely for the treatment of diabetic macular oedema [156,157], there have been no cases of fungal keratitis associated with this treatment reported in the scientific literature to date. However, other complicating local fungal infections have been reported, including fungal endophthalmitis, fungal orbital abscesses and conjunctival mycetoma [158–160].

3. Clinical Features

It can be challenging to distinguish fungal keratitis from other forms of microbial keratitis, and even more difficult to distinguish different fungal aetiologies on clinical grounds. For example, a study whereby fifteen ophthalmologists had to predict the likely microbiological aetiology found that fungal keratitis was the most challenging to diagnose, with a sensitivity and specificity of 38% and 45%, respectively [161], whilst in a separate

study using corneal photographs, corneal specialists were only able to correctly differentiate fungal and bacterial keratitis in 66% of cases [162].

There are, however, some clinical signs that have been shown to be useful predictors for filamentous fungal keratitis [2]. These are serrated margin, raised slough and colouration other than yellow. If one of these signs was present, the probability of fungal infection was 63%; if more than one of these were present the probability was 83% [2]. Without using colour as a discriminator, the probability increased to 89% [163]. Satellite lesions, which have historically been believed to be discriminatory for fungal keratitis, have been shown to occur in *Acanthamoeba* and fungal keratitis with the same frequency and are no more frequent in fungal than bacterial keratitis [164].

Some clinical features have been found to be more likely associated with *Fusarium* infection compared to *Aspergillus* infection. For example, *Fusarium* ulcers are more likely to have serrated (or "feathery", indistinct) margins or edges and non-yellow infiltrate (Figure 5A), whilst cases of *Aspergillus* keratitis are more likely to have a raised surface or presence of hypopyon (Figure 5B) [67]. Another study agreed with these findings, with *Aspergillus* cases more likely to have a raised surface, but also presence of an endothelial plaque; these were less common in *Fusarium* cases [165]. Ring infiltrates were also predictive of *Aspergillus*. Pigmented corneal infiltrates are very likely to be caused by dematiaceous fungi; in the study by Oldenburg et al. all pigmented corneal ulcers were dematiaceous [165]. Presence of a raised profile is also associated with dematiaceous fungi such as *Curvularia* spp. (Figure 5C) [15,165,166].

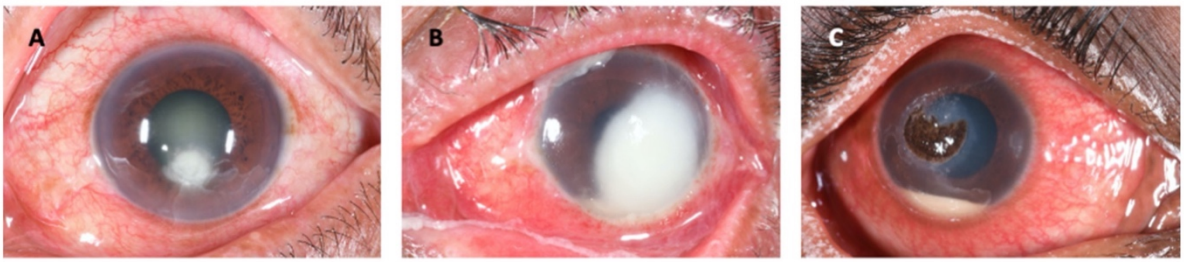


Figure 5. Differing clinical phenotypes of filamentous fungal keratitis depending on the fungal organism. (**A**): *Fusarium* sp. Note the serrated or feathery margins, satellite lesions, non-yellow infiltrate and lack of hypopyon. (**B**): *Aspergillus* sp. Note less obviously serrated margins compared to (**A**), raised profile, hypopyon. (**C**): *Curvularia* sp. Note the raised, pigmented infiltrate, in addition to the hypopyon.

Despite the above clinical signs being more frequently associated with fungal keratitis, other studies have shown a lack of statistical significance [161,164]. This adds to the challenge to accurately and confidently diagnose fungal keratitis on clinical grounds alone. Compounding this is the pleomorphic presentation as a result of late presentation, prior use of topical steroids or traditional eye remedies that unfortunately often occurs frequently in the regions where fungal keratitis is most prevalent [18].

Acutely, fungal keratitis typically leads to reduced vision due to the presence of the infection and inflammation in the cornea, blurring the vision. With treatment, the vision can improve, although often the patient is left with worse vision than they had previously due to the development of corneal scarring. At present there is no medical treatment to reverse this scarring process. Rigid contact lenses can help to a certain amount by improving the vision if there is scarring. Alternative options for severe scarring include corneal graft surgery, but this can be a technically challenging procedure and is often not available in places most in need. Fungal keratitis should therefore be considered a potentially blinding condition.

4. Making the Diagnosis

Even with all diagnostic modalities available, diagnosing fungal keratitis can be challenging. The burden of fungal keratitis globally is predominantly in low resource settings, where access to advanced diagnostic techniques is very limited. In these locations, diagnoses are still often made on clinical grounds alone (with the associated limitations as discussed above), sometimes supported by basic microscopy. However, an algorithm has been developed that uses the specific features that were systematically examined from a large case series from Ghana [163], and calculating a probability score that the microbial keratitis is fungal in aetiology, Figure 6 [2]. This can aid clinicians working in these locations and indicate the likelihood of fungal versus bacterial infection. Where diagnostic microbiology is available, however, it is best practice to rely on the results of this rather than these clinical signs, as the presence of fungal hyphae in corneal tissue is diagnostic [163].

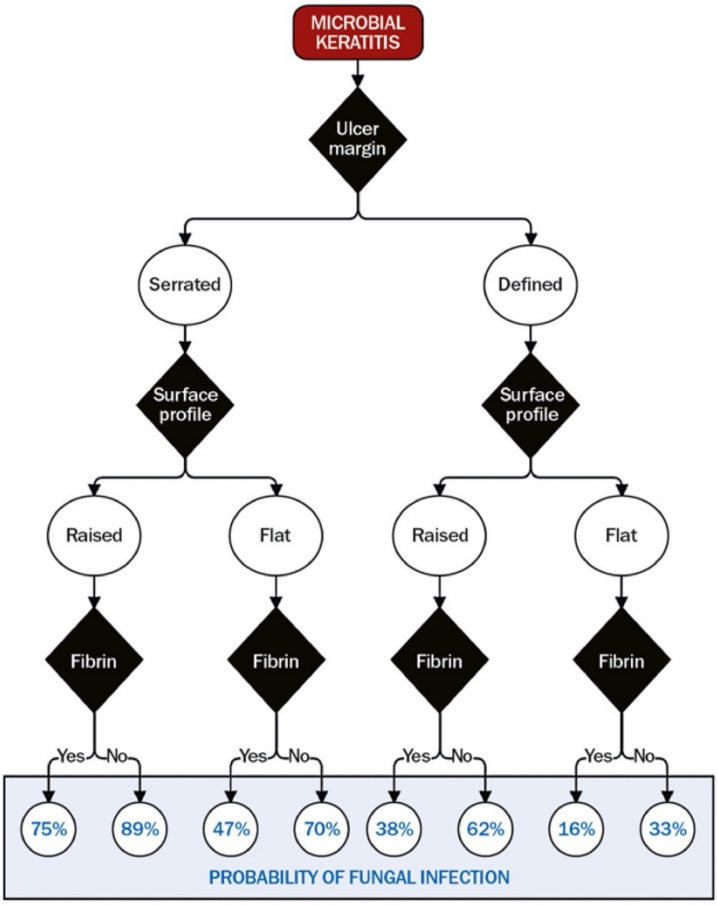


Figure 6. Algorithm for determining the probability of fungal keratitis [163]. The black diamonds are decision points about three clinical features: ulcer/infiltrate margin, surface profile, and anterior chamber fibrin. These probabilities are based on data presented in Thomas et al. [2]. This is reproduced here from [163] with permission under a CC BY-NC 4.0 license (https://creativecommons.org/licenses/by-nc/4.0/, accessed on 16 March 2021).

4.1. Laboratory

4.1.1. Microscopy and Culture

Infected corneal tissue/material is gently removed from the surface of the anaesthetised cornea using a sterile needle or scalpel blade and transferred to microscope slides and a range of solid and liquid phase culture media, including blood agars and Sabouraud dextrose agar [13].

Microscopy is still regarded as the gold standard in laboratory diagnosis of fungal keratitis and is often the only diagnostic tool available in settings where the incidence of FK is highest. The presence of fungal hyphae in corneal scrape preparations is always significant and are clearly visible using Gram stain, KOH, CFW or lactophenol cotton blue (LPCB, Figure 7) [13,167]. The ubiquitous distribution and environmental reservoirs of fungal ocular pathogens mean that positive microscopy is critical to exclude contaminants.

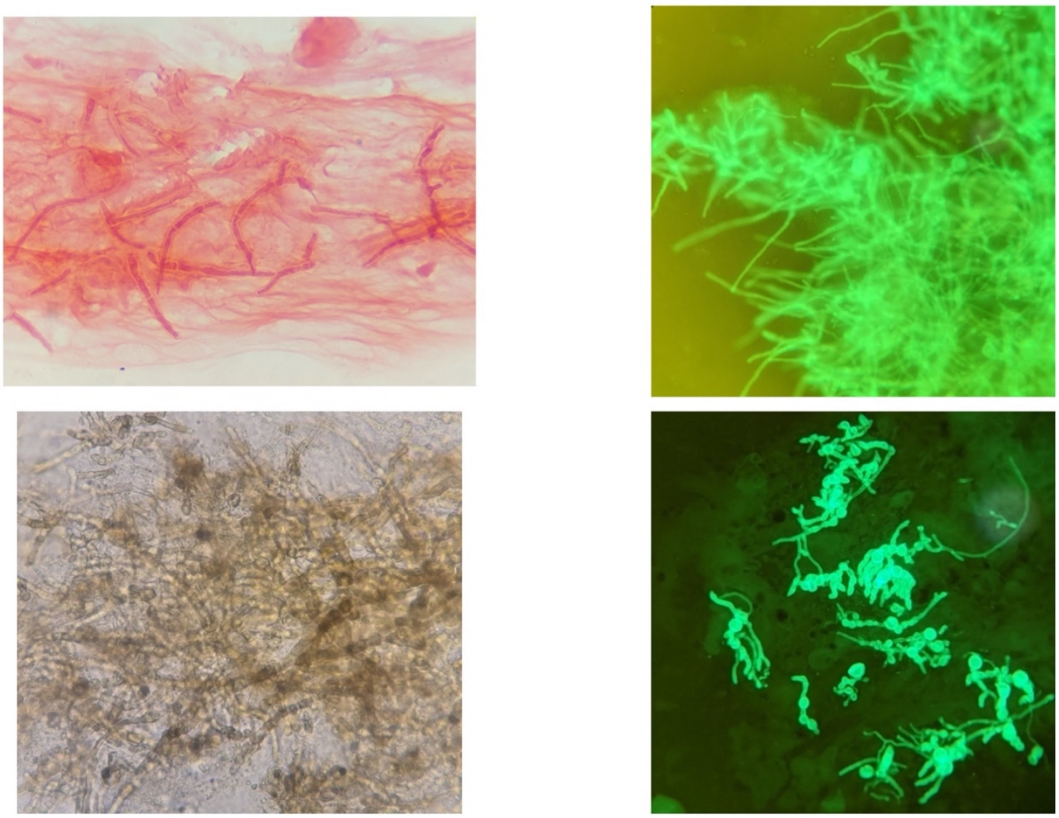


Figure 7. Microscopic appearance of filamentous fungal hyphae in corneal tissue (corneal scrape specimens) using different staining techniques. Clockwise from **top-left**: Fungal hyphae in Gram-stained corneal smear (magnification 1000x, oil immersion); fungal hyphae visible with CFW, *Curvularia* sp. stained with CFW, pigmented hyphae (*Curvularia* sp.) in a KOH preparation (magnification 400x). These images were taken using an afocal photography technique; the camera zoom was used for additional magnification.

Culture positivity rates reported vary greatly between institutions and settings [32]. Low culture positivity is attributable to the very small size of the specimen, use of antimicrobial agents by the patient prior to presentation, the quality of the corneal scrape and incorrect inoculation of media, in addition to laboratory factors [168,169]. Subculture for identification to species level may require the use of plant-based agars, most commonly examples are potato dextrose and cornmeal agars, in addition to diurnal culture methods to induce sporulation for the purpose of identification.

4.1.2. Molecular Techniques

Rapid diagnosis to inform prompt and appropriate treatment is critical to the successful clinical management of fungal keratitis. Development of molecular techniques, such as pan-fungal 16S rRNA PCR, have been favoured due to the very small size of specimen. PCR has emerged as both sensitive and specific test for the diagnosing fungal keratitis, benefiting from a high positive detection rate [14,170–174], with some evidence that it may be more sensitive than the traditional microbiological techniques of microscopy and culture [175]. However, the accuracy of PCR to diagnose fungal keratitis is dependent on adequate sampling and the primers used. Recent promising developments include evaluation of ITS primers and multiplex PCR for direct identification of fungal species from corneal tissue demonstrating high sensitivity and specificity [14].

An area currently under research that could have important therapeutic and prognostic implications is the development of genotyping methods for rapid species identification. This has shown promise for the rapid detection of *Fusarium solani* using a specific restriction site in the *EF-1a* gene [176]. *Fusarium solani* has been shown to have a worse prognosis, including higher voriconazole resistance, compared to other *Fusarium* species [177]. If rapid species identification using molecular methods were readily available, tailored treatment could be started earlier, thereby potentially improving the overall prognosis. However, the expense of molecular diagnostic methods precludes their use in many settings where FK is prevalent and further highlights the need for low-cost, point of care diagnostic tests which could be made more widely available.

Matrix-assisted laser desorption/ionization time of flight-mass spectrometry (MALDI-ToF MS) is a relatively novel rapid and reliable, high-throughput tool for the identification of microorganisms, allowing the identification of fungal isolates within minutes [178]. It also benefits from a fast turnaround time and low cost for consumables, making it potentially relevant to tertiary referral centres in LMICs where the burden of fungal keratitis is greatest. However, there are no published studies comparing MALDI-ToF MS to conventional methods for diagnosing fungal keratitis. One study has compared MALDI-ToF MS to conventional morphology and PCR sequencing which included one sample of *Aspergillus* keratitis which showed a good level of agreement between the different modalities [179]. There are a number of case reports and case series that explain how it is a useful tool in rapidly diagnosing FK, particularly for rare or unusual organisms [180–184].

4.1.3. In Vivo Confocal Microscopy

Fungal culture can have a relatively low yield-studies report a sensitivity of up to 50% [185,186]. Growth may be slow; several days, even weeks; and identification complicated due to poor sporulation in vitro. Microscopy is very helpful but can have its limitations, particularly given the infection is often deep within the stroma making yield from corneal scrapings poor [167]. Early treatment (and therefore diagnosis) is crucial in treating FK appropriately and preventing the blinding complications associated with it [1]. A potential answer to these challenges comes in the form of in vivo confocal microscopy (IVCM), which allows for real-time imaging of the cornea down to the cellular and micro-structural level. It is able to detect the presence of fungal hyphae, Figure 8 [185,186].

IVCM can be used in the diagnosis of FK as well as in monitoring the response to treatment [185–189]. Chidambaram et al. reported a sensitivity of 79.1–86.8% and specificity of 73.7–85.9%, whilst Hau et al. correctly identified fungal infection 8.3–41.2% of the time [185,190]. However, it cannot reliably differentiate the organism causing the infection, meaning culture remains the gold standard for identification.

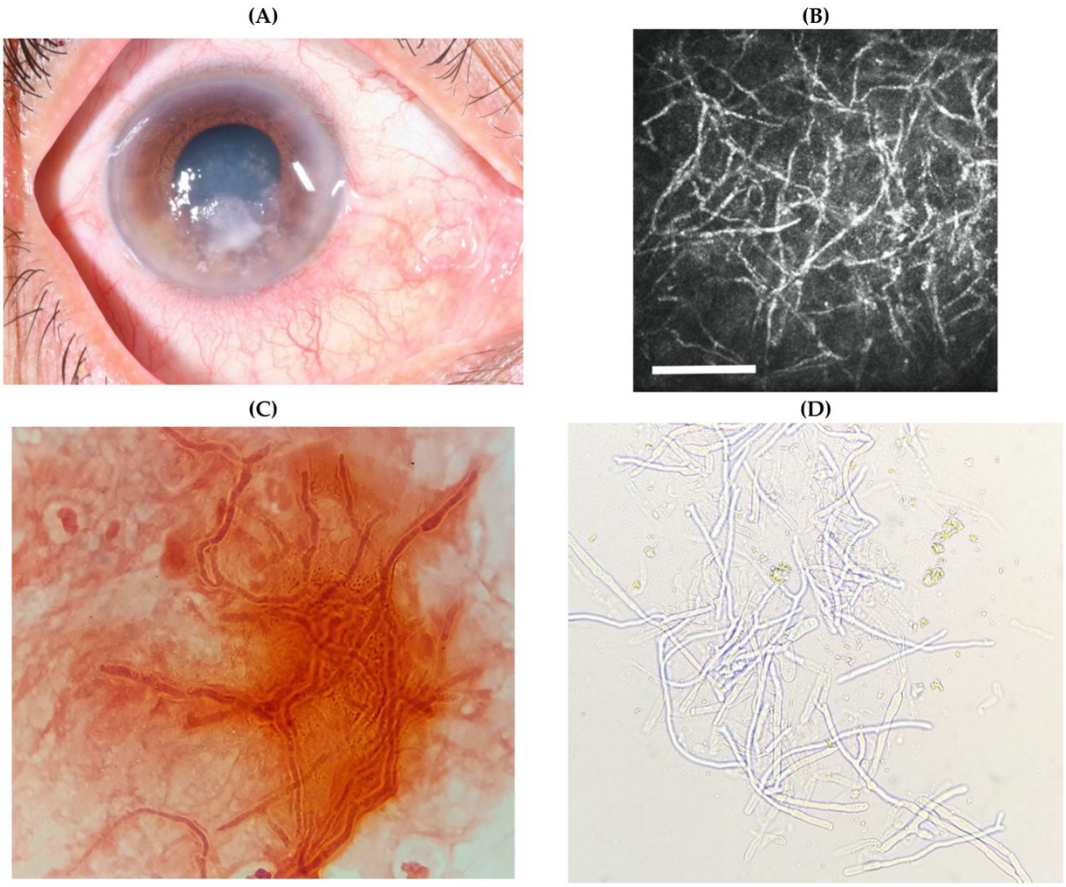


Figure 8. In vivo confocal microscopy of fungal keratitis (**A**) clinical image; (**B**) *In vivo* confocal microscopy scan of the same cornea showing extensive, branching fungal hyphae. Scale bar 100 µm. (**C**) Light microscopy demonstrated septate fungal hyphae, visible on Gram staining (magnification 1000x, oil immersion); (**D**) and KOH preparation (magnification 400x). Images (**C**,**D**) were taken using an afocal photography technique; the camera zoom was used for additional magnification.

4.1.4. Systematic Approach to Making a Diagnosis

With numerous tools available to aid in the diagnosis of fungal keratitis, it is useful to have a systemic approach. This will depend on what tools are available; as mentioned above, there are unfortunately many locations globally where access to these investigations are unavailable. In these locations, the algorithm in Figure 6 should be used. If all tests are available, we recommend following the algorithm given in Figure 9. A high index of suspicion is an important first step to diagnosing fungal infections: if a patient presents with a history of vegetative trauma, particularly if they are in a subtropical or tropical location, then fungal keratitis needs to be ruled out on the outset. As described above, if clinical signs including feathery or serrated margins, a raised profile or satellite lesions are present, then this should raise the probability of fungal keratitis. At this point a baseline corneal photograph is useful for future reference to guide future response, although staining with fluorescein should be delayed until after the PCR sample is taken to avoid theoretical interference with primers.

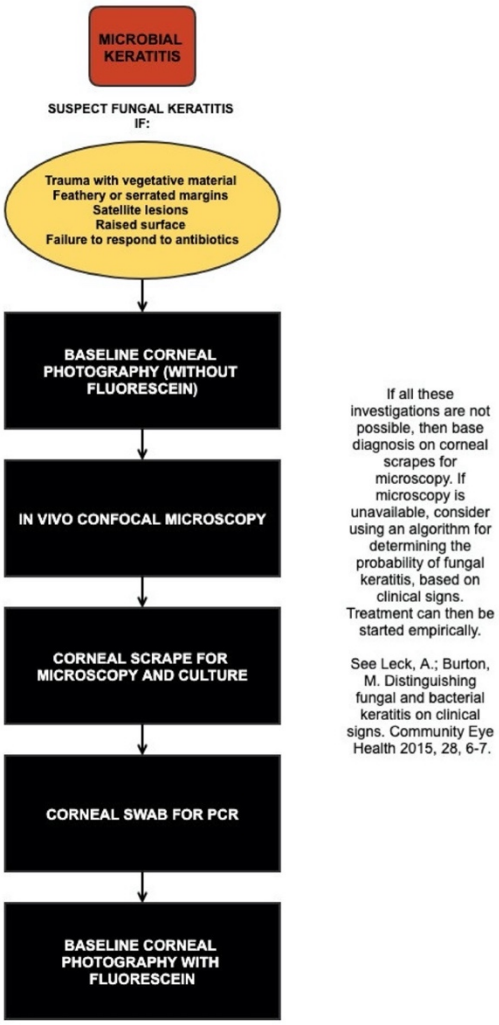


Figure 9. Algorithm for diagnosing fungal keratitis.

In these cases, the first investigation to be performed is IVCM. This should be performed before taking a corneal scrape, as taking a corneal scrape can reduce the image quality obtained by IVCM and therefore the sensitivity. Evidence of fungal hyphae are diagnostic. Ideally, the cornea should be anaesthetised with preservative free topical 0.5% proxymetacaine hydrochloride, as this is less likely to interfere with culture or PCR results. The subsequent step would be to take corneal scrapes for microscopy and culture, as described in detail in Section 4.1.1. It should be noted that a fresh sterile needle should be used for each slide or culture media being inoculated. Finally, a sample for PCR should be taken as a corneal swab. At this point, a second corneal photograph could be taken using a blue filter and topical fluorescein staining to demonstrate the size of the epithelial defect.

5. Management

Most cases of filamentous fungal keratitis are challenging to treat, requiring long-term therapy with topical, and occasionally systemic, antifungal agents. However, even when intensive appropriate topical therapy is initiated, infections frequently progress relentlessly to perforation and loss of the eye in ~25% of cases [1,22,24]. Surgery in the form of therapeutic penetrating keratoplasty (TPK) is often required. There are a limited

number of antifungals available with action against fungal keratitis, of which there are four main groups: imidazoles, triazoles, polyenes and fluorinated pyrimidines. These may be available topically, orally or by intravenous injection. Subconjunctival injection or corneal stromal injection may also be given [27,28]. The current gold standard treatment for filamentous fungal keratitis is topical natamycin 5%.

There have been a number of clinical trials comparing various treatment options for fungal keratitis over the last few decades, which have been reviewed systematically [191,192]. Natamycin (NATA), which was approved in the 1960s by the FDA for FK, has been compared to a number of newer agents. In a randomised controlled superiority trial of 116 patients from India, there was no statistical difference found between econazole 2% or natamycin 5% [26]. Voriconazole, a newer generation triazole agent, was subsequently introduced to the market and an initial prospective RCT showed no significant difference between the groups in terms of primary outcome measure (time to healing of epithelial defect). The authors therefore concluded that voriconazole was "an effective and well-tolerated drug" and larger trials were warranted to demonstrate superiority [193]. Meanwhile, Prajna et al. also compared topical natamycin to voriconazole in a therapeutic exploratory randomised clinical trial; 120 patients were randomised to either natamycin or voriconazole and either had repeated corneal epithelial scraping or not. The study also concluded that there was no significant difference between groups for the primary outcome of visual acuity at three months, with a non-significant trend favouring voriconazole. Incidentally, repeated scraping was associated with a worse outcome, although again this was non-significant ($p = 0.06$) [194]. To investigate this, the Mycotic Ulcer Treatment Trials (MUTT) were developed [22,24]. In MUTT1, topical natamycin 5% was compared to topical voriconazole 1% in a trial that was due to recruit 368 patients but was terminated earlier on recommendation by the trial Data Safety and Monitoring Committee, as the number of perforations in the voriconazole group were significantly higher than in the natamycin group (34 vs. 18 perforations, $p = 0.02$; 323 recruited). Vision was −0.18 logMAR better at three months in the natamycin group compared to the voriconazole group ($p = 0.006$) [24]. Sharma et al. also found natamycin to be superior to voriconazole in a more recent randomised controlled trial [133].

MUTT 2 compared oral voriconazole with placebo with all patients receiving both natamycin and topical voriconazole. There was no difference in primary outcome (perforation rate or corneal graft) within three months between groups, with more side effects reported in the voriconazole group ($p < 0.001$). The study therefore concluded that there was no benefit in adding oral voriconazole in the treatment of severe filamentous fungal corneal infections [22]. As a result of these studies, topical natamycin 5% without oral voriconazole remains the recommended first-line agent for filamentous FK. MUTT also investigated the susceptibility of different fungal species to either medication, and found that *Aspergillus* spp. were least susceptible to natamycin, whilst *Fusarium* spp. were least susceptible to voriconazole. In the study population where MUTT was conducted, *Fusarium* spp. was the most commonly isolated organism. However, many patients continue to progress despite treatment with natamycin 5%, meaning that alternative treatment strategies are required. In addition, natamycin 5% is difficult to formulate, expensive and often unavailable in countries where it is required, despite being on the WHO Essential Medicines List. Chlorhexidine (CHX) is an antiseptic agent, with both antibacterial and antifungal properties. It is a widely used broad-spectrum biocide, killing microorganisms through cell membrane disruption. Pilot studies from the 1990s have suggested it as a potential alternative to natamycin 5% [23,25,191,195], and a randomised controlled trial comparing natamycin 5% to chlorhexidine 0.2% for fungal keratitis is currently underway [196].

Despite MUTT 2 showing no benefit for adjunctive oral voriconazole, some ophthalmologists recommend systemic, oral therapy in severe cases of fungal keratitis, particularly if the infiltrate is larger than 5 mm or deeper than 50% corneal thickness [197]. If oral voriconazole is not available, alternative options include ketoconazole or itraconazole. A randomised controlled trial comparing oral ketoconazole with oral voriconazole found sim-

ilar healing times between groups, although patients treated with voriconazole achieved a significantly smaller scar size and better final vision [198]. It is, however, important to remember that these oral anti-fungal agents can have serious adverse effects, particularly in terms of hepatotoxicity; they should be used cautiously with correct dosing depending on the patient's weight, together with liver function monitoring. Oral voriconazole has also been associated with treatment-related visual adverse events including blurred vision and colour vision changes [199], although these have been found to be non-progressive and reversible [199].

In addition to topical treatment, injections of antifungals into the corneal stroma have also been performed in severe disease [200,201]. This was investigated in a randomised controlled trial of 40 patients who were not responding to natamycin 5%, and compared topical voriconazole 1% to intrastromal injections of voriconazole 50 µg/0.1 mL. The authors found that patients receiving topical voriconazole had a mean BSCVA of −0.397 better than the intrastromal injection group (p = 0.008). Additionally, 19/20 patients receiving topical voriconazole healed with therapy. The authors concluded that topical, as opposed to intrastromal, voriconazole may be beneficial in addition to natamycin in recalcitrant disease not-responding to natamycin 5% monotherapy [202]. There is therefore no evidence indicating a benefit from intrastromal injections of voriconazole.

More recently, corneal collagen cross-linking (CXL) has been considered for the treatment of FK [203]. However, the evidence for this is limited with heterogenous protocols and conflicting results [204]. Indeed, three prospective randomised controlled trials have found no benefit of CXL over standard-of-care and, of concern, potentially worse outcomes in the CXL group [205,206].

The last intervention option for treating FK is surgical in the form of corneal transplantation or TPK. For large corneal perforations, TPK is the only option left to salvage the eye by restoring normal anatomy, with the added advantage of removing the site of the infection [207]. Unfortunately, however, recurrence of fungal infection in the graft often occurs, particularly in the presence of a hypopyon, corneal perforation, larger infiltrates and limbal involvement [208,209]. There is therefore a degree of debate around whether to perform TPK earlier in the course of the disease, rather than waiting for the eye to perforate, when future graft failure becomes more likely [207]. A recent retrospective study from India suggests that surgical intervention should be considered early in recalcitrant cases to improve the chances of graft survival [209]. However, TPK is a relatively technical procedure requiring an appropriately trained and experienced surgeon. Lack of donor graft availability is a significant challenge in large parts of the world where the need is greatest, in part due to legal and cultural barriers.

6. Ocular Mycology

6.1. *Fusarium* spp.

Fusarium keratitis is a sight-threatening condition that often affects otherwise healthy individuals during their most economically active years of life [1,210]. The infection is very challenging to treat due to resistance of *Fusarium* spp. to many antifungals. Without adequate treatment, infection progresses relentlessly to perforation [1,22,24], endophthalmitis [211], and ultimately loss of the eye in the form of enucleation [151,212].

Epidemiology

Fusarium keratitis is most common in tropical and sub-tropical locations [13]. The main risk factor for developing infection in this setting, in common with fungal keratitis with filamentous fungal aetiology, is trauma, typically with vegetative matter, resulting in a defect in the corneal epithelium [24,46,68,132–134]. This either directly inoculates the cornea with fungal conidia or allows subsequent fungal entry to the corneal stroma. There is a history of preceding trauma in 40–60% of cases [60,70]. Other risk factors include previous ocular surgery [151,152], ocular surface disease, previous use of corticosteroids [146], contact lens use [213], immunosuppression [146], or use of traditional eye medicines [142].

Fungal keratitis caused by *Fusarium* spp. accounts for between 42% and 52.5% of all cases of FK, depending on geographical location [14,28]. It typically occurs in young healthy males who are undertaking agricultural work [13].

However, *Fusarium* keratitis is not confined to the tropics. In tandem with the increased use of disposable planned-replacement contact lenses, the numbers of *Fusarium* keratitis reported in temperate countries with developed economies has also risen. As discussed above, between 2005 and 2006 there was an outbreak of contact lens-related *Fusarium* keratitis due to the contact lens cleaning solution "ReNu with MoisutureLoc" (Bausch & Lomb, Bridgewater, New Jersey, USA) [150]. The highest number of cases was seen in the Far East, with Hong Kong reporting 33 cases between January 2005 and May 2006 [214], and Singapore reporting *Fusarium* keratitis in 68 eyes of 66 patients between March 2005 and May 2006 [215]. Given the high prevalence of myopia in these industrialised locations and widespread, increasing use of soft contact lenses [216], it is not unsurprising that these countries saw the highest incidence during this outbreak. However, other countries including the USA (164 cases 2005–2006), [217] and European Nations reported a similar peak between 2005 and 2006 [27,218–220].

Irrespective of the ReNu outbreak, there appears to be an increasing incidence in *Fusarium* keratitis in temperate climates. In the UK, a London tertiary ophthalmic hospital reported an increase in the proportion of *Fusarium* spp. isolates of all fungal keratitis cases from 18% between 1994 and 2006 to 42% between 2007 and 2014 [28]. Contact-lens use was found to be a significant risk factor (OR 4.35, 95% CI 1.50–12.7). In Germany, the national reference laboratory have reported 15 cases of *Fusarium* keratitis over 2 years between January 2014 and December 2015 [10]. The majority of these were contact lens wearers (73.3%) with no cases reporting preceding trauma or immunosuppression. However, as the reference laboratory only commenced operations in 2014, comparisons to previous results was not possible. Similar reports of a rising incidence of *Fusarium* keratitis have been described from the Netherlands [11], which also finds contact lens use as a significant risk factor in this setting, as well as in Denmark where 9/10 cases were attributable to filamentous fungi between 2010–13, of which 6/9 were confirmed as *Fusarium* spp. [146]. Unlike *Fusarium* keratitis seen in tropical countries, in temperate climates it is more common in females, likely reflecting the demographics of contact lens use [10,11,28].

6.2. *Aspergillus* sp.

Aspergillus spp. are the second most frequently reported causative organisms of fungal keratitis globally. Several species have been associated with corneal infection, the commonest being *A. flavus*, *A. fumigatus*, *A. niger* and *A. terreus* [7,14,221]. Corneal trauma with vegetative or organic matter is the predominant risk factor reported [76]. The pattern of disease is similar to that seen with *Fusarium* keratitis, but in vitro susceptibility data for ocular isolates of *Aspergillus* spp. demonstrates lower MICs compared to antifungal susceptibility profiles for *Fusarium* spp. [221] although visual outcome is also determined by other factors such as the severity of the infection on presentation in clinic; deep lesions have a poorer prognosis [13].

Epidemiology

Mycotic keratitis due to *Aspergillus* spp. also predominates in tropical and sub-tropical latitudes [222]. However, within these regions and within countries there is climatic variation-wet, dry and semi-arid climes. *Aspergillus* corneal infections predominate in drier environments in sub-tropical latitudes, for example, in northern Ghana, where the environment is dry, with seasonal harmattan winds facilitating dispersal of airborne conidia; the more temperate areas of West Bengal and in northern India where the number of infections due to aspergilli eclipsed those caused by *Fusarium* spp., including in fungal keratitis in children [7,62,65,76,223].

6.3. Dematiaceous Fungi

The most commonly reported ocular pathogens after *Fusarium* spp. and *Aspergillus* spp. are representatives from the dematiaceous moulds, a diverse group of fungi characterised by their ability to produce melanin, which has long been regarded as a unique pathogenic advantage [224]. Although ubiquitous, this group of moulds are not common causes of disease in humans, but many species are plant pathogens of agricultural importance, colonising spoil and vegetation. The link with occupational risk factors and ocular trauma is as described for other types of mycotic keratitis.

Melanin pigmentation of hyphae and conidia within this heterogenous group may be useful in rapid diagnosis in this form of phaeohyphomycosis. Darkly pigmented infected corneal tissue may be obvious on direct observation of the eye, but this is not a common clinical presentation. There are few instances where morphological appearance of fungi in vivo are specific, however, direct microscopy of corneal tissue infected with some dematiaceous species may reveal pigmented fungal elements, including swollen, irregular hyphae and yeast-like structures, which are characteristic in appearance. Some species are weakly pigmented and may appear hyaline [225,226].

Curvularia spp. are the most commonly reported of the dematiaceous fungi globally. Many other genera have also been reported to cause keratitis including *Bipolaris* spp., *Exserohilum* spp., *Alternaria* spp., *Ulocladium* spp., *Lasidoplodia theobromae and Colletotrichum* spp. (Figure 10) [7,15,17,32,227].

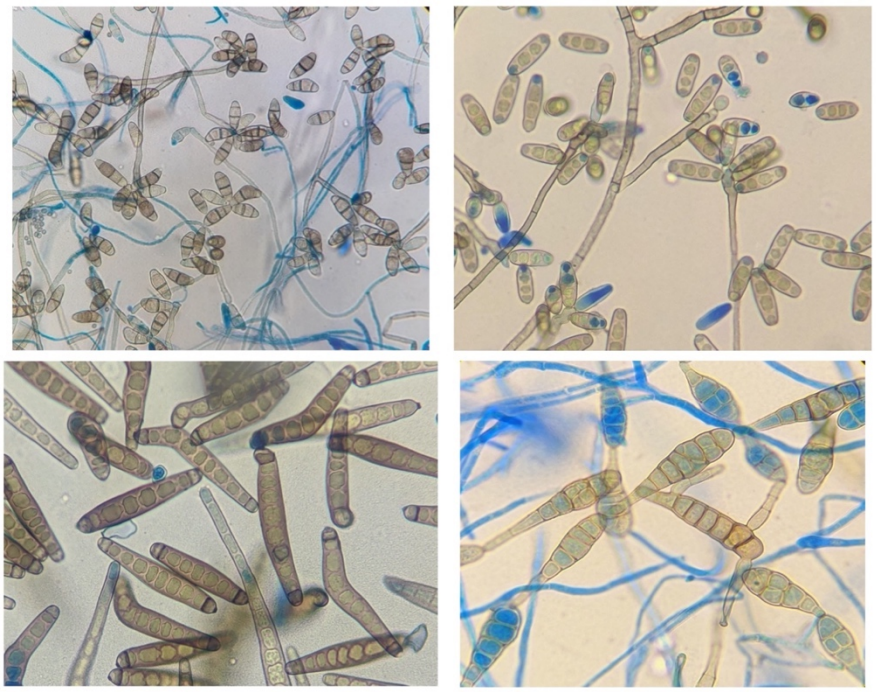


Figure 10. Examples of dematiaceous fungal genera isolated from cases of fungal keratitis stained with LPCB. Clockwise from **top-left**: *Curvularia* sp., *Bipolaris* sp. (magnification 400x); *Alternaria* sp., *Exserohilum* sp. (magnification x1000, oil immersion). These images were taken using an afocal photography technique; the camera zoom was used for additional magnification.

Epidemiology

Ocular infections due to the dematiaceous fungi have been reported from every continent. Although more commonly reported from regions with warmer, humid seasonality members of this heterogenous group have also been reported from semi-arid

regions [17,228–232]. In the terai of Nepal, the country with the highest documented incidence of fungal keratitis in the world, dematiaceous fungi such as *Curvularia* spp. are more frequently isolated than *Fusarium* spp. and *Aspergillus* spp., (personal experience & comms). *Curvularia* spp. were the most common filamentous fungi in a ten-year review of mycotic keratitis at a tertiary referral centre in North Carolina, South-eastern USA [122]. In order to understand regional patterns of causality it is important to reflect on the environmental reservoirs of many of these species, for example, *Curvularia* spp., which are pathogens of rice, maize, wheat, cassava, sorghum and grasses; common cash and subsistence crops in regions with a high incidence of fungal keratitis. To date there have been no phylogenetic studies comparing clinical (ocular) and environmental dematiaceous fungal isolates.

6.4. Other Filamentous Fungi

As previously mentioned there are more than 100 species of fungi reported as causing mycotic keratitis [13]. Other filamentous fungi less frequently reported include: *Sarocladium* spp, *Penicillium* spp., *Paecilomyces* spp., *Scedosporium* spp. and *Purepureocillum lilacinum*. Some of the least favourable therapeutic outcomes documented are mycotic keratitis cases due to *Scedosporium* spp., well characterised for their resistance to antifungal agents (Figure 11).

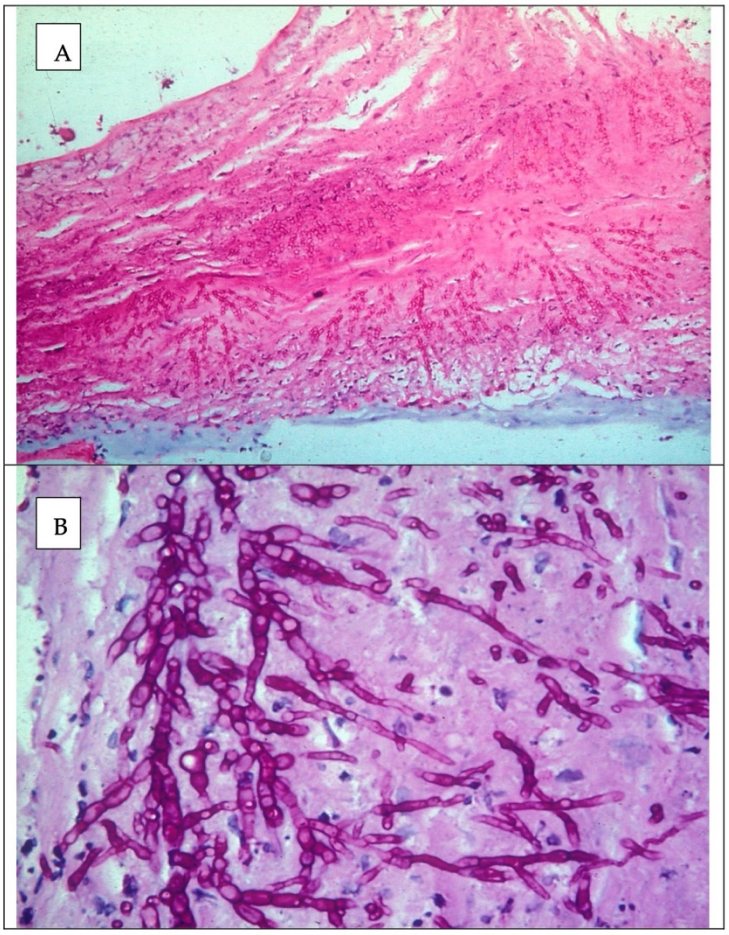


Figure 11. Histology section of a corneal button infected with *Scedosporium apiospermum* stained with H&E/PAS (A—magnification ×100, B—magnification ×1000, oil immersion).

7. Unsolved Problems and Future Work

Fungal keratitis is a disease that disproportionately affects poor people living in some of the world's poorest countries. There is evidence to suggest that the incidence of fungal keratitis is increasing globally. Unfortunately, for most people who have FK, access to appropriate diagnosis and treatment is very limited. To help address this apparent "neglect", there has been a recent push for fungal keratitis, as part of microbial keratitis, to be included in the World Health Organization's list of neglected tropical diseases (NTDs), which would help focus global attention and funding [233]. As it stands, there are a number of key areas where there are challenges and significant unmet needs, where addressing these may greatly reduce the morbidity associated with FK:

- Delay in presentation leading to poor outcomes [1,18]
- Use of traditional eye medicine and inappropriate use of conventional medicines [1,19,20]
- Limited relevant ophthalmic formal training of front-line health workers [1]
- Limited or no access to appropriate diagnostic investigations
- Topical antifungals are frequently unavailable [1]
- FK is challenging to treat, and treatment failure is common [1,22–26]

Sight-loss from severe microbial keratitis (MK) in LMIC results from a combination of these factors. In response, current and future work is focused on addressing these areas. Research projects are underway to improve the understanding of patients' health-seeking behaviour, such as that recently completed in Uganda [18,138,142]. Linked to this is implementation research into primary preventative measures, specifically how to prevent ocular injuries from occurring in the first place. Secondary preventative measures, for example antibiotic or antiseptic prophylaxis following corneal trauma, need to be enhanced. Several studies from South Asia found early antibiotic prophylaxis of uninfected corneal abrasions with chloramphenicol ointment reduced risk of MK developing [210,234–236]. However, these did not address early management of established MK presenting in the community, which still occurred in considerable numbers [210]. A suitable alternative to prevent fungal as opposed to bacterial keratitis also needs to be considered. Enhanced training of primary health workers, in addition with early referral, could potentially improve outcome.

To enhance the ability to accurately diagnose MK, microbiology laboratory capacity must be improved. This can be aided by the development of affordable point of care tests. As discussed above, the fungal species responsible (and therefore treatment susceptibility) varies with geographical location-and time-so it is essential for clinicians to be aware of the local aetiology to adjust treatment strategies. Continued microbiological surveillance is required to ensure that a change in aetiology is detected in good time.

Given that a large proportion of FK is attributed to trauma with vegetative material, and many fungal species causing FK are in fact plant pathogens, phylogenetic studies should be used to determine which plant pathogens are causing disease, specifically assessing their virulence and pathogenicity.

Regarding treatment, despite natamycin being added to the WHO Essential Medicines List in 2017 which was a huge step forward, there are still frequent shortages and in many countries is still not licensed or available [1]. When it is available, it is often too expensive for most people. Accessibility to the current gold standard treatment needs to be improved and the evidence-base into alternative treatments, such as chlorhexidine 0.2%, needs to be widened. Randomised controlled trials are currently underway to assess its efficacy [196].

8. Conclusions

Mycotic keratitis, particularly when caused by filamentous fungi, is a global problem. The incidence and main risk factors vary with geographical location and level of economic development; in tropical LMICs, trauma with organic material is the main risk factor whilst in wealthier, temperate countries contact lens use or ocular surface disease are the predominant associations. There is emerging evidence that the incidence is increasing worldwide, possibly linked in part to climate change, with other factors at play; further

research is required to explore this in detail. Unfortunately, mycotic keratitis remains a severe, sight-threatening condition for millions.

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Making the diagnosis

In my review article above, we discussed the various investigations available to diagnose the underlying microbiological cause of MK with reference to FK, namely smear microscopy and culture, molecular methods including PCR and *in vivo* confocal microscopy (IVCM).[1] Here I present further detail on IVCM as this was an integral, real-time diagnostic tool used during this study, and further background information is therefore important.

In vivo confocal microscopy

IVCM is a non-invasive, real-time, imaging tool that acquires *en face* images at increasing depths through the cornea. It works on the principle that a narrow beam of light (typically a laser) through the objective lens of a light microscope excites a specimen (in this case corneal tissue) within a narrow focal plane. Light emitted from out-of-focus planes is rejected by the confocal (pinhole) aperture. This technology allows for a volume or series of high-resolution and high-contrast images to be captured *in vivo*, which can be used to identify certain diseases and pathogens. Although mainly used in ophthalmology, which is ideally suited due to the optical clarity of the cornea allowing for images to be acquired for the full corneal thickness, IVCM has also been used in dermatology and dentistry.[96, 97]

Ophthalmic IVCM technology has evolved over the years since its introduction to clinical practice in 1990 with the tandem-scanning confocal microscope.[98] Currently there are two IVCM devices in routine clinical use: one that uses a non-contact, white light slit-scanning confocal microscope (Confoscan, manufactured by Nidek, Japan) and the laser-scanning confocal microscope (Heidelberg Retina Tomograph II or III with the Rostock Corneal Module, manufactured by Heidelberg Engineering, Germany), which uses a diode laser and direct corneal contact through a single-use sterile cap, giving a lateral resolution of 1 micron.[99, 100] The latter system is generally considered to give higher contrast, better resolution, and improved image illumination.[100, 101]

In terms of MK, IVCM is used to identify two key pathogenic groups of micro-organisms: fungi (both yeasts and filamentous fungi) and *Acanthamoeba* spp.[102-104] Its use in diagnosing bacterial keratitis is limited as bacteria are too small to be detected, except for *Nocardia* spp.[105] The presence of highly-reflective filaments or hyphae, with a filament diameter of between 3 and 8 microns and hundreds of micrometers in length, visible on confocal microscopy **(Figure 3**) is indicative of fungal keratitis,[100, 103] whilst round or ovoid hyperreflective objects (diameter <30 μm), target images (hyperreflective objects with hyporeflective halo, diameter <30 μm), and trophozoite-like objects (diameter >30 μm), are suggestive of *Acanthamoeba* keratitis

(**Figure 1**).[106] Although *Candida* albicans has been shown to have a round, budding body associated with pseudohyphae,[107] accurately distinguishing yeasts from filamentous fungi is challenging.[100]

A recently published systematic review into the use of IVCM for FK considered IVCM in the diagnosis, prognosis and follow-up of fungal keratitis.[100] This identified 10 studies that presented the diagnostic accuracy of IVCM for FK in terms of sensitivity and specificity (**Table 2**). Although the studies were heterogenous in their design, the reported sensitivities and specificities ranged from 55.8%-94% and 78%-100%, respectively, excluding results from inexperienced observers.[102, 103, 108-115] Two studies highlighted a known limitation of IVCM being observer-experience dependent – IVCM in the hands of observers with limited experience had relatively poor diagnostic accuracy (sensitivity and specificity of 27.9%-42.9% and 42.1%-87.5%, respectively).[112, 114] The challenge for these studies relates to what the "gold standard" to which they are compared to is, and introduces a considerable degree of bias. As a result, most of the authors concluded that IVCM is a useful adjunctive tool but should not be used instead of the traditional "gold standard" of smear microscopy and culture, although some studies found IVCM to outperform this.[108, 113] Bakken and colleagues concludes that future work should look evaluate IVCM using artificial intelligence for a less biased assessment; some studies have already reported promising results with high sensitivities and specificities (89.3%-91.9% and 95.7-98.3%, respectively).[109, 111]

Authors have tried to define levels of diagnostic certainty based on specific morphological features of filamentous FK on IVCM.[100, 113] One such study categorised results of IVCM into either a) clearly positive for fungi, b) inconclusive, and c) negative for fungi,[113] and recommended that patients in the "clearly positive for fungi" category commence anti-fungal treatment without delay. The review by Bakken and colleagues updated this guidance as follows:

- **Category 1, consistent with FK**. Highly reflective, branching/bifurcating, well-defined, interlocking structures (hyphae / elements), measuring 3-10 microns in diameter and not seen in isolation.
  Recommended management: immediate initiation of anti-fungal treatment
- **Category 2, possible FK**. Features resembling fungal elements visible e.g. isolated or several linear / curvilinear structures, of varying reflectivity, lacking the well-defined branching and smooth features described in Category 1**.**
  Recommended management: follow-up examination, if a Category 1 image is obtained, then follow Category 1 guidance.

- **Category 3, unlikely FK**. Only normal structures or features associated with a typical inflammatory response are visible, with no structures suspicious for fungal hyphae. **Recommended management**: only repeat IVCM examination in cases refractive to treatment where diagnosis by other methods is unsuccessful.

The authors also recommend that ten 100-image volume scans should be captured, which should include the central and peripheral parts of the infiltrate at various depths. Furthermore one should distinguish between the confocal microscope operator (person performing the IVCM scan) and the confocal assessor (person assessing the images), specifying their cadre and level of experience.[100]

Finally, IVCM has been used as a tool in monitoring the response to treatment. Building on smaller, earlier studies that suggested that IVCM was a useful tool at monitoring progress, with patients responding to treatment exhibiting reducing IVCM fungal hyphal density and length,[116, 117] Chidambaram and colleagues performed a large, prospective study, evaluating FK patients with IVCM at days 7, 14, and 21 after starting anti-fungal therapy.[118] Features associated with poor outcome included the presence of stellate inter-connected cells with absent nuclei, and/or at the final visit a honeycomb distribution of inflammatory cells and/or detection of fungal hyphae. Similarly, dendritiform cells in the basal epithelial layer at the final visit was associated with deterioration. However, the authors discussed the challenge of re-imaging the exact same location at repeat visits as a significant limitation of using IVCM to monitor progression.

The current consensus is that IVCM is a useful tool at ruling in or ruling out FK and therefore a guide to starting anti-fungal therapy, as well as indicating whether a change in therapy is needed. Examining the density of fungal hyphae, in addition to some of the features identified by Chidambaram,[118] can help clinicians make an informed decision for the ongoing management of patients. However, it is likely here that the experience of the confocal operator and assessor are crucial.

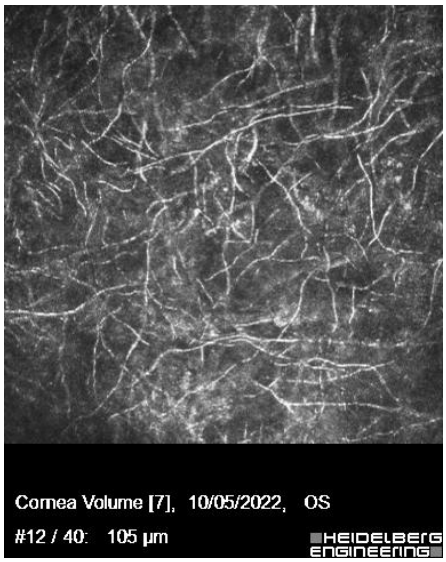

Figure 3: IVCM image from a patient with Aspergillus keratitis in Nepal showing presence of numerous hyperreflective filaments or hyphae

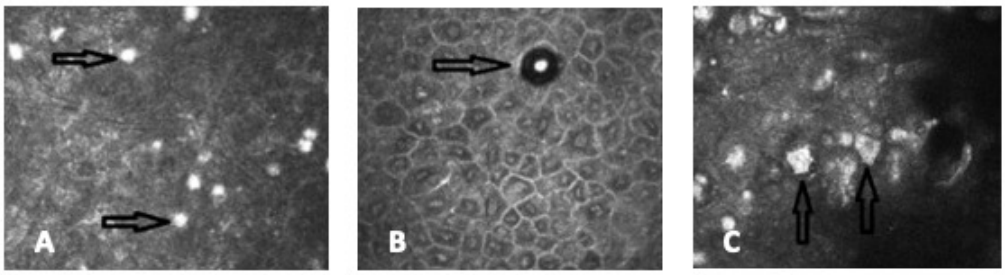

Figure 4: IVCM image from patients with Acanthamoeba keratitis. A, round or ovoid hyperreflective objects (bright spots); B, round or ovoid hyperreflective objects with hyporeflective halo (target sign); C, trophozoites. Reproduced with permission from De Craene S, Knoeri J, Georgeon C, Kestelyn P, Borderie VM. Assessment of Confocal Microscopy for the Diagnosis of Polymerase Chain Reaction–Positive Acanthamoeba Keratitis: A Case-Control Study. Ophthalmology 2018; 125(2): 161-168.

Table 2: Studies that have investigated the diagnostic accuracy of in vivo confocal microscopy for fungal keratitis. Adapted from Bakken et al.[100]

First author, year	Number of eyes	Location	Sensitivity	Specificity	Study design	Comments / key findings
Hoffman et al., 2021 [108]	15	London, UK	81%	100%	Retrospective	IVCM was the most sensitive method to diagnose FK
Lv et al., 2020 [109]	688 images^	Nanning, China	91.9%	98.3%	Retrospective	AI system based on ResNet, good results in diagnosing FK
Wang et al., 2019 [110]	12	California, USA	66.7%	100%	Retrospective	IVCM is as good as clinical assessment in diagnosing FK
Wu et al., 2018 [111]	56	Jinan, China	89.3%	95.7%	Prospective	AI guided AHD superior to corneal smear for FK
Kheirkhah et al., 2017 [112]	21	Massachusetts, USA	42.9%-71.4%	87.5%-89.6%	Retrospective	IVCM is highly dependent on of the observer's experience
Chidambaram et al., 2016 [103]	176	Madurai, India	85.7%	81.4%	Prospective	IVCM is a valuable tool in detecting FK
Nielsen et al., 2013 [113]	6	Copenhagen Denmark	86%	N/A	N/A	IVCM is superior to culture in FK
Vaddavalli et al., 2011 [102]	93	Hyderabad, India	89.2%	92.7%	Prospective	IVCM is an accurate diagnostic method for AK and FK
Hau et al., 2010 [114]	12	London, UK	27.9%-55.8%	42.1%-84.2%	Retrospective	IVCM diagnostic accuracy is dependent on observer experience
Kanavi et al., 2007 [115]	16	Tehran, Iran	94%	78%	N/A	IVCM is useful in diagnosing FK

AHD, automatic hyphae detection; AI, artificial intelligence; FK, fungal keratitis; IVCM, *in vivo* confocal microscopy.

^ 688 images included, (number of patients unknown) 1400 images negative for fungi

Management of Microbial Keratitis

An overview of the management of FK is given in the review above, with a more detailed review of the latest literature given in Chapter 2 of this thesis. An overview of the management of bacterial and *Acanthamoeba* keratitis is given here.

Bacterial keratitis

Antibiotics

The first-line treatment for bacterial keratitis is topical antibiotics.[119] There have been several high quality, randomised controlled trials investigating topical antibiotics for the treatment of bacterial keratitis; 16 of these trials were included in a Cochrane systematic review in 2014,[120] and subsequently discussed in a review on the management of bacterial keratitis.[121] The main finding of these reviews was that there was no difference in the relative risk of treatment success (complete re-epithelialisation or time-to-cure) between the "fortified" aminoglycoside-cephalosporin regime and fluoroquinolone monotherapy (

Figure 5).[120] Although there was no difference in serious complications, there were more minor side effects (including ocular discomfort and/or chemical conjunctivitis) in the aminoglycoside-cephalosporin group.[120, 122-125] Treatment with topical antibiotics is usually administered hourly initially, typically for 48 hours, and then tapered according to the patient's response. It is important not to reduce the dosing to less than four times daily as this is subtherapeutic and may lead to antibiotic resistance developing.[119]

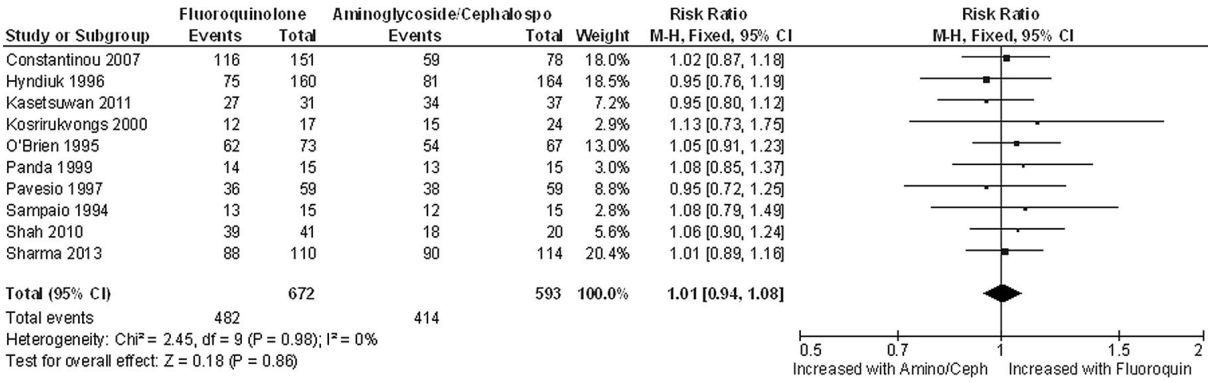

Figure 5: Forrest plot for topical fluoroquinolone compared with topical fortified aminoglycoside–cephalosporin indicating no difference in chance of treatment success.[120] Reproduced from McDonald EM, Ram FS, Patel DV, McGhee CN. Topical antibiotics for the management of bacterial keratitis: an evidence-based review of high quality randomised controlled trials. *Br J Ophthalmol* 2014; **98**(11)**:** 1470-1477 with permission from BMJ Publishing Group Ltd.

Given there is no evidence of a difference in efficacy between antibiotic treatment regimens, ophthalmologists' empirical antibiotic choice is largely based on, amongst others, the spectrum of coverage, side effects and corneal toxicity, availability and cost, resistance patterns, and the epidemiology and global distribution of the micro-organisms these factors.[126] An international survey sent to 1009 corneal specialists (with 140 responses) found that clinicians in the US favoured a more broad-spectrum regime taking into account antibiotic resistance (the fortified vancomycin regime) compared to "international" clinicians, who favoured fluroquinolone monotherapy, as these clinicians tended to be more concerned about availability and toxicity.[126]

Steroids

Using topical corticosteroids as an adjunct to the treatment of bacterial keratitis is somewhat controversial. The Steroids for Corneal Ulcer Trial (SCUT) was a large, multi-centre double-masked randomised controlled trial that compared topical moxifloxacin 0.5% plus topical steroid (prednisolone 1%) against topical moxifloxacin 0.5% plus placebo for the treatment of bacterial keratitis with 250 patients in each arm, which aimed to determine "whether topical corticosteroids as adjunctive therapy for bacterial keratitis improves long-term clinical outcomes".[127] In terms of primary analysis of the primary outcome (best spectacle corrected visual acuity (BSCVA) at 3 months), the authors reported no evidence of a difference between treatment arms. There was also no difference in scar size or time to re-epithelialisation. However, subgroup analysis found that for patients with a vision of counting fingers or worse at baseline, or central corneal ulcers at baseline, patients in the steroid-containing arm had significantly better visual acuity (-0.17 Logarithm of the Minimum Angle of Resolution (logMAR) (95% CI, −0.31 to −0.02; P = .03); and -0.20 logMAR (−0.37 to −0.04; P = .02), respectively). Secondary outcome data from BSCVA at 12 months found evidence of improved vision in non-*Nocardia* bacterial keratitis cases in patients treated with adjunctive topical corticosteroids (−0.10 logMAR, 95%CI, −0.19 to −0.02, P=0.02).[128] A further subgroup analysis found adding topical corticosteroids at two to three days after starting antibiotics (as opposed to after four or more days), resulted in a 1-line better visual acuity at three months compared to placebo.[129]

Judicious use of topical corticosteroids, such as by restricting use to patients with central corneal ulcers and presenting poor vision, only starting at between 2-3 days following starting antibiotics and once *Nocardia*, fungal or *Acanthamoeba* infection has been ruled out, may be appropriate and may reduce morbidity.[127]

Therapy in complex cases

When the integrity of the globe is compromised, for example in cases of severe, progressive corneal thinning or perforation (including descemetocele), or when there is unresponsive disease, or endophthalmitis, additional treatment is indicated. Oral tetracycline antibiotics (e.g. doxycycline) may have a role in counteracting stromal thinning by inhibition of matrix metalloproteinases, although robust evidence in terms of clinical outcomes is currently lacking.[130] Surgical intervention in the form of corneal gluing (application of tissue adhesive), lamellar keratoplasty, or penetrating keratoplasty are often used to manage severe thinning or perforations. Amniotic membrane grafts (AMG) may be helpful to prevent the need for emergency corneal grafts and may improve clinical outcomes; one randomised controlled trial found significant evidence of improved BSCVA in patients who received AMG compared to controls,[131] whilst another prospective controlled study in *Pseudomonas* keratitis found improved outcomes including improved vision and reduced pain in patients receiving AMG compared to controls.[132] Conjunctival flaps may also be beneficial in cases of microbial keratitis refractory to medical treatment.[133]

Emerging therapies

Povidone-iodine is a potential emerging treatment for bacterial keratitis and herpes simplex keratitis that has the advantage of being inexpensive to produce. A multi-centre randomised controlled trial comparing povidone-iodine 1.25% to topical antibiotics (either neomycin-polymyxin B-gramicidin (Philippines) or ciprofloxacin 0.2% (India)) for the treatment of bacterial keratitis found no evidence of a difference between groups in terms of healed epithelial defect.[134] One should note, however, that this study may have lacked sufficient power to detect a small difference between groups, the choice of antibiotic control may not have been appropriate, and the primary outcome, unlike other microbial keratitis trials, was not BSCVA at 3 months.

Corneal collagen cross-linking (CXL) is another technique that has been used with some relative success for treating moderate cases of bacterial keratitis. A systematic review and meta-analysis found that CXL is potentially effective for bacterial keratitis, reducing corneal melting.[135] It is likely that CXL is more useful for shallow corneal ulcers, as ultraviolet energy is absorbed within the first 100 micrometres.[136] Although one small study has shown cross-linking alone resolved 14 out of 16 cases of bacterial keratitis without antibiotics,[137] there is better evidence for successful treatment with CXL for anterior infiltrates as adjunctive therapy to standard antibiotics, particularly for more complex cases.[138]

Bacterial keratitis management in Nepal

The preferred first-line therapy for bacterial keratitis in Nepal is currently with topical fourth-generation fluoroquinolone antibiotics, such as moxifloxacin 0.5%. A recent prospective study from western Nepal, where 31.0% of isolates yielded bacterial organisms on culture, in addition to 9.5% mixed fungal-bacterial organisms, with *S. aureus* (47%) and *Pseudomonas spp.* (11.7%) most commonly isolated, found all isolates were susceptible to moxifloxacin 0.5%.[139] Similarly, a cross-sectional study from Kathmandu where 56% of cases were bacterial in aetiology (*S. pneumoniae* (56%) and *Strep. Viridians* (12%) most commonly), found all isolates to be susceptible to moxifloxacin, whilst 92% and 88% were susceptible to ofloxacin and ciprofloxacin, respectively.[140] In an earlier retrospective study of 414 patients with MK from western Nepal, of which 121 of the 300 culture positive cases were bacterial (40.3%), cephazolin was found to be the most effective antibiotic for gram-positive infection (84.9% susceptible) and ciprofloxacin and ofloxacin were most effective for gram-negative infection (79.3%).[141] This study was conducted prior to the routine use of moxifloxacin.

It is important to bear in mind when reviewing the results of susceptibility testing for corneal infection that these results are based on data from systemic administration and serum concentration, as opposed to topical corneal application and corneal penetration.[142]

Acanthamoeba keratitis

AK is challenging to treat due to the parasite's lifecycle: *Acanthamoeba* cysts are very resistant to treatment, whilst trophozoites are more susceptible.[85] Although there have only been a handful of case reports of AK in Nepal, it is likely that this is a significant underestimate given the lack of robust investigations.[143-145] The prevalence of AK is likely to be similar to 2% of MK isolates reported in India.[146] Treatment options for AK include diamidines (propamidine 0.1%, hexamidine 0.1%, and dibromopropamidine 0.1%) and biguanides (polyhexamethylene-biguanide (PHMB, in varying concentrations 0.02%-0.08%) and chlorhexidine 0.02%.[147]

Treatment is usually intense and prolonged, and traditionally involves combination therapy of one diamidine and one biguanide. Initially, drops are instilled hourly day and night following corneal epithelial debridement for the first few days of therapy, reducing to hourly during waking hours depending on the clinical response. If there is continued clinical improvement, frequency can be reduced to every three hours. It can take considerably longer (up to two weeks) than in cases of bacterial and fungal keratitis for clinical improvement to be observed, and treatment should continue for at least three to four weeks. Once treatment has stopped, patients should be monitored closely to ensure there is no recurrence.[85]

More recent research has suggested that biguanide antiseptics (PHMB or chlorhexidine) are the most efficacious therapies for AK,[85] possibly as they are effective against both trophozoites and cysts.[148] A double-masked randomised controlled trial comparing chlorhexidine 0.02% to PHMB 0.02% found no difference in treatment success (defined as favourable clinical response within two weeks) between the two arms (P = 0.71).[149] A clinical trial is currently under way comparing a higher concentration of PHMB (0.08%) as monotherapy to PHMB 0.02% plus propamidine 0.1% combination therapy.[150] Higher strength PHMB 0.08% has been shown to be safe and tolerated in a phase 1 clinical trial.[151]

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A bottle of natamycin 5% manufactured in India and dispensed in Nepal

General management of fungal keratitis

Treatment for filamentous fungal keratitis (FK) is usually with topical antifungal agents. Surgical intervention, usually in the form of corneal transplant or therapeutic penetrating keratoplasty (TPK), is generally reserved for cases of corneal perforation, relentless progression (despite medical treatment) and for visual rehabilitation once the acute infection has resolved and the cornea is scarred. There are a limited number of antifungals available with action against fungal keratitis, of which there are four main groups: imidazoles, triazoles, polyenes and fluorinated pyrimidines. These may be available topically, orally or by intravenous injection. Subconjunctival injection or corneal stromal injection may also be given.

Medical management of FK

Topical treatment

Most antifungal agents used for fungal keratitis belong to either the polyene (natamycin and amphotericin) or azole (voriconazole (VCZ), ketoconazole (KCZ), fluconazole, itraconazole, miconazole, and poscaconazole). Polyenes work by binding to ergosterol (essential for maintaining cellular membrane integrity) and inhibiting its cellular functions; additionally, amphotericin B permeabilises the fungal membrane (natamycin does not).[1-3] Azoles, on the other hand, act by inhibiting the biosynthesis of ergosterol.[4]

There have been several clinical trials comparing various treatment options for filamentous fungal keratitis over the last few decades, which have been reviewed systematically.[5, 6] All trials have to date been conducted in South Asian countries with a high incidence of fungal keratitis.

Natamycin, voriconazole and econazole

Natamycin (NATA) was approved in the 1960s by the FDA and still remains the only licensed drug in the USA for FK. It is produced naturally by the bacteria *Streptomyces natalensis.*[7] When manufactured, it is produced as a preserved suspension formulation at a 5% concentration (50mg/ml). Given it is a suspension, it must be shaken well before application. It is active against a wide range of fungal organisms including *Fusarium*, *Aspergillus*, *Alternaria*, *Candida*, *Cephalosporium*, *Colletotrichum*, *Curvularia*, *Lasiodiplodia*, *Scedosporium*, *Trichophyton*, and *Penicillium*.[8] Patients are usually treated initially with one drop hourly, typically for a week, with the frequency reduced depending on the clinical response. Once the infection has resolved, it is advisable to continue with natamycin four times daily for four more weeks.[9]

NATA has been compared to several other topical agents including voriconazole and econazole. [10-15] Voriconazole, a triazole antifungal agent licensed for the treatment of invasive aspergillosis, is used off-label as a topical formulation for fungal keratitis. It is not currently routinely available as a pre-prepared eyedrop, and instead must be reconstituted from the powder for injection to achieve a concentration of 10mg/ml.[16] Once reconstituted, it is advisable to be refrigerated and used within 48 hours.[16] Its antifungal activity and dosing regimen is similar to NATA.[17] In an initial prospective randomised controlled trial (RCT) showed no significant difference (P=0.837) between the groups in terms of primary outcome measure (time to healing of epithelial defect). The authors therefore concluded that voriconazole was "an effective and well-tolerated drug" and larger trials were warranted to demonstrate superiority.[10] Meanwhile, Prajna *et al.* also compared topical natamycin to voriconazole in a therapeutic exploratory randomised clinical trial; 120 patients were randomised to either natamycin or voriconazole and either had repeated corneal epithelial scraping or not. The study also concluded that there was no significant difference between groups for the primary outcome of visual acuity at three months, with a non-significant trend favouring voriconazole (0.98 logMAR better, 95%CI: -0.28 to 0.83, P=0.29). Incidentally, repeated scraping was associated with a worse outcome, although again this was non-significant (p=0.06).[11] To evaluate the efficacy of voriconazole thoroughly, the Mycotic Ulcer Treatment Trials (MUTT) were developed.[12, 13] In MUTT1, topical natamycin 5% was compared to topical voriconazole 1% in a trial that was due to recruit 368 patients but was terminated earlier on recommendation by the trial Data Safety and Monitoring Committee, as the number of perforations in the voriconazole group were significantly higher than in the natamycin group (34 vs. 18 perforations, p = 0.02; after 323 recruited). Vision was -0.18 logMAR better at three months in the natamycin group compared to the voriconazole group (P=0.006).[12] Sharma *et al*. also found natamycin to be superior to voriconazole in terms of best spectacle corrected visual acuity (BSCVA) vision at final follow-up in a more recent randomised controlled trial that recruited 119 patients (NATA mean BSCVA LogMAR 0.6 (CI 0.4-0.8), compared to voriconazole mean BSCVA LogMAR 1.1 (CI 0.9-1.2) P=0.01).[14]

There have been two published metanalyses studies comparing natamycin to voriconazole with the primary outcome being best spectacle corrected visual acuity at three months.[5, 18] These included the pilot study by Prajna and colleagues (2010), the study by Arora and colleagues, and MUTT1.[11, 12, 19] The Cochrane review found a non-significant trend favouring natamycin (**Figure 6**),[5] whilst McDonald et al found evidence for natamycin being superior to voriconazole (**Figure 7**).[18] The discrepancy between these two results is likely due differences in inclusion criteria and analysis strategy; the total number of subjects in the analysis by McDonald was 473, compared to 434 in the Cochrane review. The Cochrane review analysis

used a more conservative random-effects model rather than a fixed- effect model, as there were only three studies included. There has been no further published peer-reviewed metanalysis that includes the more recent study by Sharma et al (2015), although if this study were also to be included, then the pooled estimate of effect would be clearly in favour of natamycin 5% (standardised mean difference 0.34 logMAR, 95% CI 0.17 to 0.50; I^2 = 73.3%, P=0.011; personal communication from Dr Simon Arunga).

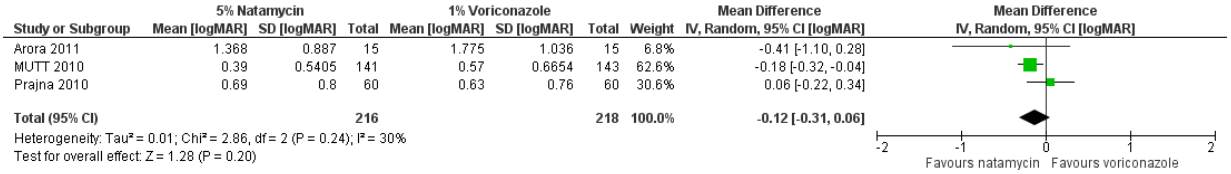

Figure 6: Forest plot of topical natamycin 5% versus voriconazole 1% (outcome: best spectacle corrected visual acuity, logMAR). Reproduced from FlorCruz NV, Evans JR. Medical interventions for fungal keratitis. *The Cochrane database of systematic reviews* 2015; **4**: CD004241 with permission from John Wiley and Sons.[5]

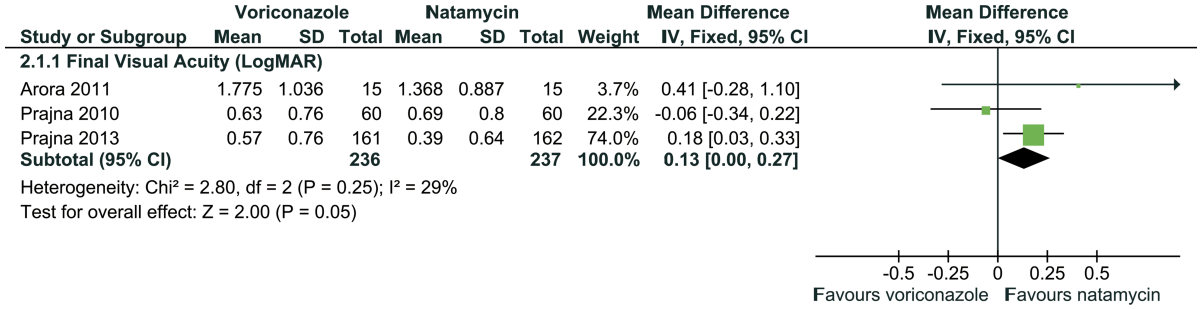

Figure 7: Forest plot of topical natamycin 5% versus voriconazole 1% (outcome: best spectacle corrected visual acuity, logMAR). Reproduced from McDonald EM, Ram FS, Patel DV, McGhee CN. Effectiveness of Topical Antifungal Drugs in the Management of Fungal Keratitis: A Systematic Review and Meta-analysis of Randomized Controlled Trials. *Asia Pac J Ophthalmol (Phila)* 2014; **3**(1)**:** 41-47 with permission Wolters Kluwer Health, Inc.[18]

If one considers perforations (or need for TPK) as the outcome measure, there are significantly fewer perforations or TPKs in patients treated with natamycin 5% compared to voriconazole,[5, 18] as reported by the two metaanalyses (risk ratio favouring natamycin 1.89, 95% CI 1.14 to 3.12; I^2 = 0%, P=0.01; **Figure 8** and **Figure 9**)[18].

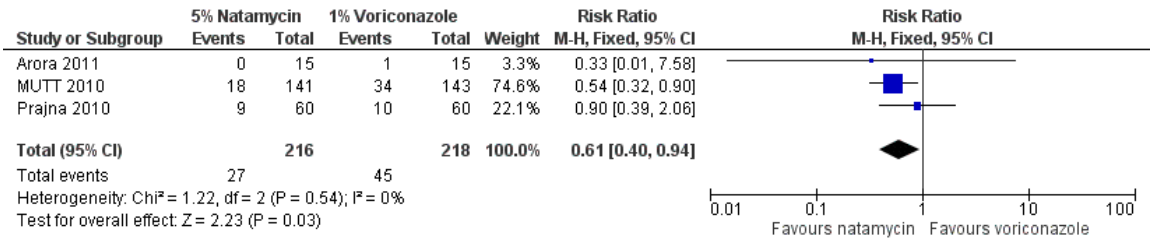

Figure 8: Forest plot of topical natamycin 5% versus voriconazole 1% (outcome: corneal perforation). Reproduced from FlorCruz NV, Evans JR. Medical interventions for fungal keratitis. *The Cochrane database of systematic reviews* 2015; **4**: CD004241 with permission from John Wiley and Sons.[5]

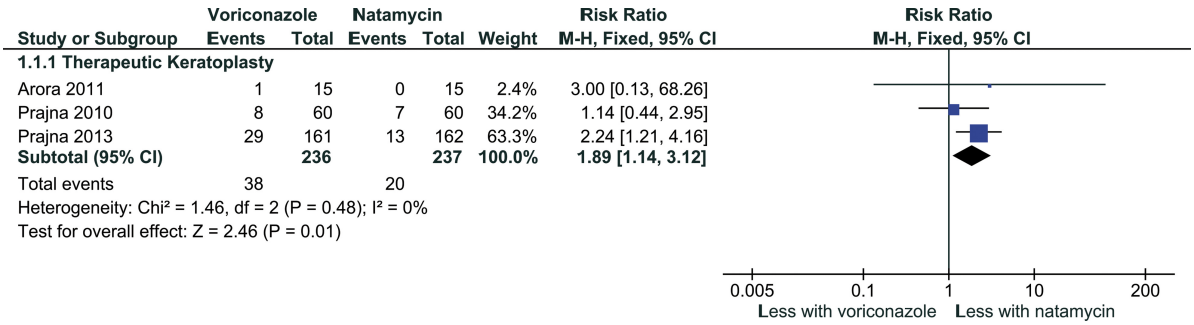

Figure 9: Forest plot of topical natamycin 5% versus voriconazole 1% (outcome: therapeutic penetrating keratoplasty). Reproduced from McDonald EM, Ram FS, Patel DV, McGhee CN. Effectiveness of Topical Antifungal Drugs in the Management of Fungal Keratitis: A Systematic Review and Meta-analysis of Randomized Controlled Trials. *Asia Pac J Ophthalmol (Phila)* 2014; **3**(1)**:** 41-47 with permission Wolters Kluwer Health, Inc.[18]

Subgroup analysis in MUTT1 demonstrated that the effect of natamycin compared to voriconazole was significantly greater in those infected with *Fusarium* species (40% of patients) compared to those with non-*Fusarium* species (*Aspergillus* 17%, other 43%), for whom there was no evidence of a difference between treatment arms.[12] However, this subgroup analysis was not prespecified in the MUTT1 protocol. Similar findings were reported by the 2015 trial by Sharma.[14]

NATA has also been compared to econazole 2%, an imidazole antifungal agent, investigating time to healing and proportion healed ("success") at four weeks.[15] In this trial, 116 patients were recruited in India, of whom the majority were infected with *Fusarium* (55.2%) followed by *Aspergillus* (25.9%), and randomised to either natamycin 5% or econazole 2%. There was no significant difference between the two arms in terms success (log rank 0.52, P = 0.47) or for time to healing (epithelial defect, log rank 0.82, P = 0.37; infiltrate, log rank 0.86, P = 0.35). This study may have been inadequately powered to detect a difference and did not consider vision or perforation rate, unlike the more recent trials, limiting the usefulness of these results.

Although the authors suggested that econazole 2% could be an alternative treatment to natamycin, there have been no subsequent randomised controlled trials, likely due to the emergence of voriconazole to the market. A retrospective study by the same group comparing NATA monotherapy with NATA plus econazole 2% found no difference between the two arms in terms of "success" (healed or healing ulcer at four weeks). Despite this, it should be borne in mind that econazole is considerably cheaper and more widely available than voriconazole and is often the only topical antifungal available in many Low- and Middle-Income Countries (LMICs).[20]

Similar to many other eyedrops, when used for a prolonged duration, natamycin can be toxic to the corneal epithelium. Other side effects include burning or stinging when applied, ocular discomfort including foreign body sensation, conjunctival hyperaemia, and epiphora. In addition to a similar mild side effect profile to natamycin, topical voriconazole has been reported to cause periocular dermatitis.

A new soluble form of topical natamycin (natamycin 1% w/v, "Natasol") has been developed, with initial experimental animal studies suggesting it is safe with no cases of ocular toxicity reported, as well as being non-inferior to natamycin 5% suspension in terms of its pharmacodynamics and ocular penetration.[21] Further research is warranted, but the authors hope that patient compliance may be increased in this formulation.

Given the strong evidence for natamycin, it is generally considered the first-line agent for fungal keratitis, particularly in areas with a high prevalence of *Fusarium.* For this reason it has been added to the WHO essential medicine list,[22] although its cost outside of South Asia (where it is made as an off-patent generic medication), remains prohibitively expensive for many and availability remains very limited.

Amphotericin B, Fluconazole, and echinocandins

Although amphotericin B has been used as an alternative, often second-line, agent to treat fungal keratitis, there is very limited evidence with regards to its efficacy other than case reports and case series, some of which have used it in addition to other antifungals.[17, 23, 24] In its standard form amphotericin B cannot penetrate the intact cornea. Amphotericin B has been shown to be more effective against yeasts compared to *Fusarium* species.[25, 26] There have been no head-to-head trials published to date comparing topical amphotericin B to other topical agents.

Fluconazole is generally considered to be much more effective against yeast infection than filamentous fungal infection. A randomised controlled trial comparing natamycin 5% to

fluconazole 0.2% was terminated early after only eight patients were enrolled, as all four patients in the fluconazole arm showed no signs of improvement.[27] However, rather surprisingly, a more recent case series from Paraguay of filamentous fungal keratitis patients treated with either fluconazole 0.2% monotherapy or fluconazole 0.2% and oral ketoconazole 200mg suggested that fluconazole 0.2% may be effective in filamentous fungal infections with 70% of patients showing resolution of their disease, with no evidence of benefit from the addition of oral ketoconazole.[28] There is currently no convincing evidence to recommend the use of topical fluconazole in filamentous fungal keratitis.

The echinocandins are a relatively novel class of antifungal agent that act by blocking β-(1,3)-D-glucan synthesis, which is an important structural component that maintains the fungal cell wall integrity,[29] and consists of three agents: caspofungin, micafungin, and anidulafungin. They have been shown to be successful at treating systemic fungal infections,[30, 31] and *in vitro* and animal studies have suggested a potential role for their use in fungal keratitis.[29, 32-36] However, other than several case reports,[29, 37-41] there is very limited published evidence regarding their efficacy (particularly as monotherapy), and their use at present is best considered experimental with larger clinical trials warranted.

Oral treatment

Adjunctive oral treatment for FK, with either itraconazole, ketoconazole or voriconazole, has been investigated and remains controversial.[9] In MUTT 2, again in conducted in South Asia (India and Nepal), 240 patients with severe fungal keratitis (BSCVA logMAR 1.3 or worse) were randomised to either oral voriconazole or placebo, with all patients receiving topical treatment (initially topical voriconazole monotherapy prior to the results of MUTT 1, then in combination with natamycin, two years into recruitment). There was no difference in primary outcome (perforation rate or corneal graft) within three months between groups (hazard ratio, 0.82; 95% CI, 0.57–1.18; P = 0.29), with more side effects reported in the voriconazole group (P < 0.001). The study therefore concluded that there was no benefit in adding oral voriconazole in the treatment of severe filamentous fungal corneal infections.[13] A prespecified subgroup analysis suggested that *Fusarium* infected patients treated with oral voriconazole may have a reduced rate of perforation, although this was not statistically significant.

An earlier RCT of 54 FK patients treated with topical itraconazole compared oral itraconazole to no oral treatment (i.e. no placebo in the control arm).[42] In terms of healing by six weeks, there was no evidence of a difference between arms (RR 1.0, 95%CI 0.37 – 2.71).

More recently, an RCT of 50 patients (all treated with topical natamycin) with severe FK were randomised to either oral voriconazole or oral ketoconazole.[43] There was evidence that

patients treated with oral voriconazole had 0.26 LogMAR better BSCVA at three months compared to those treated with oral ketoconazole (95% CI, 0.04–0.48; P = 0.02). There was no difference in perforation rates between these two groups (P= 0.45).

Oral antifungal therapy has the potential to cause severe, even life-threatening, adverse effects, primarily in the form of hepatotoxicity. Dosing for voriconazole should be body weight dependent, with adults with a body weight under 40kg receiving 200mg twice a day for two doses (as loading doses) and then 100mg twice a day for the duration of therapy.[44] Adults 40kg and above should receive double this dose. Bioavailability of voriconazole is good, with peak plasma concentration achieved between 1.43 and 1.81 hours after ingestion, although it should be taken 1 hour before or 2 hours after eating.[45, 46] Baseline and three-monthly liver function tests is necessary due to the risk of hepatotoxicity.[9] In addition, oral voriconazole can cause visual disturbance, change in colour vision, and photophobia. These are typically transient and occur in one third of patients, occurring approximately 30 minutes after administration and lasting for 30 minutes. Rarely, visual hallucinations, confusion, and psychosis have been reported.[47]

Given the limited evidence for any additional adjunctive effect and potential for systemic side effects, adjunctive oral voriconazole in FK remains controversial and should not be given routinely, even for severe disease. The need for liver function testing is an additional challenge to many settings where FK is common but such facilities may not be easily accessible or affordable.

Targeted drug delivery

In addition to topical treatment, injections of anti-fungals into either the corneal stroma (i.e. intrastromal injection) or anterior chamber (i.e. intracameral injection) have also been performed in severe disease where the response to topical treatment has been inadequate.[48, 49] Options that have been reported in the literature include intracameral amphotericin B or voriconazole, and intrastromal amphotericin B or voriconazole.[9, 47, 50] Natamycin has generally been avoided in a targeted manner as it is usually formulated as a suspension and initial animal studies did not recommend its use,[51] however there have been recent experimental studies using a new soluble form of the drug (sterile unpreserved Natasol 0.01% intrastromal injection), that suggest it may have a role to play;[21, 52] further evaluation is necessary.

Intrastromal injections are performed by injecting a suitable antifungal (e.g. voriconazole 50 µg/0.1 ml) loaded within a 1ml tuberculin syringe with a 30-gauge needle, which is inserted obliquely into the clear, uninvolved cornea to reach just adjacent to the ulcer within the mid-stromal level.[48] The drug is deposited circumferentially around the ulcer by giving five divided

doses, resulting in the drug surrounding the ulcer in each meridian. This can be repeated, with 72 hours between injections. There are inherent risks involved, including spreading infection to new foci, intraocular inflammation, cataract formation, perforation, raised intraocular pressure, hyphaema, and damage to the corneal endothelium.[9]

Intrastromal voriconazole injections have been compared to topical therapy alone in two randomised controlled trials.[53, 54] The first was a randomised controlled trial of 40 patients who were not responding to initial therapy with natamycin 5%. Patients were randomised to either topical voriconazole 1% alone or to intrastromal injections of voriconazole 50 µg/0.1 ml.[53] The authors found that patients receiving topical voriconazole had a mean BSCVA of -0.397 better than the intrastromal injection group (P = 0.008). Additionally, 19/20 patients receiving topical voriconazole healed with therapy. The authors concluded that topical, as opposed to intrastromal, voriconazole may be beneficial in addition to natamycin in recalcitrant disease not-responding to natamycin 5% monotherapy.[53] The second, more recent, clinical trial compared natamycin 5% monotherapy to natamycin 5% and intrastromal voriconazole in 70 patients with fungal keratitis.[54] The authors found no evidence of a difference between the groups for all outcome measures investigated (BSCVA, scar size, perforation rate, and microbiological cure) and concluded that "studies consistently suggest voriconazole has a limited role in the treatment of filamentous fungal ulcers".[54] Based on these two studies, there is currently no clinical trial-level evidence to support intrastromal injections of voriconazole. There have, however, been several case series that found intrastromal voriconazole to be beneficial in treating deep infiltrates or stromal abscesses that have been unresponsive to first- and second-line topical and medical therapy (natamycin 5%, voriconazole 1%, oral itraconazole or ketoconazole).[49, 55-57] There therefore may be a limited role for intrastromal voriconazole injections in select cases refractory to initial medical therapy.

There is very limited published data on the use of intracameral voriconazole. One case series evaluated five fungal keratitis patients with endoexudates who were treated with a single dose of intracameral voriconazole 50mcg/0.1ml, followed by voriconazole 1% topical therapy.[58] All cases demonstrated a complete resolution of infection within 3 weeks to 3 months. Another case series assessed intracameral voriconazole amongst 10 patients with fungal endophthalmitis resulting from keratitis in China.[59] This used 100mcg/0.1ml intracameral voriconazole, and was repeated up to 8 times over the course of the disease. The authors reported that "all cases … of the fungal anterior chamber invasion … resolved after treatment",[59] and four eyes required therapeutic penetrating keratoplasties. There may therefore be a limited role for intracameral voriconazole injection in patients with very deep fungal keratitis with anterior chamber spread of the fungus.

There is emerging evidence for the use of intracameral amphotericin B (ICAB) for patients with deep and/or recalcitrant filamentous fungal infection. There have been three published small randomised controlled trials evaluating this treatment. In the first trial of 42 patients, conducted in China, patients received topical and oral fluconazole and either intracameral amphotericin B or sham injection, with those receiving intracameral amphotericin B exhibiting faster healing time (p=0.001).[60] However, in the second trial, ICAB (5µg in 0.1ml 5% dextrose) injection, in addition to topical natamycin 5% and oral ketoconazole, was evaluated in 45 patients with deep fungal keratitis.[61] There were three arms in this trial: medical therapy alone; medical therapy and intracameral amphotericin B; and, medical therapy, intracameral amphotericin B and anterior chamber washout. This found no difference between the arms in terms of treatment success (primary outcome measure), time to disappearance of hypopyon, time to healing, final visual acuity, or healing. However, it should be noted that this study may have been underpowered to detect a difference, with only 15 patients in each arm. In contrast, two other non-randomised prospective interventional studies showed improved outcomes for patients treated with ICAB.[62, 63] Non-responding (to medical therapy) patients treated with ICAB had a significantly greater mean improvement in BSCVA (P<0.01) compared to responding patients not treated with ICAB, faster clearance of hypopyon (P<0.01), fewer scars and complications (P<0.05).[63] In the other study, all patients were given ICAB in addition to medical therapy, with one arm receiving it early (2 weeks) in contrast to the other arm receiving it late (4 weeks). Healing time was significantly faster in the early group (p<0.001) compared to the late group, whilst there were no perforations (0/25) in the early group, compared to 10/25 in the late group (p=0.006).[62] Several case series also suggest good outcomes for patients treated with intracameral amphotericin B.[64-67] ICAB may therefore an appropriate treatment in select cases including recalcitrant deep filamentous fungal keratitis cases with hypopyon or anterior chamber involvement. An adequately powered randomised controlled trial would be helpful to answer this more clearly.

Interestingly there is only limited published evidence on the use of intrastromal amphotericin B injection on its own, as several case series include it in combination with another intrastromal antifungal (either voriconazole or fluconazole). One audit from Egypt that retrospectively reviewed cases of FK not responding to topical therapy, found those treated with a single intrastromal injection of fluconazole and amphotericin B healed faster than those who received amphotericin B monotherapy.[68] A case series of 32 patients with progressive FK despite 10 days of combined topical voriconazole 1% and amphotericin B 0.15% found 87.5% of patients to demonstrate complete resolution of their fungal keratitis, with the remainder requiring TPK.[69] Patients received between 1 and 18 intrastromal injections (mean 9.3 ±6.4).

Intrastromal amphotericin B (5mcg/0.1ml), intrastromal voriconazole (50mcg/0.1ml), and intrastromal natamycin (10mcg/0.1ml) injections have recently been compared in a three-way randomised controlled clinical trial in fungal keratitis patients not responding to two weeks of topical natamycin 5% therapy.[70] Included patients had more than 50% stromal involvement and an ulcer size of more than 2mm. This found the mean duration of healing to be faster in the intrastromal natamycin group compared to the other two groups (p=0.02). There was no evidence of a difference between the groups in terms of healing success, although there was significantly more deep vascularisation in the intrastromal amphotericin arm.

Surgical treatment

Therapeutic penetrating keratoplasty

Surgical management, typically in the form of TPK, is an important step in managing a significant proportion of patients with fungal keratitis, predominantly for patients with perforation, impending perforation, and cases not improving despite maximal medical therapy.[71] The aim of surgery is to maintain globe integrity, whilst reducing the infectious burden. The percentage of patients requiring TPK is somewhat variable, with reports in the literature suggesting between 15-55% of FK patients required a TPK during the course of their disease.[13, 71-75] Risk factors for requiring TPK include presence of hypopyon, increased infiltrate size and infiltrate depth at baseline,[13] with the odds ratio for TPK or perforation found to be 2.28 (95% CI 1.18-4.40, p=0.01) in patients with hypopyon, 1.37 (95% CI 1.12-1.67, p=0.02) for each 1mm increase in infiltrate size (geometric mean), and 1.69 (95% CI 1.12-2.53, p=0.01) with increasing infiltrate depth in MUTT2.[13]

Unfortunately graft survival is often poor for patients with FK due to the active infection and inflammation, which increases the likelihood of graft rejection, secondary or re-infection, and secondary glaucoma.[71] Recurrence within the graft has been reported from 0-47%,[73, 76-80] whilst secondary glaucoma in 2-64% of cases.[71, 73, 74, 76, 81]

TPK success can be considered in terms of anatomical integrity, graft clarity, and visual improvement. These have been considered by several retrospective studies. Anatomical integrity has been reported to range from 64-97%,[71, 76, 77, 79, 82, 83] graft clarity from 26-94%,[73, 76-80] and visual improvement in 6-88% of cases.[74, 82] However, one should bear in mind that these studies did not specify whether secondary optical keratoplasties were performed.

TPK has a crucial role to play in FK by saving the eye and controlling the infection. Unfortunately, however, there is a significant lack of donor cornea material in LMICs where

the need is greatest. Where tissue is available, the quality is often poor with low endothelial cell counts.[84] It has also been suggested that outcomes of TPK in LMICs may be poor due to delayed use of topical steroids post-operatively due to the fear of re-infection, meaning inflammation, graft decompensation, and vascularisation are more commonly encountered.[71, 84]

Lamellar keratoplasty

Lamellar keratoplasty (LK) as an alternative surgical intervention to treat fungal keratoplasty has been reported in the literature.[85-89] Unlike TPK, which is most frequently performed for perforations (either impending or frank), in the context of FK lamellar keratoplasty is usually performed to surgically resect infections not extending into the anterior chamber. LK has the advantage over TPK in that corneal donor material can be preserved in glycerol or dehydration,[90, 91] meaning that supplying graft material for LMICs where there is a shortage of fresh corneal donors is possible. There have also been some published case series of using acellular porcine donor corneas, with promising results including low recurrence rates and good visual recovery.[92, 93] By preserving the host endothelium, the risk of endothelial immune rejection post-operatively is reduced.[94] However, LK is a technically more challenging procedure than TPK and surgeons will still need to convert to a TPK in the event of perforation, meaning that the advantage of using preserved donor material is lost.

LK has been compared to TPK in two retrospective studies for fungal keratitis.[85, 95] An early study from India in the 1960s concluded that “lamellar keratoplasty invariably fails, because the fungus is able to penetrate Descemet’s membrane” after it found that 6/7 LK cases developed re-infection, compared to only 1/10 cases in the TPK group.[95] However, a recent retrospective study of 94 LK cases and 161 TPK cases from China using modern surgical techniques found no difference in recurrence rates between groups in terms of recurrence, whilst immune rejection rate was significantly lower in the LK group (1.1% vs 18.6% , $p<0.001$), along with secondary glaucoma ($p=0.018$). There was no difference in visual acuity or refractive outcome between groups.[85] Another comparative study conducted for infective keratitis cases (including non-fungal cases) found no significant difference between LK and TPK in terms of therapeutic success ($p=0.74$), whilst LK patients had a significantly greater mean improvement in visual acuity compared to TPK patients (7.27 lines versus 4.76 lines, $p=0.01$).[96]

Whilst these results suggest a potential role for LK in the surgical management of superficial medically recalcitrant FK, large-scale prospective studies are needed to evaluate this more definitively. LK may prove useful in settings where human donor corneal material is

unavailable, but this needs to be balanced against the more technically challenging nature of the procedure.

Amniotic membrane grafts and conjunctival flaps

The amniotic membrane is the innermost layer of the placenta, consisting of a single layer of epithelium, a thick basement membrane, and an avascular stromal matrix.[97] It exhibits a diverse range of beneficial biological properties, including anti-inflammatory and antimicrobial, as well as promoting wound healing.[98] Unlike corneal tissue, amniotic membrane donor tissue is widely available, easy to store, and not prone to graft rejection. As a result, it has been used to treat a wide range of ocular surface disorders including microbial keratitis, chemical eye injury, corneal perforation, and limbal stem cell deficiency, amongst others.[98-100]

In managing microbial keratitis, amniotic membrane grafts (AMG) have typically been used as second-line therapy to promote corneal healing in cases of persistent epithelial defect following the sterilisation phase. However, there is emerging evidence from a recent systematic review and meta-analysis that there is a clear benefit in early adjuvant AMG in terms of more rapid corneal healing and improved visual outcome for moderate-to-severe fungal keratitis.[100] This review highlighted two RCTs and one non-randomised controlled study that compared AMG to standard antimicrobial treatment,[101-103] and calculated the pooled estimate of the time to complete corneal healing to be 6.90 days faster in the AMG group (mean difference − 6.90 days; 95% CI − 11.58 to − 2.21; P = 0.004).[100]

Conjunctival flaps are often used in LMICs and particularly sub-Saharan Africa (SSA) for recalcitrant fungal keratitis as it is a relatively straightforward, low-cost procedure and does not require any donor tissue. It is believed that healing is promoted by placing a vascular bed over the ulcer, as well as controlling infection and protecting against small perforations.[104, 105] Despite conjunctival flaps often being used, there is limited published work regarding their efficacy. There has been one randomised controlled trial that compared AMG to conjunctival flaps and found no difference between arms in terms of re-epithelialisation time, persistence of infection, complications and visual improvement.[106] Conjunctival flaps have also been used in addition to AMG. One case series from China of 17 patients with FK refractory to medical treatment who underwent conjunctival flaps in addition to AMG found that the globe was preserved in 15/17 (88%) of cases, whilst there were no cases of raised intraocular pressure.[107] Conjunctival flaps may therefore be an appropriate treatment – either alone or in addition to AMG – for severe recalcitrant FK without large perforations.

Corneal collagen cross-linking

Corneal collagen cross-linking (CXL) is a well-established technique that is typically used in preventing progression of corneal ectasias such as keratoconus and pellucid marginal degeneration. In brief, CXL involves ultraviolet-A irradiation of the cornea primed with riboflavin (vitamin B2, a photosensitiser), resulting in the formation of oxygen free radicals which then form covalent bonds between the stromal collagen fibrils, increasing the corneal biomechanical stability. Recently, CXL has been considered for the treatment of FK.[108] There are several claimed mechanism of action including:

- Direct anti-microbial action by damaging the pathogens' DNA / RNA.[109]
- Increasing the resistance to the micro-organisms' enzymatic destruction of the corneal stroma.[110]
- Enhanced corneal penetration of antifungals.[111]

However, the evidence for this is limited with heterogenous protocols and conflicting results. There have been four RCTs to date comparing CXL and medical treatment to medical treatment alone. The first study from India of deep, recalcitrant FK cases was ended early after significantly more patients in the CXL perforated than in the medical arm.[112] A second study from China showed better results for the CXL arm, but this was a small trial and the baseline severity of the cases was not reported.[111] The CLAIR trial, a larger, more recent RCT from India, found that patients who underwent CXL plus topical therapy had significantly higher culture re-positivity at 24 hours and worse vision at 3 months, regardless of whether they received natamycin or amphotericin B as topical therapy.[110] A subsequent analysis concluded that the reason for the worse vision in the CXL group was due to corneal scarring and astigmatism.[113] These findings are in contrast to another recent trial from India of superficial cases, that found patients treated with CXL healed faster and had better final vision. However, the study was small and unmasked, whilst this analysis was unadjusted for baseline characteristics.[114] These trials have been included in a systematic review and meta-analysis investigating the role of CXL in infectious keratitis, with a subgroup analysis specifically for fungal keratitis performed following the publication of the CLAIR trial.[115, 116] This found that CXL did not confer any additional benefit or harm in terms of infiltrate size or adverse events, although an unequivocal recommendation regarding CXL use could not be made due to the insufficient number of trials and uneven covariate distribution. Taken together, these studies suggest that there is no role for CXL in severe, deep-seated FK due to the increased risk of perforation, whilst there is limited evidence for any benefit in superficial cases.

Argon laser for fungal keratitis

There have been a few case reports on the use of argon laser as adjunctive treatment for refractory fungal keratitis unresponsive to topical and systemic therapy.[117] Argon laser irradiation is performed using argon blue-green wavelengths, a spot size of 500μm, pulse duration of 0.10 seconds, and power ranging from 500 to 900 mW until blanching is observed of the stroma, together with small cavitations that reach the mid-stroma.[117] It is postulated that argon laser may have a similar mechanism of action to corneal epithelial debridement, enhancing the penetration of antifungals.[117] In addition, it may be fungicidal as a result of the thermal effect on the infected tissue,[117] as the temperature rises to over 90°C when treated.[118]

There have been two small RCTs conducted that included argon laser as a treatment arm: one that compared argon laser to intrastromal voriconazole injection,[119] and the other comparing argon laser to AMG.[120] Both included patients that were not responding to topical therapy by day 7. Both trials showed a significantly faster healing time with argon laser. There was no statistically significant evidence of a difference in vision between the two groups.[119, 120]

Whilst these studies show some promise for the role of argon laser, larger, more robust RCTs are warranted to investigate this further before the use of argon laser can be recommended routinely for treatment-refractory cases of FK; at present it remains experimental.

Management strategy

Developing standardised guidelines applicable to all settings for the management of FK is challenging, due to in part to differences in what treatment options and facilities are available, the prevailing fungal organisms, and cultural factors. Effectively evaluating any management strategy is also difficult. In spite of this, the so-called TST Protocol (Topical, Systemic and Targeted Therapy)[57] has been developed based on the evidence outlined above, with the authors reporting the outcomes after four years of implementation on 223 cases (**Figure 10**). This protocol recommends initial monotherapy with natamycin 5% hourly for 48 hours, then two-hourly during waking hours until complete re-epithelialisation. At this point the dose is reduced to approximately four times a day for a further three weeks. Cycloplegia and pain relief are given. Patients with deep (>50% stromal depth), large (>5mm) ulcers are prescribed a systemic anti-fungal in addition (oral KCZ; administered 200 mg administered twice daily with meals or oral VCZ; 200 mg twice daily 2 h after a meal). If there is poor response by day 7-10 after starting treatment, then topical voriconazole 1% is added, following the same dosing regime as natamycin. If patients were still responding poorly after a further 7-10 days, then

intrastromal and/or intracameral injections of anti-fungals were performed. These are repeated up to a maximum for four injections, 72 hours apart. Patients still not-responding despite targeted therapy, patients with significant corneal thinning contraindicating intrastromal injections, or those with frank corneal perforations undergo TPK. The treatment "success" rate in this group (healing without perforation or requiring TPK) was 79.8% overall, i.e. 20.2% of patients required TPK. This compares to 16% and 43.8% in MUTT 1 and 2 respectively.[12, 13] Treatment success was 89% in patients receiving intrastromal voriconazole, compared to 63.1% in the medical management group. There was no comparative arm to this study, limiting conclusions, but it does highlight a potential rational, step-wise approach to managing FK.

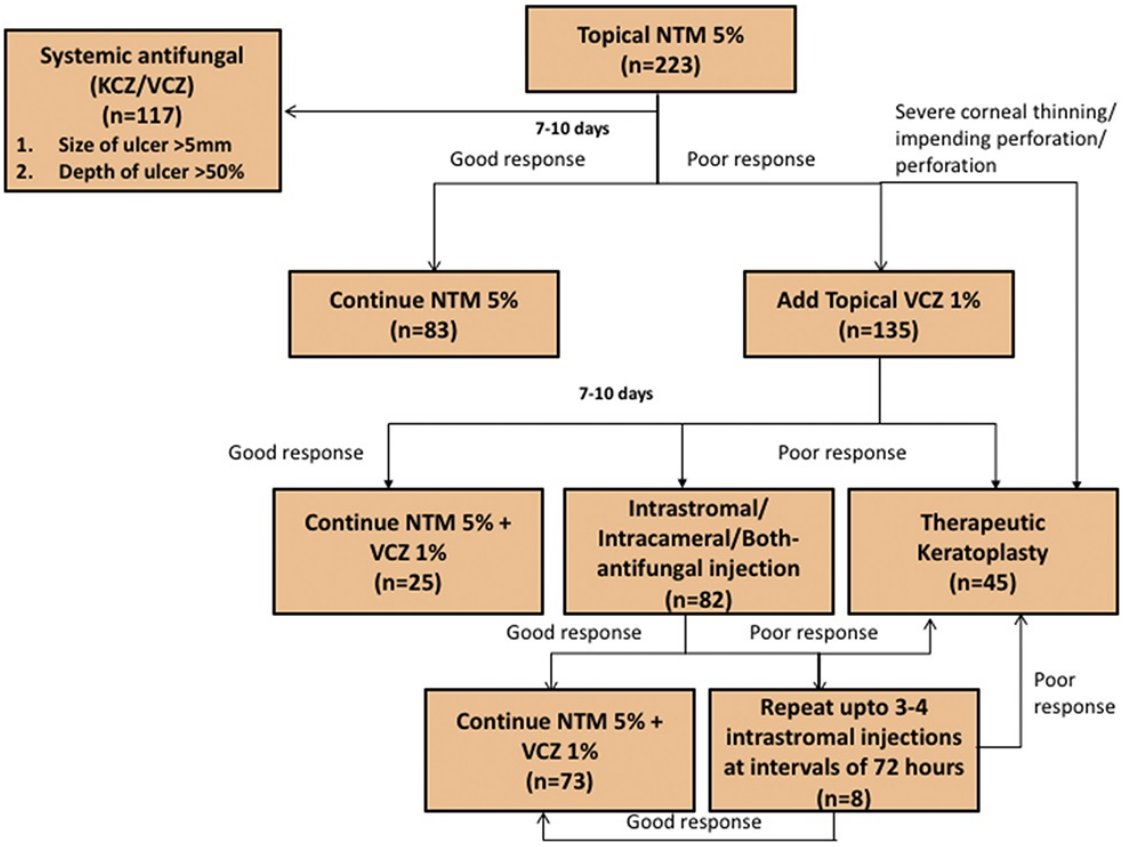

Figure 10: Tropical, Systemic and Targeted Therapy (TST) protocol for the management of fungal keratitis. Reproduced from Sharma N, Sahay P, Maharana PK, Singhal D, Saluja G, Bandivadekar P *et al.* Management Algorithm for Fungal Keratitis: The TST (Topical, Systemic, and Targeted Therapy) Protocol. *Cornea* 2019; **38**(2)**:** 141-145 with permission from Wolters Kluwer Health, Inc.[57] KCZ, ketoconazole; VCZ, voriconazole; NTM, natamycin.

In view of the above evidence, first line management of filamentary FK is usually with topical natamycin 5% when it is available. It was added to the WHO Essential Medicines List in 2017 for this indication. However, even when intensive topical natamycin is initiated, infections frequently progress relentlessly to perforation and loss of the eye in ~25% of cases, **Figure 11**.[12, 13, 20]

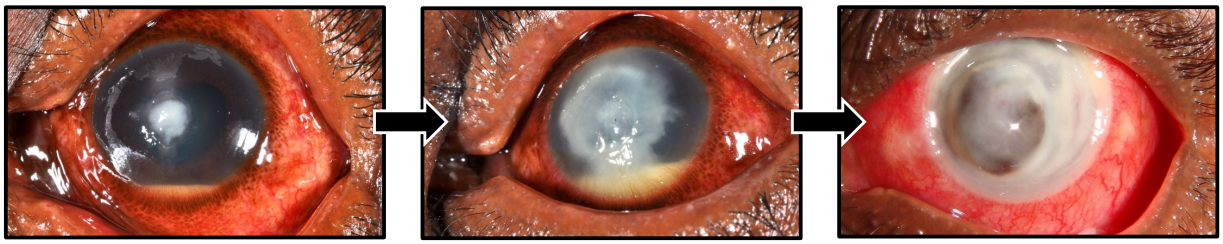

Figure 11: Progression of Fusarium fungal keratitis in a Tanzanian patient despite prompt treatment with topical natamycin 5%. First photograph, baseline presentation; second photograph, progression of infiltrate and increasing hypopyon at one week despite admission and intensive natamycin 5% treatment; third photograph, corneal perforation at three-weeks following presentation.

Additional and alternative drugs are clearly needed if the outcome of these infections is to improve. Moreover, in many countries antifungal eye drop treatments are simply not available. This includes most countries in SSA, some Asian countries and some countries in Europe. Natamycin is relatively expensive even if it is available.

Chlorhexidine for Fungal Keratitis

Chlorhexidine (CHX) is an antiseptic agent, with both antibacterial and antifungal properties. It is a widely-used broad-spectrum biocide, killing microorganisms through cell membrane disruption.[121-123] For example, chlorhexidine 0.2% w/v solution is very widely used as a long-term mouth wash for the prevention and treatment of oral candidiasis (a fungal infection) and for general oral hygiene.[124-126] Chlorhexidine 0.2% mouth wash is considered to be locally and systemically safe.

CHX has been used in ophthalmology for more than 30 years as an eye-drop preservative, sterilizing contact lenses, pre-operative topical antiseptic and for treating *Acanthamoeba sp.* and fungal keratitis.[127-132] It is very important to note that all chlorhexidine solutions used topically in ophthalmic practice are aqueous preparations: i.e. they do not contain any detergents or alcohol.

In a study evaluating potential affordable anti-fungal treatments for keratitis, chlorhexidine digluconate was compared *in vitro* with propamidine (Brolene), povidone iodine and polyhexamethylene biguanide (PHMB).[133] Several concentrations of these agents were tested against a panel of 95 fungal keratitis isolates from Ghana and India. The chlorhexidine 0.2% gave the best results *in vitro*, with inhibition of 90/95 isolates. The investigators then conducted a pilot case series study in India of chlorhexidine digluconate 0.2% in 11 patients with fungal keratitis (7 non-severe and 4 severe cases). They found that 10/11 cases healed on CHX; one severe case did not respond. The study also included a non-randomised comparison group of

8 patients with fungal keratitis (7 non-severe, 1 severe) who were treated with topical econazole (a frequently used treatment at that time). They reported that 7/8 responded to econazole; the severe case did not respond to the econazole.

Subsequently two pilot RCTs of CHX for fungal keratitis were conducted. In the first trial, involving 60 patients conducted in south India, three chlorhexidine gluconate concentrations (0.05%, 0.1%, 0.2% w/v) were compared to each other and to natamycin 5%.[128] There was evidence suggestive that chlorhexidine 0.2% might be better than natamycin 5% both in terms of the proportion showing a favourable response by 5 days (75% vs. 44%) and cure by 21 days (83% vs. 50%). The CHX 0.2% performed better than both the 0.05% and the 0.1% concentrations. The chlorhexidine 0.2% w/v concentration used in this trial is the same as that is used in mouthwash; and is systemically safe for oral mucosal application.

In the second trial, involving 70 patients conducted in Bangladesh, CHX 0.2% was compared to topical NATA 2.5% (half standard concentration). There was evidence CHX 0.2% was associated with a favourable response in more cases than NATA 2.5% by 5 days (89% vs. 51%; RR=0.23, 95%CI 0.09-0.63).[129] By 21 days 44% of the CHX treated group were cured compared to 28% of the NATA group.

Overall, a Cochrane systematic review of treatments for fungal keratitis found a non-significant trend favouring CHX over NATA in "curing" by 21-days (RR=0.70, 95%CI 0.45-1.09), when the data from these two trials was combined, **Figure 12**.[5]

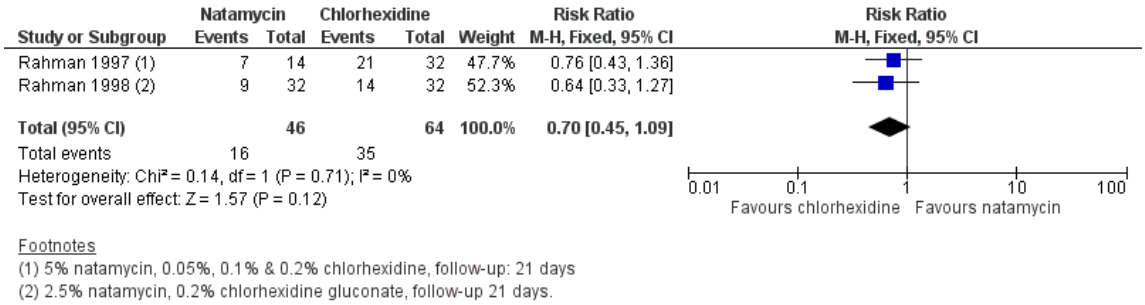

Figure 12: Forest plot of topical natamycin 5% versus chlorhexidine 0.2% (outcome: clinical cure). Reproduced from FlorCruz NV, Evans JR. Medical interventions for fungal keratitis. *The Cochrane database of systematic reviews* 2015; **4**: CD004241 with permission from John Wiley and Sons.[5]

In the first RCT from India, no toxicity effects were observed. In the second RCT from Bangladesh both CHX 0.2% and NATA 2.5% were well-tolerated; no treatment was discontinued because of allergy or toxicity. One patient in the chlorhexidine arm developed short-lived punctate corneal epithelial changes, which resolved when the drop frequency was

reduced. This a common finding when many different antibiotic drops are used very frequently. There was no early cataract development up to one year. Overall, CHX is safe and well-tolerated at these concentrations when used as a topical treatment for corneal infections.[128, 129, 132, 134] There is also extensive experience from using topical CHX for other indications. For example, CHX is applied to the ocular surface for antisepsis before giving intravitreal injections. A case series from Australia of 40,535 intravitreal injections which used CHX 0.1% or 0.05% for antisepsis reported that it was well tolerated, with only one suspected mild local allergic reaction noted.[135]

There have been several reports in the dermatology surgery literature of corneal toxicity following the use of skin antisepsis solutions containing chlorhexidine (concentrations 0.5 – 4%) applied to the face.[136-139] However, in all of these cases the solutions contained alcohol and detergent, which are known to be harmful to the eyes and were the likely cause of the toxicity. Solutions containing alcohol or detergent must not be used near the eye as these are harmful.

It is important to note that the chlorhexidine 0.2% eye drop solution used in the earlier trials and in clinical practice only contains water and no excipients.

CHX is used for treating fungal MK in the UK, EU, US and SSA.[6, 20, 127] We use CHX for this indication, and have seen responses in eyes deteriorating on NATA. In our experience it is usually well-tolerated by patients.

Management of fungal keratitis in Nepal and associated challenges

There are very few published reports on management strategies from Nepal. Experience from our collaborating institution in south-eastern Nepal (Sagarmatha Choudhary Eye Hospital) is that natamycin 5% is used as first-line monotherapy for confirmed cases of fungal keratitis, together with prophylactic topical moxifloxacin four times daily and cycloplegia. Similar to what is recommended in the TST Protocol,[57] patients who fail to respond after 7 days are commenced on topical voriconazole 1%. Ulcers that are more than 50% deep may receive oral antifungals. TPK is performed for cases of corneal perforation.

Thanks to the generic production of natamycin by several Indian pharmaceutical companies coupled with ease of import into Nepal, compared to other LMICs (in particular those in SSA) natamycin 5% is relatively easily available and costs approximately 150 Nepali Rupees for a bottle (approximately £1.50). Although this sounds reasonable, given the average daily wage is only £1.80,[140] this can still be unaffordable for many people. There have also been instances

where the supply of natamycin from India is halted, due to political tensions between the two countries, strikes, or border restrictions resulting from global pandemics.

Conclusions

Fungal keratitis is challenging to treat. The current first-line treatment for filamentous fungal keratitis is with topical natamycin 5% monotherapy, supported by a robust evidence base. Additional agents can be used either topically, as targeted injections, or systemically in more challenging cases, but evidence for their benefit is less established. There remains a clear role for surgical intervention usually in the form of therapeutic penetrating keratoplasty in eyes that have perforated; whilst earlier surgical intervention may be helpful but requires further research before clear recommendations can be made.

Unfortunately, there remain significant challenges for the management of fungal keratitis, largely attributed to the fact that most patients who suffer with this blinding condition are some of the poorest in the world, frequently neglected and marginalised. Early anti-fungal treatment is hampered by poor access to services and inappropriate treatment with traditional medicine and steroids. Compounding this, natamycin is frequently unavailable, or if it is, unaffordable. Alternative agents are therefore warranted, with a clear need for well-designed clinical trials to provide definitive evidence for their use.

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Chapter 3: Research Setting

Visual acuity testing station at Sagarmatha Choudhary Eye Hospital, Nepal

Geography of Nepal

Figure 13: Political map of Asia, showing location of Nepal relative to neighbouring China and India. Reproduced with permission from Nations Online Project.[1]

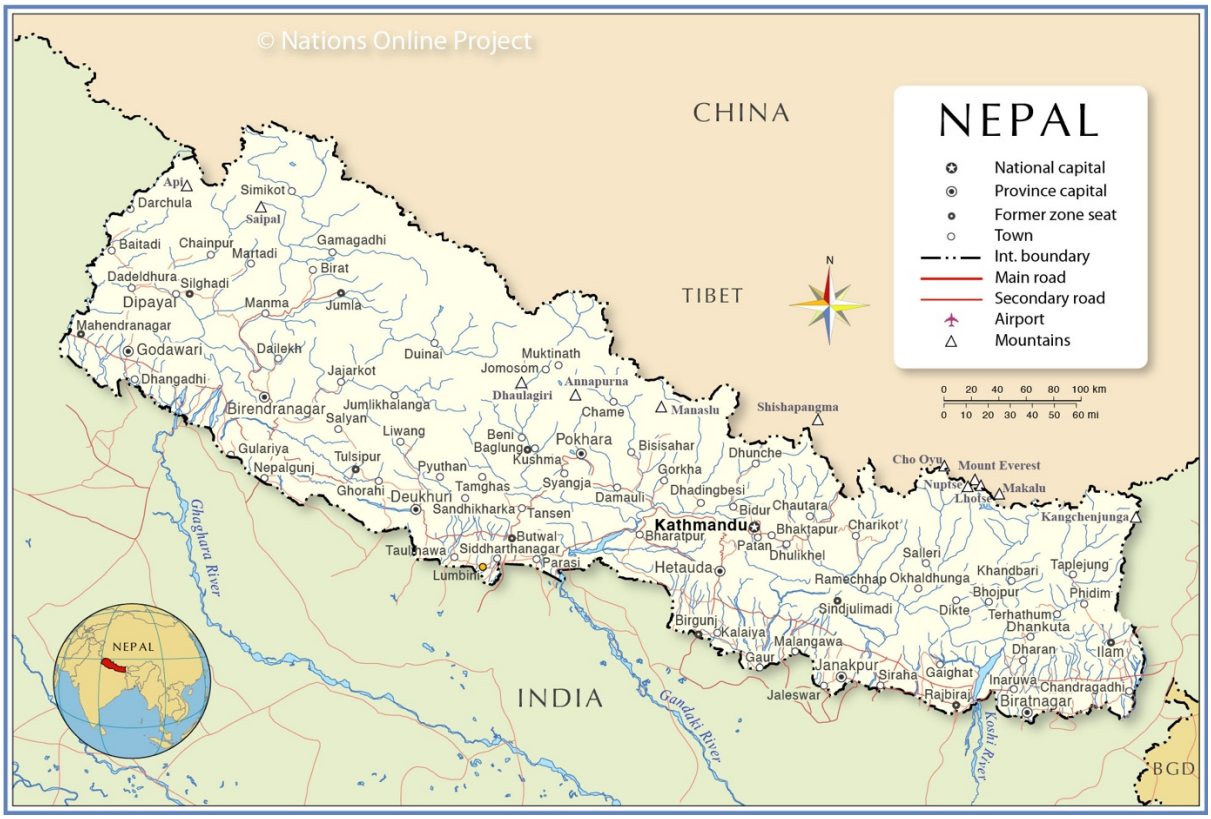


Figure 14: Political map of Nepal. Reproduced with permission from Nations Online Project.[1]

Nepal is a landlocked, trapezoidal-shaped country in South Asia, sandwiched between the Tibet Autonomous Region of China to the north, and India to the south, west, and east. It is approximately 800km long and 200 km wide, with an area of 147,181km^2, lying between latitudes 26° and 31°N, and longitudes 80° and 89°E (**Figure 13** and **Figure 14**).[2] Although it does not share any borders, Nepal is geographically close to Bangladesh (separated by the Siliguri Corridor) and Bhutan (separated by the Indian state of Sikkim). Although famed for the Himalayas and home to eight of the world's ten tallest mountains, including Mount Everest, Nepal is in fact a geographically diverse country, consisting of three distinct physiographic belts known as Himal-Pahad-Terai: the fertile, low-land plains, known locally as the "Terai" at less than 250m above sea level situated to the south of the country and bordering India; the subalpine forested hills where it rarely snows, the "Pahad", of less than 4000m above sea level, where the capital Kathmandu is located; and the high mountainous, snow-containing "Himal" zone above this making up the northern part of the country (**Figure 15**).[3]

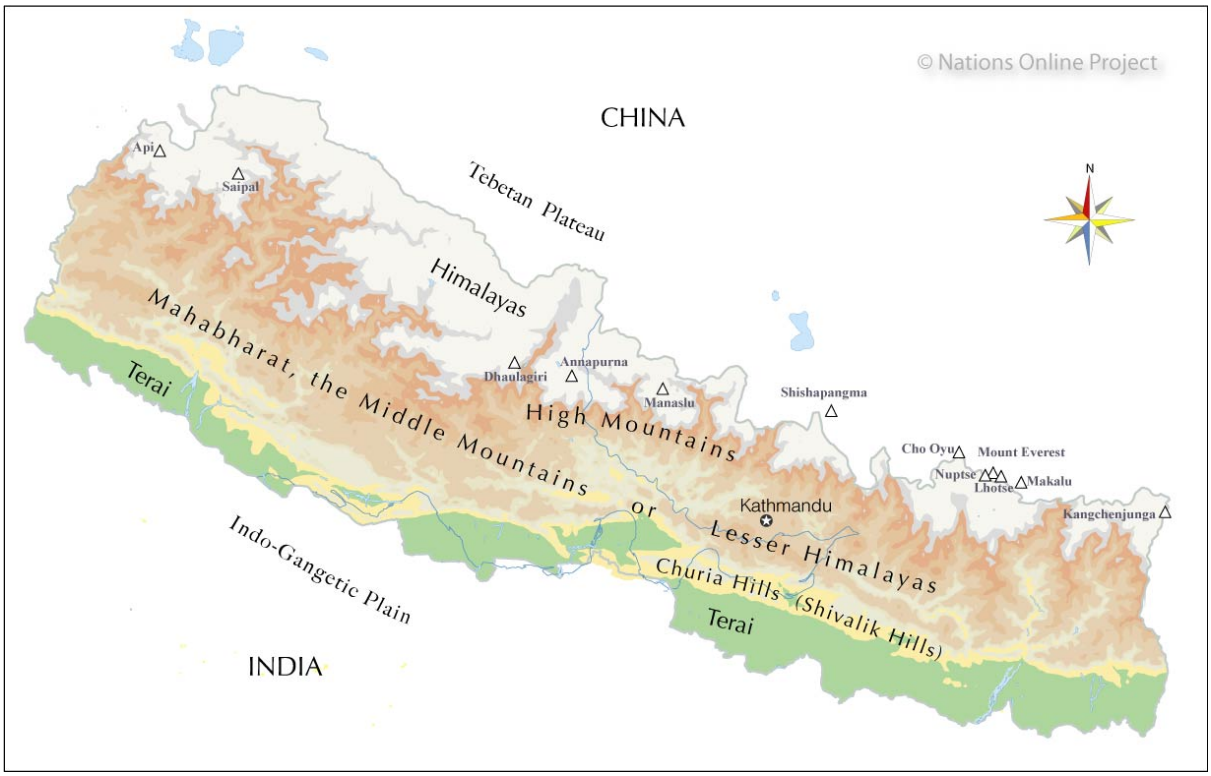


Figure 15: Nepal's geographic regions. The Terai are the fertile plains located in the south of the country, bordering India to the south. The Pahad zone (also known as the Mahabharat, Middle Mountains, or simply "hills" or "hilly" area) is located north of the Terai. The Himal or Himalaya Zone is located to the north of the country and borders Tibet. Reproduced with permission from Nations Online Project.[1]

There are five climatic zones within Nepal that correspond to altitude:

- Tropical and sub-tropical zones: below 1,200m
- Temperate zone: 1,200m to 2,400m
- Cold zone: 2,400m to 3,600m
- Subarctic zone: 3,600m to 4,400m
- Arctic zone: above 4,400m

In addition, there are five seasons: spring, summer, monsoon, autumn, and winter. Most of the rainfall occurs during the monsoon season.[3]

Demographics of Nepal

The population of Nepal was reported as 29.19 million in the 2021 census, with a growth rate of approximately 0.93% per year.[4] The median age in Nepal is 25.3 years (**Figure 16**), with a life expectancy at birth of 72.4 years.[2] The majority of Nepali people live in the central Pahad zone, whilst the Terai have experienced a significant amount of immigration in recent years, both from within Nepal and from neighbouring India.[5] It is one of the ten least urbanised countries, whilst conversely being one of the ten fastest urbanising countries, with high rates of urbanisation occurring in the eastern Terai and Kathmandu Valley.[5]

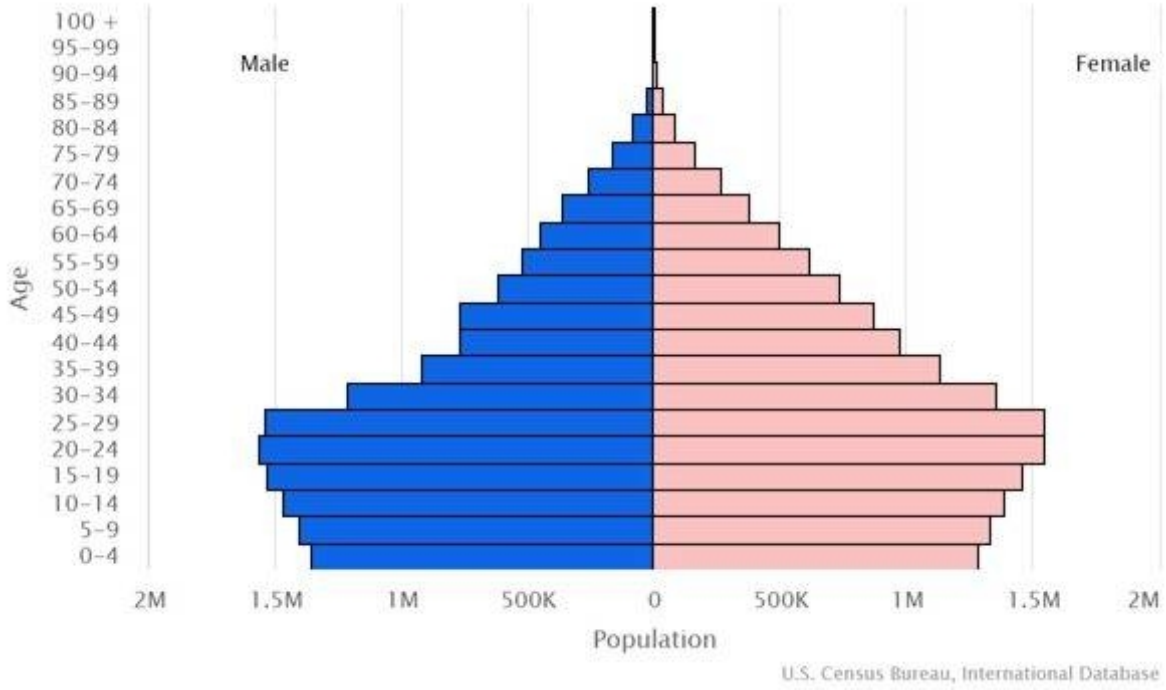


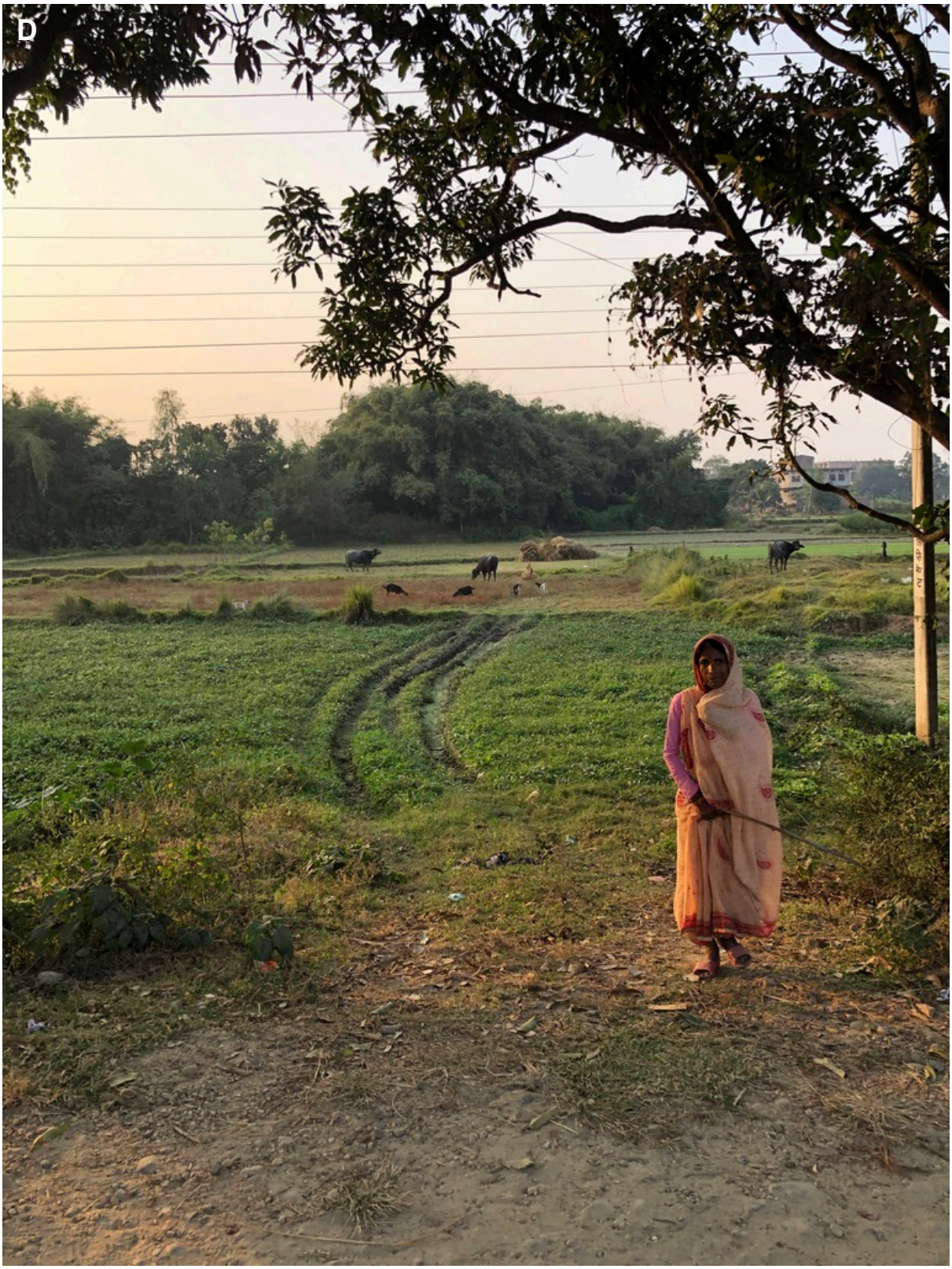
Figure 16: Population pyramid of Nepal, 2021. U.S. Census Bureau International Database. Available from *The World Factbook* 2021. Washington, DC: Central Intelligence Agency, 2021.[2]

Nepal's economy is heavily dependent on agriculture, with 76% of the workforce employed in agriculture, but providing 31.7% of the country's gross domestic product (GDP) (**Figure 17**).[2, 3] Rice, wheat, fruits and vegetables are the main food crops, with the fertile lowland Terai region producing a surplus of food that is sold elsewhere in the country (**Figure 17F**). Only 20% of the total area of Nepal can be cultivated, most of this is found within the Terai.[3] Nepal is a lower-middle income economy, with 25.2% living below the poverty line of US$1.90 per day.[2] Nepal ranks 186th in the world (out of 229 states) in terms of real GDP per capita ($1,380).[2]


A


B


C


D


E


F

Figure 17: A: Arable agriculture in the Terai during the harvest season; B: View across the Terai, with Pahad hills in the distance; C: Subsistence farming in the Terai in 2018, with a man riding an Ox; D: Subsistence farming on the outskirts of Lahan, in the Terai zone; E: Typical rural housing in the Terai; F: Produce grown in the Terai being sold elsewhere in Nepal; G: Paddy fields in the Terai following the monsoon rains

Madhesh Province (formerly Province No. 2)

Until the establishment of federalism and the adoption of the new constitution in 2015, Nepal was subdivided into fourteen administrative zones. Since 2015, this system was replaced by provinces, formed by grouping together existing districts; Nepal is now therefore made up of seven provinces (**Figure 18**).[6]

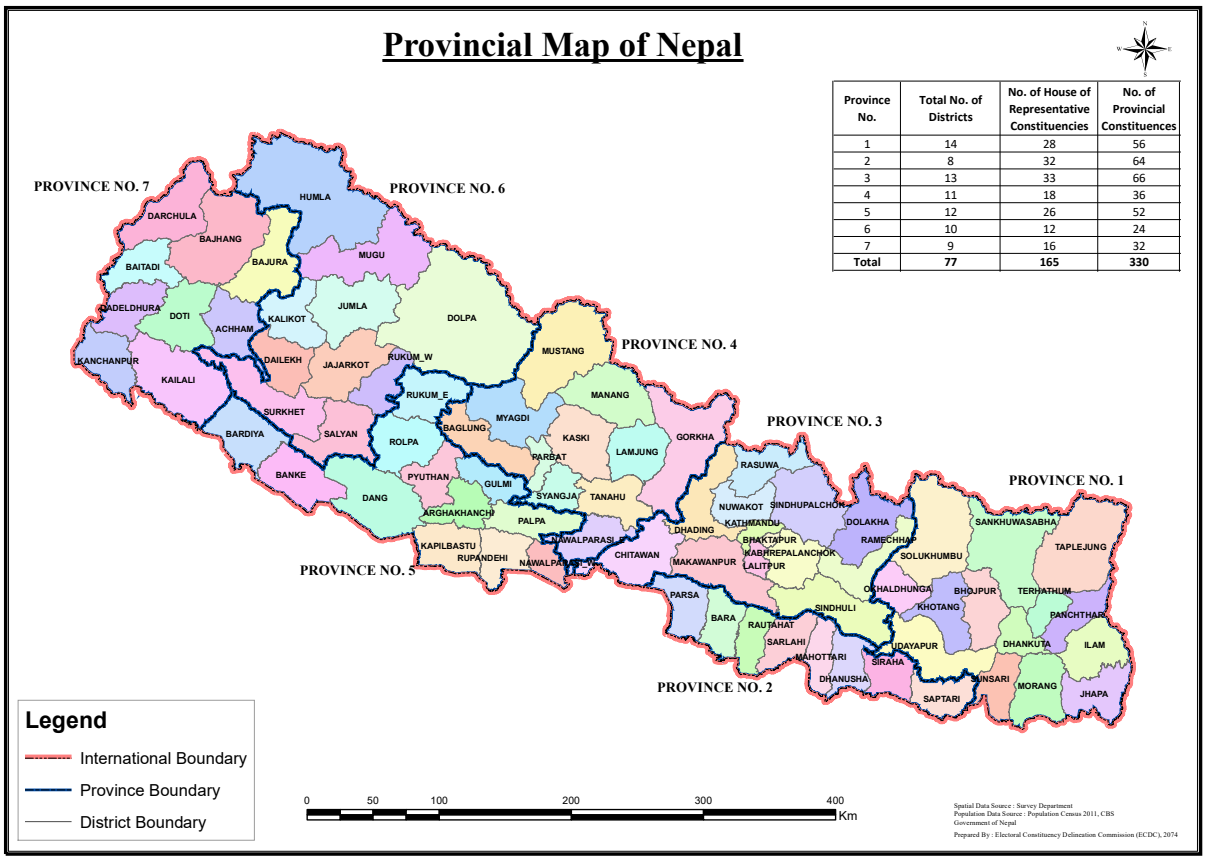


Figure 18: Provincial map of Nepal. Reproduced with permission from the Election Commission, Nepal

Madhesh Province (formerly Province No. 2), in the south-eastern region of Nepal, is the smallest province by area (9,661 km2) but the country's most populous (6.1 million). It is located in the flat plains of the Terai, bordered to the north by the Shiwalik Hills and Bagmati Province, the east by the Koshi River and Province No. 1, the west by Chitwan National Park and Bagmati Province, and to the south by the Indian state of Bihar (Figure 6). It is formed of eight districts, from the Saptari District in the east, to Parsa District in the west. Madheshis are the largest ethnic group in the province (**Figure 19**), with the Yadav caste the largest group among the Hindu Madhehsis at 14.8% of the population.[4] Madheshis share many cultural traditions, educational and family ties with people living south of the international border in the neighbouring Indian state of Bihar. The literacy rate of this region is 52.3% and most of the population are subsistence farmers or agricultural workers.[4]

Figure 19: Three Nepali Madhesi women in Lahan, Terai zone, during the celebration of a Hindu festival.

Nepali Health System

Nepal has a mixed health service delivery system that includes the public sector, non-governmental organisations (NGOs), and private-for-profit sector. The public sector, governed by the Ministry of Health (MOH), predominantly serves the rural poor, whilst private providers tend to cater for the urban populations.[7]

The MOH develops and standardises health policy at the central level. Public health services are then delivered at the five lower levels, from the regional to the community (**Figure 20**).[7] The community level includes the two political levels (administrative district, of which there are 75, and electoral constituency), the village development committee (VDC) and the ward. Services at the VDC are delivered through health posts (HPs) or primary health clinics (PHC), which are staffed by auxiliary health workers (AHWs) and auxiliary nurse midwifes (ANMs), paid health workers who are employed by the MOH. These cadres will have received between one year and 18 months of training, with no provision for ophthalmic training. At the ward level, services are provided in the community by female community health volunteers (FCHVs). Each level of the public health system is linked by a formal, coordinated referral system.[8]

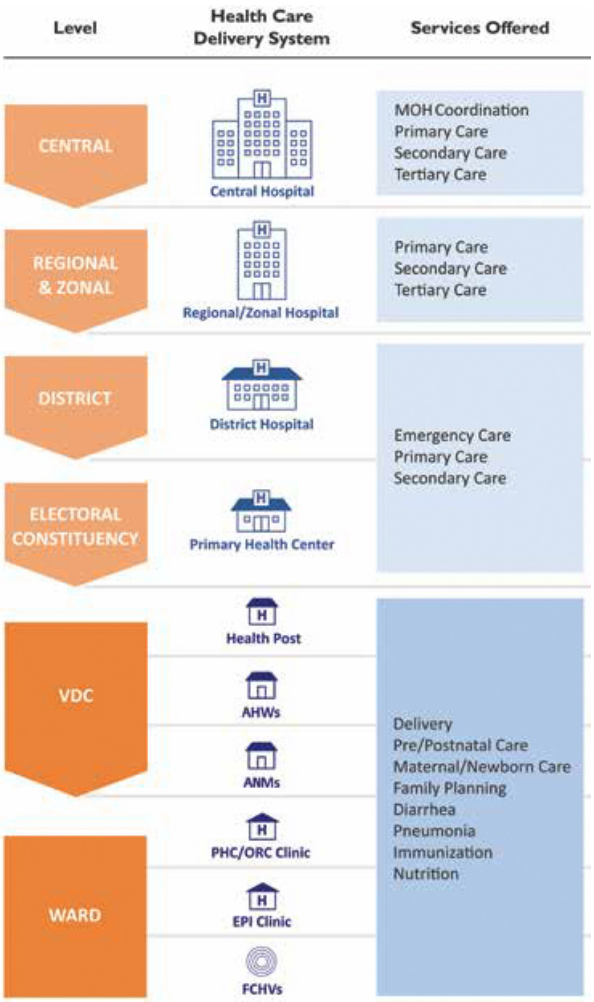


Figure 20: Structure of the Nepali Health System. VDC, village development committee; AHW, auxiliary health worker; ANM, auxiliary nurse midwives; PHC/ORC, primary health care outreach centre; EPI, expanded programme of immunisation; FCHVs, female community health volunteers. Adapted with permission from Merchant HF, Devlin K, Egan KF *Nepal's Community-based Health System Model: Structure, Strategies, and Learning*. Advancing Partners & Communities: Arlington, VA; 2016.[7]

In addition to the public health system described above, patients seek primary health care from many, often unregulated, private providers including pharmacies and traditional healers.

Eye Care in Nepal

Nepal has a well-established, integrated eye care system. Despite being one of the poorest countries globally, Nepal has developed a well-functioning model with an impressive track record. Following the first national blindness prevalence survey in 1981 and the subsequent expansion of eye care services, the prevalence of blindness in the country has significantly decreased.[9, 10] This is largely thanks to the establishment of Nepal Netra Jyoti Sangh (NNJS, the National Society for Comprehensive Eye Care) in 1978, at which point there were only

three ophthalmologists working outside of Kathmandu.[9, 10] NNJS is supported by the Government of Nepal with the remit of providing ophthalmic services across the country. Currently 90% of all eye services in Nepal are delivered through NNJS-affiliated NGO providers, with most eye hospitals located within the Terai. There are now 254 ophthalmologists throughout the country, working at 35 secondary or tertiary eye hospitals, and supported by 96 community eye care centres (ECCs), available across 11 of the 14 Administrative Zones.[11, 12] This equates to approximately 8.4 ophthalmologists per million population. The ECCs are usually affiliated to an eye hospital and exist as satellite clinics that can diagnose, manage and refer eye conditions as appropriate. They are staffed by ophthalmic assistants, optometrists and eye health workers. There is approximately one ECC per district. There is currently no provision for eye care at the electoral constituency, VDC, or ward level, meaning that access to primary eye care is limited.[13]

Eye care in Madhesh Province

Eye care in Madhesh Province is managed by the NNJS-affiliated Eastern Region Eye Care Programme (ERECP), which includes one tertiary eye care hospital (Sagarmatha Choudhary Eye Hospital, SCEH) and 10 ECCs (**Figure 21** and **Figure 22**). Founded in 1983 as a not-for-profit organisation, SCEH is one of the busiest eye hospitals in the country. SCEH is located in the Terai town of Lahan in the Siraha District of Madhesh Province (formerly the Sagarmatha Zone) on the East-West Highway, approximately 18km from the Indian border. As a result, between 30-50% patient of patients are Indian nationals.[14]

Figure 21: Signage at the entrance to SCEH indicating the locations of satellite eye care centres (ECCs).

Figure 22: Staff members stand outside the entrance to an ECC in Madhesh Province

Outside the NNJS programme, other than a few private providers in the main cities including Janakpur, there are no other formal ophthalmic services. Many patients with eye conditions initially seek treatment from local pharmacists, traditional healers, PHCs, and HPs. Interestingly, 69% of patients attending SCEH with microbial keratitis initially visited a primary care level worker (including traditional healers and pharmacies) first, whilst only 14% had attended a secondary care provider such as an optometrist initially.[15]

Epidemiology of eye disease in Nepal

Conducted between 1979-1980, the first national blindness survey in Nepal found the prevalence of blindness (BSCVA <3/60) to be 0.84%, with cataract the leading cause (66.7%), followed by the complications of cataract surgery (5.3%).[9] The situation was reassessed between 2006 and 2010, with the prevalence of blindness (presenting visual acuity (PVA) <3/60) having reduced to 0.35%, with visual impairment (PVA <6/18 to ≥3/60) found to be 6.97%.[10] The leading cause of blindness remained cataract (65%), followed by retinal (9%) and corneal (6%) pathology.

Epidemiology of eye disease in Madhesh Province

The most recent survey of blindness to be conducted was the 2020 Rapid Assessment of Avoidable Blindness (RAAB), which was the first to occur following federalism and therefore the first to analyse blindness at the provincial level.[16] The results of this survey are yet to be published. Prior to this, the most recently published survey of blindness that included the districts surrounding SCEH was conducted in 2009, reporting on results from the Sagarmatha Zone. This found that 1.3% of the population were blind (PVA < 3/60) (**Table 3**), with the leading causes of blindness being cataract (66.7%), retinal disease (10.3%) and non-trachomatous corneal opacity (5.1%).[17]

As discussed in detail in Chapter 1, Nepal has some of the world's highest incidence of fungal keratitis, particularly in the Terai zone, with studies reporting between 61% and 70% of all microbial keratitis cases being fungal in aetiology.[18-20] Although these studies are from the neighbouring Province No.1, the geography and ethno-demographics of these locations is very similar to Madhesh Province. A retrospective audit from SCEH evaluating five years of microbial keratitis patient presentations found that fungal keratitis was responsible for 78.1% of cases.[15] Over these five years, there were on average 1874 cases per year of microbial keratitis, equating to over 150 cases per month. Assuming three quarters are fungal, then approximately 112 cases of fungal keratitis are seen at SCEH per month. This makes SCEH an ideal location to conduct research into FK.

Table 3: Blindness and visual impairment in Sagarmatha zone reported in the 2009 RAAB [17]

Vision category	**WHO Definition***	**Prevalence age 50+ (% (95% CI))**
Moderate visual impairment	<6/18 to 6/60	10.3 (8.4-12.3)
Severe visual impairment	<6/60 to 3/60	2.5 (1.8 – 3.3)
Blind	<3/60	1.3 (0.7-1.8)

* Presenting visual acuity

Fungal keratitis in Nepal

Although FK is of global concern, as discussed above it is a disease that predominantly affects LMICs in tropical and subtropical latitudes. Nepal is a country with one of the highest rates of fungal keratitis globally, with FK accounting for between 61-70% of microbial keratitis cases in the lowland plains region.[18-20] Interestingly, there is variation within the country in terms of prevalence of FK; bacterial keratitis is more common in urban locations or those at higher altitude, such as Kathmandu, where FK only accounts for 25% of cases.[21]

This has several implications for research. Firstly, given the high prevalence of fungal keratitis, it is a perfect setting to undertake studies investigating management options for fungal keratitis, such as clinical trials into the treatment. Secondly, research that leads to a better understanding of the barriers to accessing appropriate care within the country has the potential to lead to an improved health system and ultimately better outcomes for patients. Thirdly, it is a setting that shares many similarities with other countries where there is a high burden of FK, allowing results of research to be generalisable.

Research Partners

Sagarmatha Choudhary Eye Hospital

The mission for SCEH is to provide "high quality, high volume, affordable, and comprehensive eye care services" (**Figure 23**). Since it was founded in 1983 with only 12 beds, SCEH has grown considerably and has now treated over 11 million patients, with over 1.9 million surgical procedures performed (**Figure 24**). In 2021, despite some ongoing restrictions due to the COVID-19 pandemic, 889,335 patients were examined, with 102,186 surgeries performed (73,591 cataract operations including 44,216 small incision cataract surgery). In the same period, 133 penetrating keratoplasties and 105 amniotic membrane grafts were performed. Currently SCEH and its satellite clinics employ 466 members of staff including 16 ophthalmologists, one microbiologist, 37 ophthalmic assistants, 17 optometrists, and 48 eye health workers. Subspecialties covered include cornea, retina (medical and surgical), paediatrics, oculoplastics, and glaucoma.[14]

Figure 23: Entrance to Sagarmatha Choudhary Eye Hospital, Lahan

Figure 24: Patients queue for outpatients at Sagarmatha Choudhary Eye Hospital, Lahan

In 2018, a collaboration agreement was setup between SCEH, NNJS and the London School of Hygiene & Tropical Medicine. As part of this collaboration, a dedicated research team was recruited, equipment procured, and a dedicated microbiology laboratory created at SCEH, Lahan (**Figure 25Figure 29**). The microbiology laboratory at SCEH will perform smear microscopy, culture, and sensitivities.

Figure 25: The research team at SCEH, Lahan

Figure 26: A blessing took place after the delivery of the Corneal Research Project vehicle

Figure 27: Unloading of laboratory equipment for the new microbiology laboratory at SCEH

Figure 28: *In vivo* confocal microscopy in use at SCEH

Figure 29: A new microbiology laboratory was constructed at SCEH. This photograph was taken prior to the installation of the new equipment.

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Chapter 4: Overview of Study Design

The Corneal Ward at Sagarmatha Choudhary Eye Hospital, Lahan, where enrolled patients were admitted

Rationale for the study

In view of the limited efficacy and availability of eye drops to treat fungal keratitis (FK) there is a clear need for additional alternative treatments. The pilot trial data suggests that chlorhexidine (CHX) 0.2% has a similar effect to natamycin (NATA) and may even be superior.[1-3] However, the combined size of the two pilot trials comparing chlorhexidine and natamycin is not sufficient to reach firm conclusions; presently according to this meta-analysis there is clinical equipoise in terms of which treatment is best for treating fungal keratitis.

Chlorhexidine is cheap, stable and easily prepared by aqueous dilution. If chlorhexidine is found to be non-inferior (or even superior) to natamycin this offers the potential of an effective, affordable and accessible treatment for fungal keratitis, which could benefit millions of people each year who currently have no treatment options. This work is a response to this expressed need from both clinicians and patients for a readily available and affordable medication for fungal infection.

In order to answer the question of whether CHX is effective at treating FK definitively, full-scale trials of chlorhexidine 0.2% are warranted in regions with high burden of fungal keratitis, to provide the evidence for its use. Nepal, with a high burden of fungal keratitis, is one such region. There are various potential primary outcome measures that can be considered when designing a trial investigating corneal infection, including vision (typically best spectacle corrected visual acuity, BSCVA), healing time, proportion of cases healed by at a certain interval (and conversely proportion of patients perforating or requiring penetrating keratoplasty), and microbiological parameters such as proportion of cases remaining culture positive at day 7. As previous landmark corneal infection trials have used BSCVA as the primary outcome measure,[4-6] to aid comparison we would do the same. Three months is the time at which clinical experience suggests most corneal ulcers have usually healed. BSCVA is chosen as it is easy to measure and is of functional significance. The non-inferiority design of clinical trials is a clinically pragmatic way to compare two treatments that have additional benefits other than the primary effect of treatment, such as reduced cost or better tolerability. Therefore, to address this important question of whether CHX is a potentially useful weapon in the armoury against fungal keratitis, we chose to conduct a non-inferiority randomised controlled trial, with the primary outcome as BSCVA at three months. If CHX is found to be within the pre-specified non-inferiority margin of 0.15 logMAR (about 1.5 Snellen lines) then CHX may prove to be a sustainable solution for the treatment aspect of the complex problem of fungal keratitis.

Given this, the main purpose and focus of this PhD and associated research programme was to conduct a randomised, non-inferiority clinical trial comparing chlorhexidine 0.2% to natamycin 5% for the treatment of fungal keratitis in Nepal. The hypothesis we would be testing is that chlorhexidine 0.2% is non-inferior to natamycin for the treatment of fungal keratitis in terms of BSCVA at three months. We would also be comparing the two treatments in terms of several secondary outcome measures, including healing time, scar size, ulcer depth, hypopyon height, and proportion of patients perforating or requiring an emergency corneal graft. As discussed in Chapters 1 and 3, the Terai region of Nepal has amongst the highest proportion of fungal keratitis cases as a subset of microbial keratitis (MK) globally. Sagarmatha Choudhary Eye Hospital (SCEH), in the Terai, reports treating on average over 100 MK patients per month and has an established model of care,[7] making SCEH and ideal study site and collaborating partner to conduct this trial.

In addition to this overall purpose, in order to better understand fungal keratitis in Nepal and devise ways to better tackle it, we will be conducting several ancillary studies investigating the microbiology, aetiology, epidemiology, and diagnostic strategies of MK in Nepal.

Our hope is that what is discovered through this research will improve the management, and ultimately the outcome, of patients with fungal keratitis in Nepal and across tropical lower- and middle-income countries where FK is endemic in the following ways:

1. If chlorhexidine 0.2% is found to be non-inferior (or indeed superior) to natamycin 5% for treating fungal keratitis, then there will be robust evidence for a new treatment option that is not only effective but affordable, available, and easy to formulate, resulting in improved access to treatment for many people who currently do not have access to current first-line agents.
2. By better understanding what clinical signs are associated with fungal keratitis, improved early diagnosis and treatment could lead to better outcomes for patients.
3. Comparing the different diagnostic tools available (such as microscopy, culture and *in vivo* confocal microscopy, IVCM) can help units to allocate and rationalise resources appropriately, to allow for improved, faster diagnosis, and ultimately better clinical outcomes for patients.
4. Knowledge of the barriers to prompt presentation of patients with microbial and fungal keratitis in Nepal will assist stakeholders in designing and implementing strategies to improve early presentation.

5. Establishing the local causative organisms will help direct stakeholders to appropriate empirical therapy for the region and improve the understanding of why current strategies may be failing.

Research aims

The specific research objectives are given in **Table 4**.

Primary Objective:

To test the hypothesis that topical chlorhexidine 0.2% is non-inferior to topical natamycin 5% for treating fungal keratitis, in terms of visual acuity at three months.

Secondary Objectives:

1. To determine whether either treatment (chlorhexidine 0.2% or natamycin 5%) is superior to the other for treating fungal keratitis, in terms of vision at three months.
2. To determine whether there is a difference between chlorhexidine 0.2% and natamycin 5% in terms of secondary clinical outcomes: infiltrate / scar size, time to re-epithelialisation, re-culture rates at one week.
3. To determine the different genera and species of microorganism causing bacterial and fungal keratitis and to characterise their susceptibility patterns and the relationship to clinical outcomes.
4. To compare alternative methods of detecting fungal organisms in corneal infections (including *in vivo* confocal microscopy, light microscopy, and culture), relative to a composite reference standard.
5. To determine what clinical signs (if any) are associated with fungal keratitis.
6. To evaluate what factors can contribute to delayed presentation to appropriate care and the care-seeking pathway of patients with microbial keratitis.

Table 4: Specific research objectives

Study	***Specific objectives***	***Study Design***
Topical Chlorhexidine 0.2% versus Topical Natamycin 5% for the Treatment of Fungal Keratitis in Nepal	1. To design a suitable study to test the hypothesis that chlorhexidine 0.2% is non-inferior to natamycin 5% for the treatment of fungal keratitis in Nepal 2. To determine if topical CHX 0.2% is non-inferior to topical natamycin 5% for treating filamentous FK, in terms of vision at 3 months. 3. To determine whether either treatment (CHX 0.2% or natamycin 5%) is superior to the other, in terms of vision at 3 months 4. To determine whether there is a difference between CHX 0.2% and natamycin 5% in terms of secondary clinical outcomes including infiltrate/scar size, time to re-epithelialisation, and re-culture rates at 1 week	Randomised controlled, non-inferiority trial
Microbial Keratitis in Nepal: Predicting the Microbial Aetiology from Clinical Features	1. To report the aetiology of MK in patients presenting to SCEH in Nepal 2. To assess for an association between clinical signs and patient presentation and the aetiology of MK 3. To explore which clinical signs are predictive of FK or BK 4. To formulate a clinical score that can be used to make a predictive diagnosis of FK in the absence of further investigations 5. To further distinguish distinct mycological groups of FK based on clinical signs	Cross-sectional study nested within a prospective cohort study
Diagnosis of fungal keratitis in low-income countries: evaluation of smear microscopy, culture, and in vivo confocal microscopy in Nepal	1. To estimate the sensitivity in detecting fungal keratitis using in vivo confocal microscopy, smear microscopy, and culture in a setting with a high prevalence of FK. 2. To estimate the sensitivity of different smear microscopy stains in detecting fungal keratitis in a setting with a high prevalence of FK	Cross-sectional study nested within a prospective cohort study
Delay in accessing definitive care for patients with microbial keratitis in Nepal	1. To describe the presentation journey of patients with MK to SCEH in south-eastern Nepal and their demographics 2. To investigate factors associated with direct presentation. 3. To investigate factors associated with delayed presentation 4. To calculate the cost of care for patients and analyse for an association with number of visits	Prospective cohort study

Methods overview

The overall design of this study was a **randomised non-inferiority controlled trial** of patients with fungal keratitis presenting to Sagarmatha Choudhary Eye Hospital in Nepal. We designed a two-stage consent process to allow us to enrol non-fungal microbial keratitis patients into the research programme, to allow us to capture data on their demographics, clinical features and history, treatment pathway, and microbiology. This formed the basis of the **prospective cohort study**, that primarily evaluated the patients' journey and factors associated with delay. Nested within this, we were then able to conduct **nested cross-sectional studies**, that investigated clinical features associated with the microbiological diagnosis and sensitivity of the different diagnostic tools. Patients with fungal keratitis who were eligible for the main trial would then undergo a second, separate consent. **Figure 30** graphically presents how the project was conducted.

The design of the clinical trial is presented in detail in Chapter 7. **Figure 31** presents an overview of the clinical trial. In brief, we tested the hypothesis that g-chlorhexidine 0.2% was non-inferior to g-natamycin 5% in a two-arm, single-masked RCT. We used a pre-specified non-inferiority margin (Delta) of 0.15 logMAR units.

The sample size was calculated to be 500 participants with fungal keratitis. The presence of the infection was confirmed by fungal elements detected on smear microscopy and/or IVCM. Participants were randomised 1:1 to either topical chlorhexidine 0.2% or topical natamycin 5%. The treatment was given hourly for one week, then two-hourly for a further two weeks. Ongoing treatment duration was then tailored to clinical response. Follow-up assessments were conducted at 2 days, 1 week, 2 weeks, 3 weeks, 2 months and 3 months, to determine the outcome.

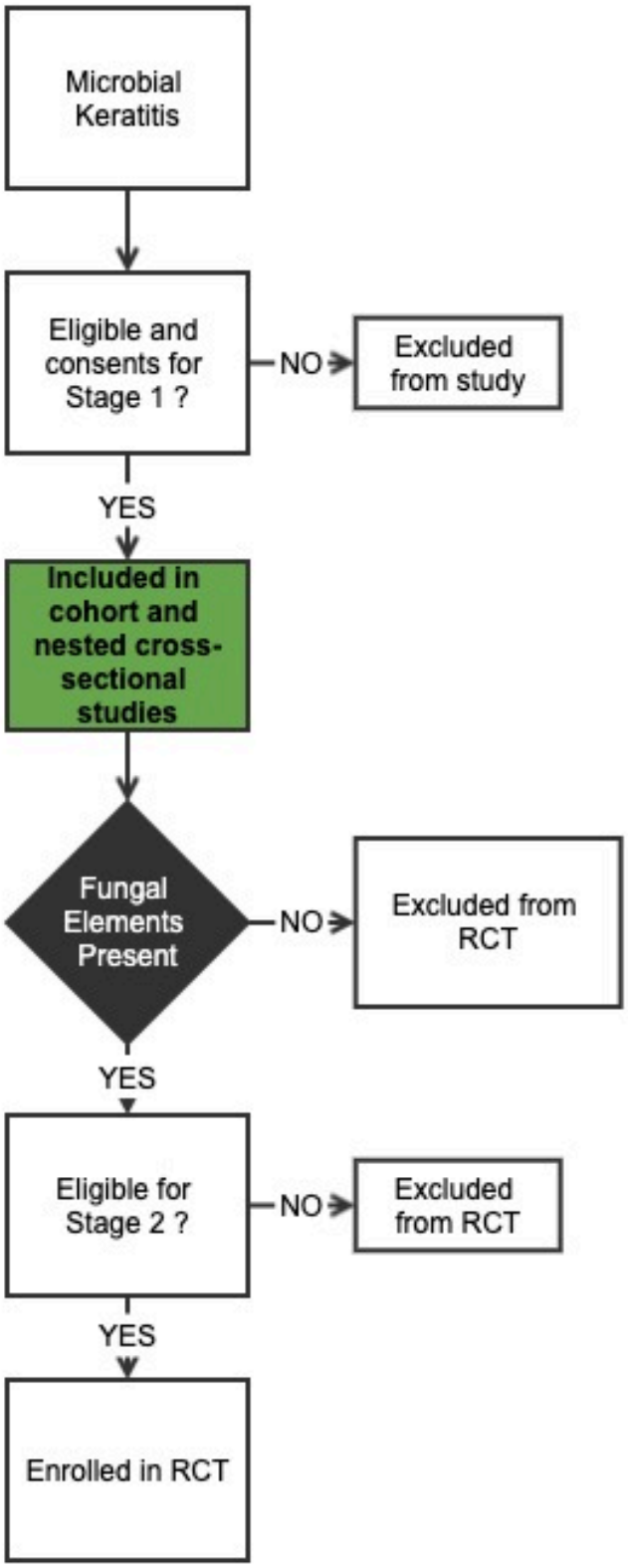

Figure 30: Overview of research project. All patients presenting with acute MK are reviewed and enrolled into stage 1, following written, informed consent. This involves history, examination, and investigations (corneal scrapes for microbiological assessment and in vivo confocal microscopy (IVCM)). If there is evidence of fungal hyphae on smear or confocal microscopy, patients then proceed to stage 2.

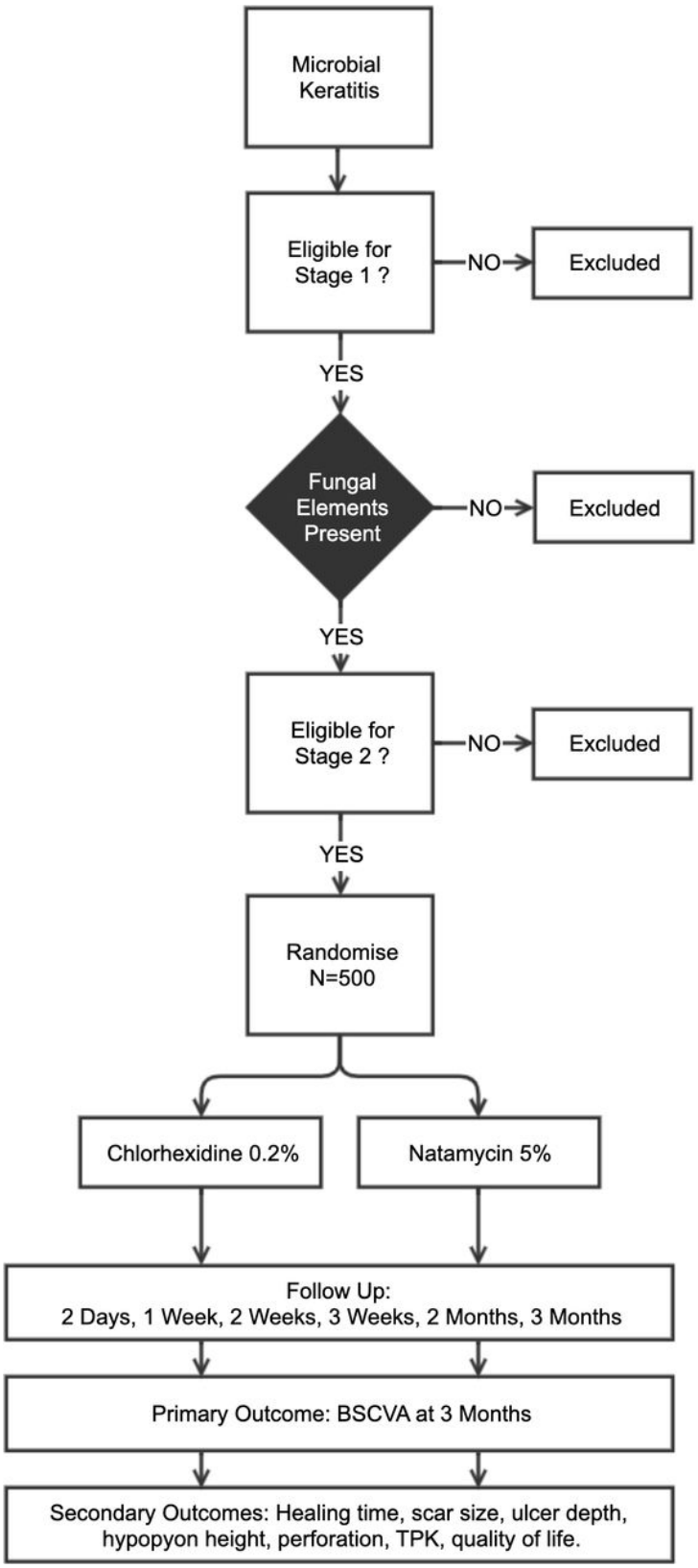

Figure 31: Overview of the clinical trial. Microbial keratitis is defined as presence of corneal epithelial ulceration (>1 mm in diameter), corneal stromal infiltrate and signs of acute inflammation (eg, conjunctival injection, anterior chamber inflammatory cells, hypopyon). Fungal elements were detected by smear microscopy and/or confocal microscopy. Those eligible were randomised 1:1 to CHX or natamycin (n=500). BSCVA, best spectacle corrected visual acuity; CHX, chlorhexidine; TPK, therapeutic penetrating keratoplasty.

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Chapter 5: Microbial keratitis in Nepal: Predicting the Microbial Aetiology from Clinical Features

Clinical examination of a study participant during the early stages of the COVID-19 pandemic in Nepal

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SECTION A – Student Details

Student ID Number	324283	**Title**	DR
First Name(s)	JEREMY JOHN STANTON LUNN		
Surname/Family Name	HOFFMAN		
Thesis Title	Managing Fungal Keratitis in Nepal: A Randomised Controlled Trial Comparing Chlorhexidine 0.2% to Natamycin 5%		
Primary Supervisor	PROF. MATTHEW J. BURTON		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	JOURNAL OF FUNGI		
When was the work published?	19th February 2022		
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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Conception of this work was mine. I wrote the entire first draft of the manuscript and revised it following comments from my supervisors. I designed the methodology, carried out the investigations and clinical examinations along with the co-authors, curated the data, and co-adminstered the project.

SECTION E

Student Signature	[redacted]
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Microbial Keratitis in Nepal: Predicting the Microbial Aetiology from Clinical Features

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Abstract: Fungal corneal infection (keratitis) is a common clinical problem in South Asia. However, it is often challenging to distinguish this from other aetiologies, such as bacteria or acanthamoeba. In this prospective study, we investigated clinical and epidemiological features that can predict the microbial aetiology of microbial keratitis in Nepal. We recruited patients presenting with keratitis to a tertiary eye hospital in lowland eastern Nepal between June 2019 and November 2020. A structured assessment, including demographics, history, and clinical signs, was carried out. The aetiology was investigated with in vivo confocal microscopy and corneal scrape for microscopy and culture. A predictor score was developed using odds ratios calculated to predict aetiology from features. A fungal cause was identified in 482/642 (75.1%) of cases, which increased to 532/642 (82.9%) when including mixed infections. Unusually, dematiaceous fungi accounted for half of the culture-positive cases (50.6%). Serrated infiltrate margins, patent nasolacrimal duct, raised corneal slough, and organic trauma were independently associated with fungal keratitis ($p < 0.01$). These four features were combined in a predictor score. The probability of fungal keratitis was 30.1% if one feature was present, increasing to 96.3% if all four were present. Whilst microbiological diagnosis is the "gold standard" to determine the aetiology of an infection, certain clinical signs can help direct the clinician to find a presumptive infectious cause, allowing appropriate treatment to be started without delay. Additionally, this study identified dematiaceous fungi, specifically *Curvularia* spp., as the main causative agent for fungal keratitis in this region. This novel finding warrants further research to understand potential implications and any trends over time.

Keywords: microbial keratitis; fungal keratitis; dematiaceous fungi; clinical diagnosis; microbiology; Nepal; *Curvularia* spp.; *Fusarium* spp.

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1. Introduction

Microbial keratitis (MK) is responsible for over 2 million cases of monocular blindness annually in Africa and Asia [1]. It can result in significant morbidity, including stigma and pain. Recently, there have been calls for MK to be recognised as a neglected tropical disease by the World Health Organization [2]. Causative organisms include bacteria, fungi, protozoa, and viruses. In tropical regions, fungal infections may account for more than half

of reported cases, so a key distinction for management is whether the cause is bacterial or fungal [3]. Effective treatment relies on accurately and promptly diagnosing the organism responsible, usually through smear microscopy and confirmation by culture. However, anecdotally many eye care practitioners in low- and middle-income countries (LMICs) do not have access to microbiology, relying on clinical signs alone to guide treatment [4,5]. Empirical treatment is often used, but typically this is antibiotic monotherapy, as antifungals are expensive and frequently in short supply, leading to treatment delay for fungal keratitis patients [6]. Microbial keratitis typically presents with pain, conjunctival hyperaemia (redness), corneal stromal infiltration, and epithelial ulceration. Unfortunately, differentiating between bacterial, fungal, and other types of infection clinically is challenging.

Several studies have described clinical features that are more likely to be associated with fungal versus bacterial keratitis. Earlier studies have suggested that fungal keratitis (FK) might be associated with features such as Descemet's membrane folds, serrated margins, elevated surfaces, hypopyon, and satellite lesions; however, these were limited in the authors' sample sizes [7,8]. Subsequent, larger cross-sectional studies have reported the frequency of clinical features for bacterial keratitis (BK) and FK, rather than attempting to objectively quantify the predictive value of each sign to make an accurate diagnosis [9,10]. One study by Thomas et al. developed a scoring tool to aid in the diagnosis of FK in the absence of laboratory tests [4]. However, this tool did not incorporate other potentially useful relevant factors, including the patient's history. Furthermore, it did not attempt to distinguish between the major fungal genera based on clinical signs. No subsequent scoring tools have been published since.

In this study, we report the aetiology of MK from a large prospective cross-sectional study in Nepal in relation to the patient presentation and clinical signs. The incidence of MK in Nepal is amongst the highest reported in the world, at 799/100,000/year [11]. Using this dataset, we explored which clinical signs are predictive of FK or BK, formulating a clinical score that can be used to make a predictive diagnosis in the absence of further investigations. We also attempted to further distinguish three clinically distinct mycological groups of FK based on clinical signs: *Fusarium* spp., *Aspergillus* spp., and infection caused by the most commonly identified ocular dematiaceous fungal pathogens (predominantly *Curvularia* spp.).

2. Materials and Methods

2.1. Ethical Statement

This study followed the tenets of the Declaration of Helsinki. It was approved by the London School of Hygiene and Tropical Medicine Ethics Committee (Ref. 14841) and the Nepal Health Research Council Ethical Review Board (Ref. 1937). Written informed consent was obtained in Nepali before enrolment. If the patient was unable to read, the information was read to them and they were asked to indicate their consent by the application of their thumbprint, which was independently witnessed.

2.2. Study Design and Setting

We prospectively recruited patients at Sagarmatha Choudhary Eye Hospital (SCEH) in Lahan, Nepal, between 3 June 2019 and 9 November 2020 for a cross-sectional analysis. This formed part of the triaging assessment used to enrol eligible patients with FK into a randomised controlled trial comparing natamycin 5% to chlorhexidine 0.2%. The full protocol for this study has been published in [12]. SCEH is a tertiary ophthalmic referral hospital in southeastern Nepal that serves a population of approximately 5 million people.

2.3. Study Participants

Eligible patients were adults (>18 years) with acute MK, defined as having corneal epithelial ulceration > 1 mm in diameter, corneal stromal infiltrate, and signs of acute inflammation (conjunctival hyperaemia, anterior chamber inflammatory cells, or hypopyon).

2.4. Clinical Findings

Demographic details and ophthalmic clinical history were collected using a structured case record form. This included the duration of symptoms, any preceding trauma, medication (conventional or traditional), and past medical and ophthalmic history. Baseline clinical assessment included visual acuity (best spectacle-corrected visual acuity, BSCVA; presenting and pinhole visual acuity), slit-lamp examination using a structured protocol including eyelid assessment, corneal ulcer features, anterior chamber (flare, cells, hypopyon shape, and size), and perforation status. The infiltrate and epithelial defect size was calculated as the mean of the maximum diameter of the infiltrate and the widest perpendicular diameter [13]. High-resolution digital photographs with and without fluorescein staining were captured.

2.5. Investigations

In vivo confocal microscopy (IVCM) was performed, prior to corneal sample collection, by experienced operators using the HRT II/RCM confocal microscope (Heidelberg Engineering, Dossenheim, Germany) with a previously described technique [14,15]. All the images were reviewed during the procedure in real-time and classified by type of keratitis by one experienced observer. IVCM was used in this study to detect either fungal or amoebic keratitis; the unequivocal presence of fungal hyphae or cysts on IVCM was considered diagnostic.

Laboratory diagnosis was determined using microscopy and culture. Corneal scrape specimens were collected from the base and edge of the ulcer using a slit lamp and 21G needles after the application of topical proxymetacaine. Samples underwent processing for Gram, potassium hydroxide, calcofluor white, and lactophenol blue preparations as well as direct inoculation on solid culture media (fresh blood agar, chocolate agar, and Sabouraud dextrose agar). Media were incubated and read daily at 35–37 °C for up to 7 days for bacteria and at 25–27 °C for up to 21 days for fungi. Organism identification was performed using standard microbiological techniques.

We followed a previously described approach for reporting positive microbiological results [4]. In brief, culture results were significant if one of the following conditions were met:

- growth of the same organism was demonstrated on two or more solid culture media;
- semi-confluent growth at the site of inoculation or growth on one solid medium consistent with microscopy;
- semi-confluent growth at the site of inoculation on one solid medium (if bacteria);
- growth of the same organism on repeated scraping.

Culture positivity is the "gold standard" for the diagnosis of BK. As such, microscopy alone was not considered to be conclusive evidence if only a few organisms were seen; the exception to this rule was if many bacteria were observed in multiple fields of view. However, if fungal hyphae were visible by microscopy, the causative organism was reported as fungal (regardless of the culture results).

An overall "composite" diagnosis of definite fungal, bacterial, and mixed fungal–bacterial keratitis, or unknown aetiology, was obtained by combining the results of IVCM with cases meeting the microbiological diagnostic criteria described above.

2.6. Statistical Analysis

Data were analysed in STATA 17 (STATA Corp., College Station, TX, USA). Only patients with confirmed bacterial or fungal keratitis were included in the analysis to determine the diagnostic scoring. Mixed bacterial–fungal infections were included, with a sensitivity analysis performed for non-mixed infections. Unknown cases were coded as neither bacterial nor fungal. Summary frequency tables were generated to describe the demographics, presentation time, clinical history. and features. We classified presentation time as prompt (0–3 days), early (4–7 days), intermediate (8–14 days), late (15–30 days), and very late (more than 30 days), as previously reported [16,17]. LogMAR BSCVA measurements were

converted to their Snellen equivalent and categorised according to the WHO classification system [18]. Pairwise associations between clinical features (including clinical history and signs) were investigated using univariable logistic regression. Factors with univariable associations with a p-value < 0.2 or odds ratios (OR) greater than 2 or less than 0.5 were included in an initial multivariable logistic regression model; then, factors with associations with a p-value > 0.05 were removed one by one using backwards elimination. A predictive score was derived from a count of the features independently and positively associated with fungal aetiology, similar to in previous work [4]. Diagnostic accuracy indices were calculated for each score value for diagnosing FK. The probability of fungal infection was calculated by running our logistic regression model with score as the exposure, calculating the log odds of each person (based on their score) and the standard error, and converting these to probability. The 95% confidence intervals were calculated in a similar fashion. Rainfall data were obtained for Janakpur (the capital of Province 2, 56 km from Lahan) from the Department of Hydrology and Meteorology [19].

3. Results

3.1. Participants

Between 3 June 2019 and 9 November 2020, 890 patients with suspected MK were assessed at SCEH. Of these, 643 participants consented and were included in this study. The reasons for exclusion are listed in Supplementary Table S1. One patient fainted following visual acuity assessment; some clinical data are therefore missing for this patient. All cases of keratitis were unilateral (331/643, 51.5% left eye). Recruitment was paused on 24 March 2020 and resumed on 13 June 2020 due to emergency COVID-19 legislation. Demographic characteristics are presented in Table 1. The median age was 45.9 years (IQR 35.7–57.7, total range 18.1–100.1). The majority of patients were female (61.0%), Nepali (374/643, 58.2%), agricultural labourers (332/643, 51.6%), illiterate (499/643, 77.6%), and had no formal education (494/643, 76.8%).

Table 1. Demographic characteristics and clinical history of study participants.

		n/643	Percent
Age (median = 45.9, IQR 35.7–57.7)	<30 years	80	12.4%
	30–40 years	136	21.2%
	40–50 years	139	21.6%
	50–60 years	144	22.4%
	>60 years	144	22.4%
Gender	Male	251	39.0%
	Female	392	61.0%
Nationality	Nepali	374	58.2%
	Indian	269	41.8%
Occupation	No job	263	40.9%
	Farmer	332	51.6%
	Other	48	7.5%
Education	None	494	76.8%
	Primary level	80	12.4%
	Secondary level	12	1.9%
	Tertiary level	57	8.9%

Table 1. *Cont.*

		n/643	Percent
Literacy level	Illiterate	500	77.8%
	Reads/writes limited Nepali	51	7.9%
	Reads/writes Nepali well	48	7.5%
	Reads/writes English and Nepali	44	6.8%
Marital status	Unmarried	66	10.3%
	Married	577	89.7%
Presenting time (median = 8, IQR = 4–13)	Prompt 0–3 days	90	14.0%
	Early 4–7 days	230	35.8%
	Intermediate 8–14 days	178	27.7%
	Late 15–30 days	108	16.8%
	Very late > 30 days	37	5.8%
Most important symptom (self-reported)	Pain	471	73.3%
	Vision	57	8.9%
	Other	115	17.9%
History of trauma	No history of trauma/unsure	326	50.7%
	Vegetative matter	226	35.1%
	Other	86	13.4%
	Unknown object	5	0.8%
Used treatment	No	93	14.5%
	Yes	550	85.5%
	Previous steroids	105	16.3%
	Previous antibiotics	463	72.0%
	Previous antifungals	134	20.8%
	Previous other topical medication	260	40.4%
	Previous systemic medication	353	54.9%
	Used traditional eye medicine	12	1.9%
Diabetic	No	630	98.0%
	Yes	13	2.0%
HIV-positive	No	643	100.0%

3.2. Presentation

The number of patients with MK attending SCEH varied on a monthly basis (Figure 1), with the highest numbers presenting in November and December 2019. The overall patient numbers from March 2020 were low, coinciding with COVID-19-related restrictions. Patient numbers were at their highest during the dry, winter months (October–January), which correspond to the main harvest months in the region. There was no apparent direct relationship between case numbers and the monsoon rains that occur between June and September. The median time from the onset of symptoms to presentation at SCEH was 8 days (IQR 4–13, total range 0–92 days, Table 1). Only 14% of patients presented "promptly" within 3 days of symptom onset. A definite history of trauma was reported in 49.3% (317/643) of cases. Of the cases with a history of trauma, 71.3% (226/317) reported trauma with vegetative material. Preceding use of traditional eye medication (TEM) was reported very infrequently (1.6%, 10/643), whilst 7.8% (50/643) had used topical steroids

prior to attendance. Of note, 463/643 (72%) of cases reported the use of topical antibiotics prior to presentation.

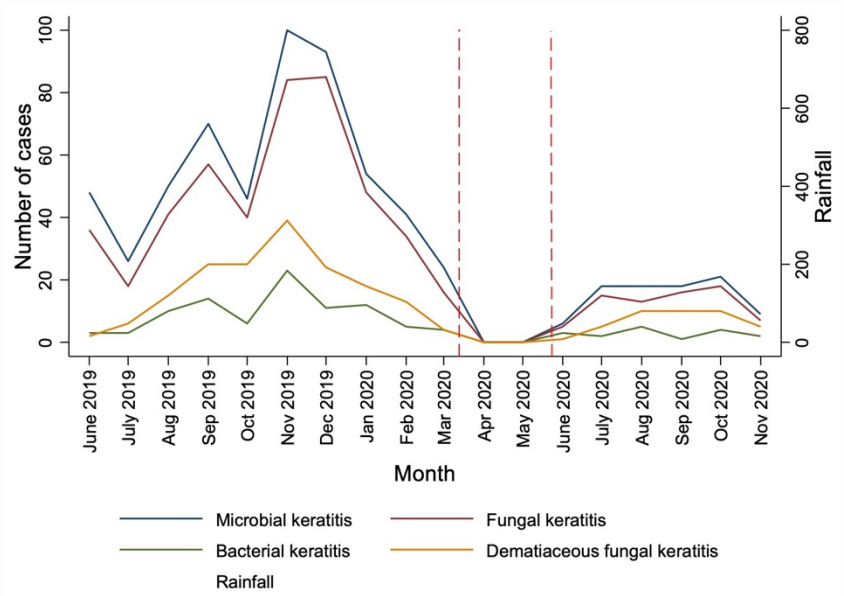

Figure 1. Number of microbial keratitis cases presenting per month and monthly rainfall within Province 2. Area between red dashed line represents when hospital was closed due to COVID-19 restrictions. Fungal and bacterial keratitis cases include mixed fungal–bacterial infections.

3.3. Clinical Features and Diagnosis

Table 2 shows the clinical features at presentation. Over one-quarter of patients (26.6%) were classed as blind in the affected eye at presentation with a BSCVA of less than 3/60. The median infiltrate size and epithelial defect sizes were 2.75 mm (IQR 1.75–4.0) and 2.90 mm (2.0–4.25), respectively.

Table 2. Clinical features and diagnosis at presentation.

		Median	**IQR (Total Range)**
Epithelial defect size (mm)		2.90	2.0–4.25 (0–12)
Infiltrate size (mm)		2.75	1.75–4.0 (0.2–11.75)
		n/642	**Percent**
Snellen BSCVA (affected eye) ~	6/5–6/18	296	46.0%
	6/24–6/60	164	25.5%
	5/60–1/60	103	16.0%
	CF-PL	80	12.4%
Slough	None	43	6.7%
	Flat	114	17.8%
	Raised	485	75.5%
Infiltrate edge	Defined	75	11.7%
	Serrated	554	86.3%
	Not visible	13	2.0%

Table 2. *Cont.*

		Median	IQR (Total Range)
Satellite lesions present	No	369	57.5%
	Yes	214	33.3%
	Unable to see	59	9.2%
Infiltrate colour	White	607	94.5%
	Cream	3	0.5%
	Yellow	1	0.2%
	Dark brown	10	1.6%
	Black	13	2.0%
	Other	8	1.2%
Fibrin	No	533	83.0%
	Yes	41	6.4%
	Unable to see	68	10.6%
Hypopyon	No	457	72.3%
	Yes	175	27.7%
	Unable to see	10	1.6%
Perforation status	No	634	98.8%
	Descemetocele	6	0.9%
	Perforated	2	0.3%

~ One patient fainted following visual acuity measurement; N = 643 for visual acuity but N = 642 for all other clinical features. BSCVA, best spectacle-corrected visual acuity; CF, counting fingers; PL, perception of light.

3.4. Aetiology and Diagnosis of Microbial Keratitis

Combining the microbiology and IVCM results, fungi alone were identified as the main causative agent of infection, being responsible for 75.1% of MK cases (Table 3). Mixed fungal–bacterial infections were present in 50/642 (7.8%) of cases, whilst bacteria alone were identified in 33/642 (5.1%) of cases. No causative agent could be identified in 77/642 (12.0%) of cases by either IVCM or microbiology. In each case of mixed infection, a single bacterial species was associated with a single fungal species. No cases of *Acanthamoeba* keratitis were identified in this study.

Table 3. Aetiology of microbial keratitis with corresponding results of investigations.

	Combined Laboratory and IVCM Diagnosis (N = 642)									
	Fungal *n* **(%)**		**Bacterial ~** *n* **(%)**		**Mixed** *n* **(%)**		**Unknown** *n* **(%)**		**Total** *n* **(%)**	
Microbiological Diagnosis										
No growth/NSS/No sample ^	41	(8.5)	0	(0)	0	(0)	70	(90.9)	111	(17.3)
Fungal keratitis	437	(90.7)	0	(0)	0	(0)	0	(0)	437	(68.1)
Bacterial keratitis	0	(0)	33	(100)	20	(40.0)	0	(0)	53	(8.3)
Mixed bacterial / fungal	0	(0)	0	(0)	30	(60)	0	(0)	30	(4.7)
Corneal scrapes not performed	4	(0.8)	0	(0)	0	(0)	7	(9.1)	11	(1.7)
IVCM Diagnosis										
No FK	50	(10.4)	33	(100)	6	(12.0)	77	(100)	166	(25.9)
FK	432	(89.6)	0	(0)	44	(88.0)	0	(0)	476	(74.1)

Table 3. *Cont.*

	Combined Laboratory and IVCM Diagnosis (N = 642)									
	Fungal *n* (%)		**Bacterial ~** *n* (%)		**Mixed** *n* (%)		**Unknown** *n* (%)		**Total** *n* (%)	
Overall composite diagnosis (prevalence) #	482	(75.1)	33	(5.1)	50	(7.8)	77	(12.0)	642	(100)
Mixed fungal–bacterial infections included §	532	(82.9)	83	(12.9)	n/a	n/a	77	(12.0)	n/a	n/a
Results of microbiology investigations										
Microscopy and culture-negative	34	(7.1)	0	(0)	0	(0)	50	(64.9)	84	(13.1)
Microscopy-positive, culture-negative	78	(16.2)	0	(0)	0	(0)	16	(20.8)	94	(14.6)
Microscopy-negative, culture-positive	5	(1.0)	4	(12.1)	0	(0)	0	(0)	9	(1.4)
Microscopy and culture-positive	349	(72.4)	29	(87.9)	50	(100)	0	(0)	428	(66.7)
Microscopy-positive, cultures not performed	12	(2.5)	0	(0)	0	(0)	0	(0)	12	(1.9)
Microscopy-negative, cultures not performed	0	(0)	0	(0)	0	(0)	4	(5.2)	4	(0.6)
Corneal scrape contraindicated	4	(0.8)	0	(0)	0	(0)	7	(9.1)	11	(1.7)
Total	482	(100)	33	(100)	50	(100)	77	(100)	642	(100)

^ Microscopy and culture-negative infections seen in 84/111 cases, microscopy was positive for bacteria but not meeting diagnostic criteria as no growth was seen on culture in 17/111 cases, microscopy was positive for bacteria but cultures were not performed in 6/111 cases, or microscopy was negative with no cultures performed in 4/111 cases. A total of 41/111 cases were confirmed as fungal keratitis by IVCM. ~ Bacterial keratitis was only diagnosed by significant growth on culture media, as described in the Methods Section. # Composite diagnosis was based on positive microbiological diagnosis and/or positive IVCM diagnosis. § Mixed bacterial–fungal infections ($n = 50$) were added to both fungal and bacterial categories. NSS, nothing significant seen; IVCM, in vivo confocal microscopy; FK, fungal keratitis.

There were 111/642 (17.3%) cases with no microbiological diagnosis. Microscopy and culture were negative in 84/111 cases, microscopy was positive for bacteria but cultures were negative in 17/111 cases, cultures were not performed for 6/111 cases, and microscopy was negative with no cultures performed in 4/111 cases. Of these "negative" results, 41/111 (36.9%) showed unequivocal diagnostic evidence of fungal hyphae visible by IVCM. Of the cases with bacteria detected on microscopy but no culture results, 15/23 (65.2%) were Gram-positive cocci. Bacterial infection alone was identified by microbiology in 53/642 (8.3%) of cases (i.e., no evidence of fungal infection was identified by microbiological investigations). However, 20/53 (37.7%) of these had evidence of fungal infection by IVCM and so were diagnosed as mixed fungal–bacterial infections.

3.5. Fungal and Bacterial Organisms

The fungal organisms identified by culture are presented in Table 4. *Curvularia* spp. was the most frequently identified fungal genus, isolated in 170/397 (42.8%) of positive fungal cultures. Dematiaceous fungi accounted for over half of all fungal organisms cultured (201/397, 50.6%). The second and third most commonly isolated genera were *Fusarium* spp. (63/397, 15.9%) and *Aspergillus* spp. (54/397, 13.6%). It was not possible to identify the fungal organism in 51/397 (12.8%) of cases because they either failed to grow or it was not possible to induce sporulation in vitro. Two cases of yeast infection were identified (0.5%), and there were two mixed filamentous fungal infections (0.5%).

Of the bacterial isolates identified, Gram-positive cocci (*S. aureus* (11.8%), coagulase-negative staphylococci (17.2%), and pneumococci (19.4%) were the most common cause of infection. There were just 3 cases (3.6%) of *Pseudomonas* spp. *Streptococcus* spp. was the most common bacterial genus identified (23/83, 27.7%), followed by *Staphylococcus* spp. (*Staphylococcus aureus* 6/83, 7.2%). Due to resource limitations, further identification was limited.

Table 4. Identification of fungi isolated from corneal samples of patients with microbial keratitis.

Fungi	*n*	Percent
Fusarium spp.	63	15.9
Aspergillus spp.	54	13.6
Dematiaceous fungi	201	50.6
Curvularia spp.	(170)	(42.8)
Bipolaris spp.	(19)	(4.8)
Exserohilum spp.	(7)	(1.8)
Alternaria spp.	(5)	(1.3)
Scedosporium apiospermum	2	0.5
Sarocladium spp./*Acremonium* spp.	8	2.0
Pestalotiopsis sp.	1	0.3
Colletotrichum spp.	6	1.5
Purpureocillium lilacinum	2	0.5
Trichoderma spp.	3	0.8
Syncephalastrum racemosum	1	0.3
Fusarium sp. and *Bipolaris* sp.	1	0.3
Mixed FFI	2	0.5
Yeast	2	0.5
Unidentified fungus	51	12.8
Total	**397**	**100.0**

FFI: filamentous fungal infection.

3.6. Clinical Features and Causative Agent

The frequency of various clinical features observed in FK and BK cases (including mixed infections) is shown in Table 5. Features significantly ($p < 0.05$) associated with fungal keratitis by univariate analysis were as follows: serrated margin, absence of hypopyon, raised slough, satellite lesions, absence of nasolacrimal duct obstruction (NLDO), vegetative trauma, delayed presentation (>3 days), previous antibiotics, and previous steroids (Table 5). There was no evidence of an association between the frequency of occurrence of fibrin, reduced corneal sensation, the presence of an immune ring, keratic precipitates, perineural infiltrates, endothelial plaque, flare or cells in the anterior chamber, or previous TEM use with FK. In a multivariable logistic regression model, the clinical features predictive of fungal infection were serrated margins, the absence of NLDO, raised slough, and vegetative trauma (Table 6). The presence of NLDO, the absence of serrated margins, and no prior use of topical antibiotics were associated with BK.

Table 5. Clinical features occurring in fungal and non-fungal keratitis (mixed infections included), with univariable analysis for features associated with fungal keratitis.

					Indices for Detecting Fungal Keratitis					
	Frequency in Fungal Cases (Including Mixed)	**(%)**	**Frequency in Non-Fungal Cases**	**(%)**	**Odds Ratio for FK**	***p*-Value**	**95% CI**	**Sens.**	**Spec.**	**PPV**	**NPV**
Serrated margins	497/527	94%	57/102	56%	13.08	<0.001	7.64–22.38	94.3%	44.1%	89.7%	60.0%
Fibrin	35/481	7.3%	6/93	6.5%	1.14	0.777	0.46–2.79	7.3%	93.5%	85.4%	16.3%
Hypopyon	136/524	26%	39/108	36%	0.62	0.033	0.40–0.96	26.0%	63.9%	77.7%	17.1%
Raised slough	439/532	83%	46/110	42%	6.57	<0.001	4.23–10.20	82.5%	58.2%	90.5%	40.8%
Satellite lesions	192/483	40%	22/100	22%	2.34	0.001	1.41–3.88	39.8%	78.0%	89.7%	21.1%
Pigmented colour	28/532	5.3%	3/110	2.7%	1.98	0.268	0.59–6.64	5.3%	97.3%	90.3%	17.5%
Nasolacrimal duct obstruction	15/486	3.1%	18/99	18%	0.14	<0.001	0.07–0.30	3.1%	81.8%	45.5%	14.7%
Reduced corneal sensation	70/532	13%	20/110	18%	0.68	0.169	0.40–1.18	13.2%	81.8%	77.8%	16.3%
Trauma with vegetative object	177/532	33%	21/110	19%	2.11	0.004	1.27–3.51	33.3%	80.9%	89.4%	20.0%
Previous antibiotics	392/532	74%	70/110	64%	1.60	0.034	1.04–2.47	73.7%	36.4%	84.8%	22.2%
Delayed presentation > 3 days	464/532	87%	88/110	80%	1.71	0.049	1.00–2.90	87.2%	20.0%	84.1%	24.4%
Previous steroids	96/532	18%	9/110	8.2%	2.47	0.013	1.20–5.06	18.0%	91.8%	91.4%	18.8%

FK, fungal keratitis; CI, confidence interval; Sens., sensitivity; Spec., specificity; PPV, positive predictive value; NPV, negative predictive value.

Table 6. Multivariable analysis of clinical features occurring in fungal and bacterial keratitis (mixed infections included).

	Odds Ratio	*p*-Value	95% CI
Fungal keratitis—clinical features			
Serrated margins	7.50	<0.001	4.09–13.78
Raised slough	4.27	<0.001	2.51–7.24
Nasolacrimal duct obstruction	0.18	<0.001	0.07–0.42
Trauma with vegetative object	2.65	0.006	1.32–5.32
Bacterial keratitis—clinical features			
Serrated margins	0.36	0.001	0.20–0.66
Nasolacrimal duct obstruction	3.08	0.006	1.38–6.87
Previous antibiotics	0.33	<0.001	0.20–0.53

A score was derived from the four clinical features associated with FK (serrated margin, raised slough, trauma with vegetative object, and absence of NLDO): a score of +1 was given for each feature present (Table 7, Figure 2). The probability of FK if only one sign was present was 30.1% (95% CI 17.8–47.2%), compared to a probability of 96.3% (95% CI 91.3–98.4%) if all four clinical features were present.

Table 7. Screening test indices for each score.

N = 574	*n*	(%)	Sensitivity	Specificity	PPV	NPV
Score > 0	572	(99.7)	100%	2.17%	84.3%	100%
Score > 1	536	(93.4)	97.7%	29.3%	87.9%	71.1%
Score > 2	417	(72.7)	81.3%	72.8%	94%	42.7%
Score > 3	134	(23.3)	26.8%	94.6%	96.3%	19.8%

Only patients who had all features examined were included in calculating the diagnostic accuracy. PPV, positive predictive value; NPV, negative predictive value.

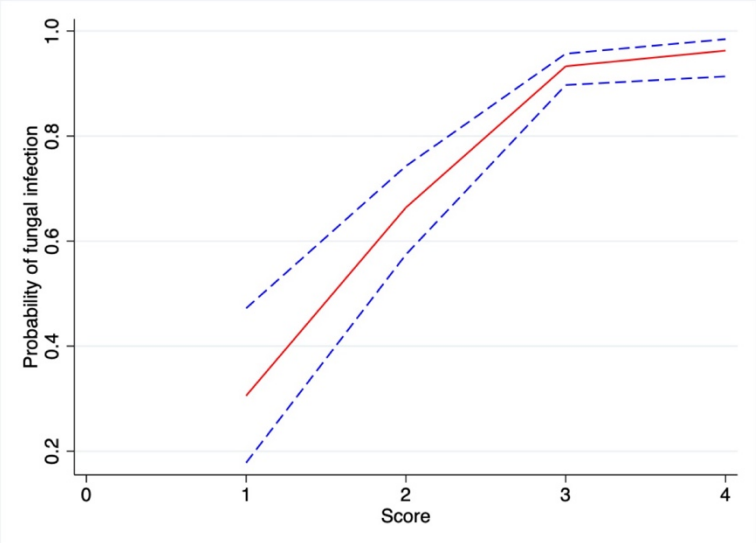

Figure 2. Operating characteristic curve showing the probability of fungal infection at different scores (95% CI dashed lines).

A sensitivity analysis that excluded mixed infections was carried out (Supplementary Tables S2–S4). This found that the same clinical parameters were statistically independent risk factors associated with FK. There was very little difference between the screening test indices calculated for each score or the probability of FK at different scores (Supplementary Figure S1).

Univariable logistic regression found that the presence of serrated margins, raised slough, and pigmented colour, in addition to a history of preceding steroids, were risk factors for dematiaceous FK (Supplementary Table S5). The presence of satellite lesions and reduced corneal sensation were more strongly associated with other aetiologies. Only 11% of all the dematiaceous fungi isolated had pigmented corneal ulcers at presentation. The multivariate logistic regression model found that clinical features predictive of dematiaceous FK were a pigmented colour and the presence of serrated margins, as well as an absence of satellite lesions and/or fibrin (Supplementary Table S6).

No clinical parameters were found to be predictive for either *Fusarium* or *Aspergillus* keratitis by univariable or multivariable logistic regression models, possibly due to the small sample size used (data not shown).

4. Discussion

In this prospective study from eastern Nepal, fungal organisms were found to be the sole cause of infection in 75.1% of patients with microbial keratitis, with fungal organisms implicated in 82.9% of MK cases when mixed fungal–bacterial infections were included. To the best of our knowledge, our study reports the highest proportion of FK cases amongst MK anywhere in the world. With the highest previous reported proportion being 81.5% in Sri Lanka (1976–1981) [5,20], this is considerably higher than the proportions reported in previous studies from Nepal (ranging from 25% in Kathmandu to 70% in Biratnagar) [21–27].

Furthermore, another surprising finding from this study was that *Curvularia* spp. was the most frequently isolated fungal genus (42.8%). We believe that this is the only study to report a dematiaceous fungus as the leading causative organism. Over half the cases in our study were dematiaceous moulds. This is in itself unusual; FK caused by dematiaceous or melanised species is usually less common than *Fusarium* spp. and other hyaline filamentous fungi, which typically account for the majority of FK cases [28–30]. The only study to date with a similar proportion of dematiaceous fungi was a prospective study from North India conducted between 1999 and 2001 on 485 cases of MK, of which 39% were found to be fungal in aetiology. Although *Aspergillus* spp. (41%) was the most common fungal isolate, followed by *Curvularia* spp. (29%), dematiaceous fungi as a group (*Curvularia* spp., *Bipolaris* spp. and *Alternaria* spp.) accounted for 43.2% of cases [31]. Our finding that *Curvularia* spp. is the most commonly isolated dematiaceous genus is supported by the majority of other studies [28,32–35], with only one study from South India and one recent study from Thailand finding *Cladosporium* spp. and *Lasiodiplodia* spp. to be the most commonly isolated dematiaceous fungal genus, respectively [36,37].

Dematiaceous fungi and *Fusarium* spp. are plant pathogens which are frequently found in soil in tropical and sub-tropical regions [28,33]. As a result, one would typically expect a history of trauma, particularly with organic material, to precede FK. Indeed, a break in the corneal epithelium is required for all but a handful of microorganisms to establish an infection, which is usually the result of trauma. However, in our study only 33% of FK cases gave a definitive history of trauma, whilst 25% of BK cases also reported such a history. This proportion was similar for dematiaceous fungi alone (32%). Despite this relatively small difference, organic trauma was independently associated with FK versus non-fungal keratitis at presentation. This relatively low proportion of patients with a definite history of trauma contrasts with an earlier study from a similar location in Nepal (Nepalgunj, 2011–2012), where 58% of patients gave a history of trauma [23]. Studies from India also typically report a history of trauma greater than that found in ours, with a range of 40–92% in all studies included in a recent review of FK [5], other than one study from Delhi which had a similar number to ours (32%) [38]. This is surprising, given that the

majority of the study participants were either farmers (51.5%) or likely subsistence farmers, given that they were unemployed (40.9%), as found in previous studies from Nepal and India [23,39]. The plains or "terai" area of Nepal is predominantly an agricultural society mainly involved in "paddy" farming, and it is likely that fungal spores are ubiquitous. One possible explanation for the lack of preceding trauma could be that some people may not recall minor abrasive events, which could be a sufficient breach for infections to develop.

In terms of patient demographics, the median age of 45.9 is similar to that found in other studies from the region [5]. We found there was also no change in the frequency of FK with increasing age, but that BK became slightly more frequent in patients aged 50 or older (60% of all BK cases including mixed infections compared to 42% of all non-BK cases, $p = 0.049$). BK was previously shown to be more common in older patients [36], and our results support this finding. In LMICs, MK has typically been more common in males [40], regardless of its aetiology [5]. However, in this study 61% of patients were female, with a similar female predominance found if stratified according to aetiology. There has been one other study from Nepal where the majority of cases occurred in women [25]. The reason for this is unclear, but may represent increased exposure amongst women in this region to agricultural work and trauma or possibly different health-seeking behaviours. Further epidemiological research is required to investigate this. Most patients were from low socio-economic groups, as evidenced by 76.7% reporting no formal education and 77.8% being illiterate. These findings highlight important opportunities and challenges for primary prevention strategies within Nepal.

There was considerable variation in the numbers of people presenting with MK per month; the highest numbers were seen between September 2019 and January 2020. This contrasts with the results of a five-year retrospective study conducted from the same institution between 2010 and 2015, which found little variation and with the peak attendance occurring between June and August [41]. Our observations obtained from March 2020 onwards are greatly affected by travel restrictions imposed to control the COVID-19 pandemic. The winter months in Nepal (October–January), when the numbers presenting in our study were at their highest, are the cool, dry harvest months, when the number of fungal spores in the atmosphere are likely to be at their highest [42]. Interestingly, we did not find any increase in cases during the monsoon rains. In particular, the number of dematiaceous fungal cases presenting to SCEH appear to follow this trend. It is likely that the seasonal distribution reflects periods of increased risk of trauma through occupational risk factors, such as corneal abrasions from the direct inoculation of plant material and dust during harvesting, threshing, and winnowing, which are seasonal activities intrinsically linked with climate. A recent study from North India reported a similar seasonal trend for dematiaceous fungi, with the majority of cases presenting between September and December [33]. Although we did not collect data on the number of non-microbial keratitis patients attending SCEH during this period, historically the number of cases attending is constant throughout the year, other than a slight increase in elective surgical procedures such as cataract surgery occurring between December and March. It is therefore unlikely that this observed increase is simply due to an inflated denominator.

Clinically differentiating FK from BK is challenging. The sensitivity of experienced ophthalmologists clinically diagnosing FK has been reported as very low (38%) [43], whilst corneal specialists have been shown to only be able to correctly diagnose fungal keratitis from clinical photographs in 66% of cases [44]. Several clinical signs have been found to be helpful in discriminating FK from BK: serrated margin, raised slough, colouration other than yellow, and the absence of fibrin [4]. In an earlier analysis, in patients who had raised slough, serrated margins, and no anterior chamber fibrin the probability of fungal keratitis was 89% [45], compared to 16% if the margins were defined with a flat surface and fibrin present. In our study, we also found serrated margins and raised slough to be independently predictive of FK, with higher odds ratios (95% CI in brackets) than those seen in the previous study (serrated margins OR 7.50 [4.09–13.78] vs. 3.45 [2.12–5.64]; raised slough OR 4.25 [2.51–7.24] vs. 2.32 [1.43–3.74]). We did not find colour or the absence

of fibrin to be independently associated with FK. However, a history of organic trauma (OR 2.65 [1.32–5.32]) was found to be associated with fungal infection, whilst nasolacrimal duct obstruction was not (OR 0.18 [0.07–0.42]). The previous study by Thomas and co-workers only included clinical signs and therefore did not assess a positive history of trauma, as they were concerned about recall bias and the fact that, pathologically speaking, nearly all microbiological organisms require a defect in the corneal epithelium in order to enter, which is usually a result of mechanical trauma [4]. The probability of FK in our series was 96.3% if all four clinical features were present. The one clinical sign most likely to distinguish FK from BK is the presence of a serrated or irregular margin, as this was the only clinical sign whose presence or absence was found to be independently significantly associated with fungal and not with bacterial keratitis, respectively.

Satellite lesions, which have previously been thought to be indicative of FK based on limited case reports, were not found to be significant predictors in our multivariate model, despite occurring more frequently in fungal than in bacterial cases. This is in keeping with the results from other cross-sectional studies [4,46].

Although NLDO has been suggested as a major risk factor for non-healing bacterial keratitis (BK) [47], until recently few studies have confirmed an association between NLDO and BK [48,49]. Recent work from India adds weight to this by demonstrating that patients with untreated NLDO and MK have a worse clinical outcome [50]. For this reason, at our institution all patients with MK undergo lacrimal syringing. In this study, 16% of BK patients had NLDO, compared to only 3.1% of FK patients ($p < 0.001$). We would therefore recommend clinicians consider lacrimal syringing as part of their MK diagnostic work-up, as this can help to differentiate BK from FK, as well as potentially detecting patients at risk of recurrence and a poor outcome.

Although microscopy and culture remain the "gold standard" in terms of diagnosing MK, a clinical score based on predictive factors for FK can help guide the clinician to start antifungal treatment promptly if these investigations are not possible. This also allows for the more rational use of antifungals, helping to reduce the cost to the patient and the risk of resistance whilst ensuring that the limited supplies of antifungals reach those most in need.

We found that raised slough, pigmentation, the absence of satellite lesions, and/or the absence of fibrin were clinical features predictive of dematiaceous fungal infection. A previous study from India also found that raised slough and pigmentation were predictive features for dematiaceous fungal infection [51], although it did not mention the other two clinical features. Consistent with our study, only 16% of dematiaceous cases were pigmented, whilst recent studies from India and Thailand found 18% and 26% of dematiaceous cases to be pigmented, respectively [33,37]. We did not find any features that were predictive of *Fusarium* or *Aspergillus* keratitis, likely due to the relatively small sample size. Given the delay between corneal scrape and culture results, using these four clinical signs may be helpful to guide preliminary diagnosis and management, although if microscopy is available with a quick turn-around time, this should remain the gold standard for diagnosing fungal keratitis.

Our study has several strengths. It was a large, prospective study that utilised IVCM to help identify cases of FK which may otherwise have been missed. We had a relatively low culture-negative rate. However, there were several possible limitations. Firstly, this series may not be fully representative of all MK occurring in this population, as it was conducted at a tertiary referral centre; the cases presenting to the hospital may be more severe if milder diseases can be managed effectively in the community. Furthermore, it is possible that BK is more adequately treated than FK in primary care settings, leading to fewer BK cases being referred for treatment. This possibility is supported by the widespread prior use of topical antibiotics amongst our participants (72%), whilst only 21% had used topical antifungals. The relatively low number of patients with BK in the analysed population reduces the ability of our method to adequately detect differences between groups. Secondly, we included mixed infections in our analyses in order to include as many bacterial cases as possible, in contrast to previous work [4]. This was decided at the outset in the analytical plan and

was deemed a pragmatic, real-world approach. However, sensitivity analyses did not find any significant difference if mixed infections were excluded (Supplementary Tables S2–S4). Thirdly, given that only conventional diagnostic resources were available (i.e., no molecular diagnostic testing was available), it was not possible to speciate some of the pathogens at our centre. This is true of many hospital laboratories in LMICs where molecular methods are unavailable. Many genera are difficult to speciate based on phenotypic characteristics alone due to inter- and intra-species variation. Fourthly, the clinical score that we calculated to aid in diagnosis needs to be replicated and assessed in other settings where the prevalence of fungal keratitis differs. Finally, we did not detect any cases of *Acanthamoeba* in this study. There were no cases which appeared clinically suspicious for amoebic keratitis. Although we used IVCM, accurately diagnosing *Acanthamoeba* infection with IVCM can be challenging and requires a highly skilled operator [52]. No *Acanthamoeba* cysts were visualised using microscopy and culture was not performed routinely due to the very low incidence of cases reported in the region.

5. Conclusions

In conclusion, this large, prospective study found dematiaceous fungi to be the most common cause of FK cases in eastern Nepal (there is variation within the country). To the best of our knowledge, this study reports the highest ever proportion of FK found amongst MK cases. Although there is significant clinical variation in presentation, certain clinical signs can help to distinguish FK from other causes: specifically serrated margins, raised slough, no NLDO, and organic trauma. We also identified raised slough, pigmentation, the absence of satellite lesions, and the absence of fibrin to be predictive of dematiaceous FK. Although microscopy and culture remain the gold standard for diagnosis, using these clinical signs may help direct clinicians without access to a microbiology service, or, in cases where microscopy and culture results are negative, to a presumptive aetiology, allowing appropriate treatment to be started without delay.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/jof8020201/s1. Figure S1: Operating characteristic curve showing probability of fungal infection at different scores (mixed infections excluded); Table S1: Reasons for exclusion of potential participants from the study; Table S2: Univariable analysis for features associated with fungal keratitis (mixed infections excluded); Table S3: Multivariable analysis of clinical features occurring in fungal and bacterial keratitis (mixed infections excluded); Table S4: Screening test indices for each score (mixed infections excluded); Table S5: Clinical features occurring in dematiaceous, Fusarium spp., and Aspergillus spp. keratitis, and univariable analysis for features associated with dematiaceous fungal keratitis; Table S6: Multivariable analysis of clinical features occurring in dematiaceous fungal keratitis.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. It was approved by the London School of Hygiene and Tropical Medicine Ethics Committee (Ref. 14841) and the Nepal Health Research Council Ethical Review Board (Ref. 1937).

Informed Consent Statement: Written informed consent was obtained in Nepali from all subjects before enrolment in the study. If the patient was unable to read, the information was read to them, and they were asked to indicate their consent by the application of their thumbprint, which was independently witnessed.

Data Availability Statement: The datasets generated during and/or analysed during the current study will be available upon request from M.J.B. (matthew.burton@lshtm.ac.uk). The full data set will be available with all patient identifiable details removed. Data will be available after formal reporting of the study findings in a peer-reviewed scientific publication. Datasets will only be available to bona fide scientific investigators. Requests should be made to the Chief Investigator in writing detailing the scientific investigators background and intended use for the data. Consideration will be given to all proposed analyses, with likely envisaged uses including investigators planning on conducting meta-analyses, for example. Patient Information Sheets and consent forms specifically referenced making anonymised data available, and this has been approved by the relevant ethic committees.

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Chapter 6: Diagnosis of fungal keratitis in low-income countries: evaluation of smear microscopy, culture, and in vivo confocal microscopy in Nepal

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Surname/Family Name	HOFFMAN		
Thesis Title	Managing Fungal Keratitis in Nepal: A Randomised Controlled Trial Comparing Chlorhexidine 0.2% to Natamycin 5%		
Primary Supervisor	PROF. MATTHEW J. BURTON		

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SECTION E

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Diagnosis of fungal keratitis in low-income countries: evaluation of smear microscopy, culture, and *in vivo* confocal microscopy in Nepal

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Abstract: Clinically diagnosing fungal keratitis (FK) is challenging; diagnosis can be assisted by investigations including *in vivo* confocal microscopy (IVCM), smear microscopy, and culture. The aim of this study was to estimate the sensitivity in detecting fungal keratitis (FK) using IVCM, smear microscopy, and culture in a setting with a high prevalence of FK. In this cross-sectional study nested within a prospective cohort study, consecutive microbial keratitis (MK) patients attending a tertiary-referral eye hospital in south-eastern Nepal between June 2019 and November 2020 were recruited. IVCM and corneal scrapes for smear microscopy and culture were performed using a standardised protocol. Smear microscopy was performed using potassium hydroxide (KOH), Gram stain, and calcofluor white. The primary outcomes were sensitivities with 95% confidence intervals [95% CI] for IVCM, smear microscopy and culture, and for each different microscopy stain independently, to detect FK compared to a composite referent. We enrolled 642 patients with MK; 468/642 (72.9%) were filamentous FK, 32/642 (5.0%) were bacterial keratitis and 64/642 (10.0%) were mixed bacterial-filamentous FK, with one yeast infection (0.16%). No organism was identified in 77/642 (12.0%). Smear microscopy had the highest sensitivity (90.7% [87.9-93.1%]), followed by IVCM (89.8% [86.9-92.3%]) and culture (75.7% [71.8-79.3%]). Of the three smear microscopy stains, KOH had the highest sensitivity (85.3% [81.9-88.4%]), followed by Gram stain (83.2% [79.7-86.4%]) and calcofluor white (79.1% [75.4-82.5%]). Smear microscopy and IVCM were the most sensitive tools for identifying FK in our cohort. In low-resource settings we recommend clinicians perform corneal scrapes for microscopy using KOH and Gram staining. Culture remains an important tool to diagnose bacterial infection, identify causative fungi and enable antimicrobial susceptibility testing.

Keywords: microbial keratitis; fungal keratitis; in vivo confocal microscopy; diagnosis; microbiology; Nepal; cornea; culture; microscopy

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1. Introduction

Microbial keratitis (MK) is an ocular emergency; without prompt, appropriate treatment, significant ocular morbidity can ensue, including blindness through corneal

scarring and even eye-loss.[1] As there is a diverse range of causative organisms – bacteria, fungi, protozoa and viruses – correct treatment depends on accurately identifying the microbe responsible through clinical examination and suitable investigations.[2] In tropical low- and middle-income countries, fungal infection can account for more than half of MK cases,[3] meaning a key consideration for management is whether the organism is bacterial or fungal. Unfortunately, making a diagnosis on clinical grounds alone is difficult as there are no pathognomonic signs unique to bacterial or fungal keratitis, meaning diverse organisms can result in similar clinical appearances.[4] Investigations including microscopy and culture are therefore necessary.

Traditionally, microbiological culture has been considered the gold standard for diagnosing microbial keratitis.[5,6] However, this is more relevant in settings where bacterial keratitis is more common compared to fungal keratitis, such as in temperate, high-income settings, as growth on one or more solid media is a diagnostic condition for bacterial keratitis. Fungal growth in a single medium with no associated hyphae visible on microscopy may represent contaminants, whilst conversely some fungal species are difficult to culture in vitro. As a result, culture has been shown to have a low sensitivity for FK,[7] and a variable sensitivity for bacterial keratitis.[8] Microscopy – both in the form of routine "smear" microscopy from corneal scrapings and *in vivo* confocal microscopy (IVCM) – therefore plays a key role in diagnosing fungal infections.[9] Smear microscopy has the advantage of being fast, inexpensive and accurate, whilst IVCM offers real-time non-invasive diagnosis, with high sensitivity and specificity for diagnosing FK.[7,10,11] A positive finding of fungal hyphae on microscopic examination of corneal epithelial tissue is a highly reliable indicator for ocular fungal infection and should always be treated as significant. However, it may be difficult to interpret the significance of scanty bacteria within corneal smear material, for example, the presence of a very small number of Gram-positive cocci may represent transient flora from the lid margin or conjunctiva, hence the recommendation for supporting cultures.[4]

There are several smear microscopy staining techniques available to identify fungal and bacterial organisms. Conventional techniques include Gram stain, potassium hydroxide (KOH) wet mount, Giemsa stain and lactophenol cotton blue.[9,12] These techniques are quick, cheap and easy to perform but their accuracy has been reported to vary considerably, largely due to potential artefacts and misinterpretation. Calcofluor white is inexpensive and quick to prepare but requires a fluorescence microscope[13]. Other techniques include Gomori's methenamine silver,[14] periodic acid-Schiff,[15] and fluorescein-conjugated lectins,[16] which may be more accurate but are more time-consuming and may require additional equipment and expense.

Although there have been several studies reporting the diagnostic accuracy of IVCM, culture and smear microscopy,[7,8,10,11,17-19] few have compared these techniques to a composite referent including results of IVCM as opposed to culture alone, particularly within a setting with a high prevalence of FK. In addition, most of these studies have not compared the accuracy of the different microscopic staining techniques. This study aimed at prospectively evaluating the sensitivity of several different smear microscopy stains, IVCM and culture at a tertiary ophthalmic referral centre in Nepal, a setting where there is a high burden of fungal keratitis.

2. Materials and Methods

2.1 Ethical statement

This study followed the tenets of the Declaration of Helsinki. It was approved by the London School of Hygiene & Tropical Medicine Ethics Committee (Ref. 14841) and Nepal Health Research Council Ethical Review Board (Ref. 1937). Written informed consent in the local language was obtained before enrolment. If the patient was unable to read, the information was read to them, and they were asked to indicate their consent by application of their thumbprint, which was independently witnessed.

2.2 Study design

This cross-sectional study nested within a prospective cohort study formed part of the triaging assessment used to enrol eligible patients with FK into a randomised controlled trial comparing natamycin 5% to chlorhexidine 0.2%. The full protocol for this study and results have already been published.[20,21] We have previously described the methodology relating to clinical findings, microbiological diagnosis, and *in vivo* confocal microscopy in our earlier work;[22] we therefore describe these briefly here.

2.3 Study setting and participants

Patients were prospectively recruited patients at Sagarmatha Choudhary Eye Hospital (SCEH) in Lahan, Nepal between 3rd June 2019 and 9th November 2020. SCEH is a tertiary ophthalmic referral hospital within Province 2 of south-eastern Nepal that serves a population of approximately 5 million people. It is located approximately 18km from the Indian border, with many patients treated in outpatients being Indian nationals. There are 22 satellite "Eye Care Centres" (ECCs) located within Province 2 that are operated by SCEH and provide routine eye examination and treatment, referring to SCEH for more complex cases and surgery.

Eligible patients were adults (>18 years) with acute MK, defined as having a corneal epithelial defect greater than 1mm in diameter associated with corneal stromal infiltration, and any/all signs of acute inflammation (conjunctival hyperaemia, anterior chamber inflammatory cells, hypopyon). All eligible patients who consented to participate in the study were included.

2.4 Clinical findings

We collected data on demographic details, ophthalmic clinical history and clinical examination findings using a structured case record form, as previously described.[22] Clinical examination included best spectacle corrected visual acuity (BSCVA) in LogMAR and slit-lamp examination, which followed a structured approach: eyelid assessment, corneal ulcer features, anterior chamber characteristics (flare, cells, hypopyon shape, and size), and perforation status.

2.5 Microbiological diagnosis

Laboratory diagnosis was determined using smear microscopy and culture, as previously described.[22] Following application of preservative-free topical anaesthesia (proxymetacaine), corneal scrape specimens were collected from the base and edge of the ulcer using a slit lamp and 21G needles Samples were processed for Gram stain, potassium hydroxide (KOH), and calcofluor white (CFW) preparations as well as direct inoculation on solid culture media (fresh blood agar, chocolate agar, and Sabouraud dextrose agar). Media were incubated and read daily at 35–37°C for bacteria for up to 7 days and at 25-27°C for up to 21 days for fungi. Organism identification was performed using standard microbiological techniques. We followed a previously described approach for reporting positive microbiological results.[4,22] Culture positivity was used to diagnose BK; smear microscopy alone was not considered to be conclusive evidence. However, if fungal hyphae were visible by smear microscopy the causative organism was reported as fungal (regardless of culture results).

2.6 In vivo confocal microscopy

In vivo confocal microscopy (IVCM) was performed prior to corneal sample collection. IVCM was performed by trained, experienced operators using the HRT III/RCM confocal microscope (Heidelberg Engineering, Dossenheim, Germany) using a previously described technique,[10,11,22] with all the images reviewed in real-time and classified into either fungal or amoebic keratitis by one experienced observer. IVCM was not used to diagnose bacterial keratitis as the resolution is inadequate to visualise bacteria other than *Nocardia* spp. or to visualise infectious crystalline keratopathy (ICK). The presence of fungal hyphae (defined as highly reflective, branching/bifurcating, well-defined, interlocking structures, measuring 3-10 microns in diameter and not seen in isolation)[23] on IVCM

was deemed diagnostic of FK; where there was any uncertainty or the diagnosis was only "possible FK", the IVCM result would be deemed as negative for the purpose of the analysis.

2.7 Disease definition

Disease definition (fungal, bacterial, amoebic or mixed fungal-bacterial keratitis) was based on positive diagnostic results from culture, smear microscopy and/or IVCM, similar to previous work.[7] Clinical findings or response to treatment were not used for disease definition to ensure we were assessing the sensitivity of investigations. An overall "composite" diagnosis of definite fungal, bacterial or mixed fungal-bacterial keratitis, or unknown aetiology, was obtained by combining the results of IVCM with cases meeting the microbiological diagnostic criteria described above and previously.[22] If all results for each investigation were negative for FK then the composite diagnosis was negative for FK; if one or more results of the individual investigations were positive for FK then the composite diagnosis was positive for FK. Missing data were treated as negative in defining the composite diagnosis and excluded for the individual analyses.

2.8 Statistical analysis

Data were analysed in STATA 17 (STATA Corp., USA). The diagnostic performance of the various tests was evaluated by determining: (1) the sensitivity (including exact binomial confidence intervals) of culture, smear microscopy and IVCM in comparison to a composite reference standard for diagnosing FK, and (2) the sensitivity of the different smear microscopy staining techniques in comparison to the composite reference standard for diagnosing FK. As we are comparing to a composite reference standard, by definition there are no false-positive results, meaning that any calculated specificities would always be 100%. We have therefore chosen not to report specificities to avoid misinterpretation.

3. Results

Between 3rd June 2019 and 9th November 2020, 890 patients with suspected MK were assessed at SCEH. Of these, 643 participants consented, with one patient fainting during examination and subsequently withdrawing consent; 642 patients were therefore included in this study. All cases of MK were unilateral (331/643, 51.5% left eye).

It was not possible to perform all investigations on all patients. For example, due to excessive corneal thinning or not enough material available for smear microscopy and/or culture. Therefore, results from smear microscopy were available in 631/642 cases, IVCM in 638/642 cases and culture for 624/642 cases. Bacterial culture results were not available for 7 cases, whilst fungal culture results were not available for 2 cases.

A causative organism was identified in 565/642 (88%) of cases when all tests were combined (any test positive). The detection method for different types of organisms are shown in Table 1. Most cases that were positive using a composite diagnosis had had all three investigations performed—the few exceptions are mentioned in the text below. Figure 1 illustrates the combination of positive tests for fungal keratitis (including mixed fungal-bacterial infection). We identified 32 cases of monomicrobial bacterial keratitis. The total number of bacterial cases (diagnosed by culture alone), including 65 mixed bacterial-fungal infections, was 97: 15.1% of cases in our study. There was one case of a polymicrobial yeast and bacterial infection, which was detected by culture alone for both the yeast and bacterial infection. There were no cases of *Acanthamoeba* keratitis detected in this study by any of the three methodologies.

We identified 468 cases of monomicrobial filamentous fungal keratitis: 72.9% of cases in our study. A further 64 mixed fungal-bacterial cases were identified (10%). Including mixed infections, fungal keratitis was diagnosed in 532/642 (82.9%) of all cases of MK in our study. Of these, culture results were not available for 10/532 cases, smear microscopy results were not available for 4/532 cases, and IVCM results were not available for 2/532 cases. Table 1 and Figure 1 show that all three investigations were positive in 355/532 (66.7%) of cases, whilst both smear microscopy and IVCM detected a similar number of

FK cases overall (425/464, 91.6% and 421/466, 90.3%, respectively). IVCM detected more cases of FK overall not identified by other modalities (45/532), whilst culture only identified a further 4 cases not detected by IVCM or smear microscopy.

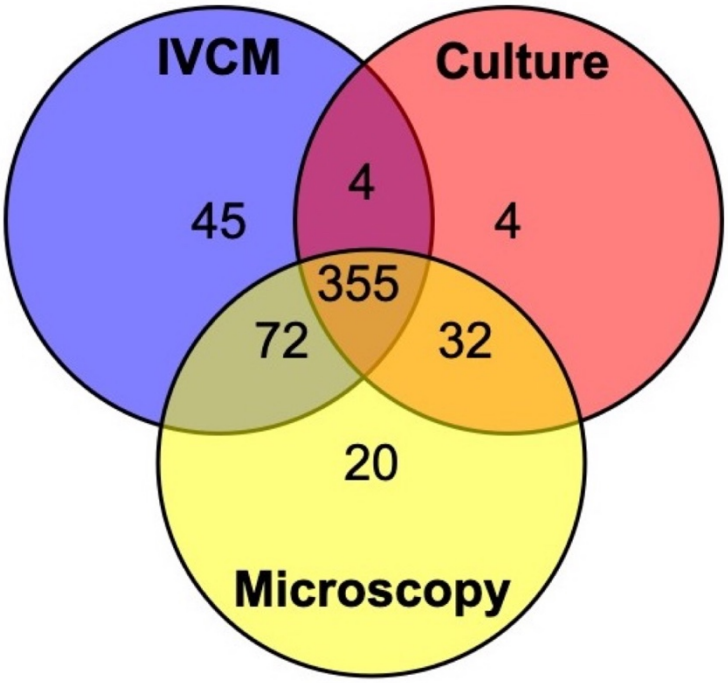

Figure 1: Venn diagram showing the number of cases that were positive for filamentous fungal keratitis (n = 532, including mixed bacterial-fungal cases) using culture, smear microscopy, and *in vivo* confocal microscopy (IVCM). Cases that were positive for more than one test are given within the overlapping areas.

3.1 Sensitivity – all investigations compared to composite referent

The sensitivity of smear microscopy, IVCM and culture for diagnosing FK were compared to the composite reference standard (Table 2). Both IVCM and smear microscopy performed similarly, with sensitivities of approximately 90%, whilst for culture this was 75% (95% CI 75.4-82.5%).

3.2 Sensitivity – different smear microscopy stains

The sensitivity for the three different smear microscopy stains used for diagnosing FK (KOH, Gram, CFW) were compared to a composite diagnosis reference standard (Table 3). KOH had the highest sensitivity (85.3%, 95% CI 81.9-88.4%), followed by Gram stain (83.2%, 95% CI 79.7-86.4%) and CFW (79.1%, 95% CI 75.4-82.5%). The three different smear microscopy staining techniques were available for 423 cases and compared in Figure 2. All three stains were positive in 336/423 (79.4%) of cases. Gram stain identified 17 cases which the other two techniques did not, KOH 8 cases and CFW 2 cases. KOH identified 396/423 (93.6%) of all cases positive by any microscopy means, Gram stain 385/423 (91.0%), and CFW identified 374/423 (88.4%). There were 34 cases of FK not detected by any of the three compound microscope techniques in the 510 instances where all tests were performed (6.7%).

Table 1: Microbial aetiology for 642 keratitis patients categorised by group of organism and diagnostic techniques, both separately and for the composite diagnosis using all methods combined.

		Diagnostic methods							
		Composite †		Microscopy		IVCM		Culture	
		n	(%)	n	(%)	n	(%)	n	(%)
Number of subjects tested by each method		642		631		638		624	
Positive tests (any organism)		565/642	(88.0)	533/631	(84.5)	476/638	(74.6)	443/624	(71.0)
Positive test by organism type									
Monomicrobial	Bacteria ‡	32	(5.0)	n/a		n/a		32/32	(100)
	Filamentous fungi	468	(72.9)	425/464	(91.6)	421/466	(90.3)	345/458	(75.3)
	Yeast	0	(0)	0	(0)	0	(0)	0	(0)
	Acanthamoeba§	0	(0)	0	(0)	0	(0)	0	(0)
Polymicrobial ‡	Bacteria ***WITH*** filamentous fungi¶	64	(10.0)	n/a		n/a		64/64	(100)
				54/64	(84.4)	55/64	(85.9)	50/64	(78.1)
	Bacteria ***WITH*** yeast	1	(0.2)	n/a		n/a		1/1	(100)
				0/1	(0)	0/1	(0)	1/1	(100)

† The composite reference standard was generated by combining the positive results from microscopy ± IVCM ± culture
‡ Only culture was used to detect bacterial keratitis
§ *Acanthamoeba* were investigated for by IVCM and/or smear microscopy only as culture facilities for *Acanthamoeba* were not available
¶ Bacteria were identified by the results of culture only (microscopy was not used for diagnosis of bacterial keratitis)
IVCM, *in vivo* confocal microscopy
Fungal infection was identified by more than one method; the numbers identified by multiple methods are described in Figure 1

Table 2: Sensitivity values for detecting filamentous fungi (n=532) using smear microscopy, in vivo confocal microscopy, and culture compared to a composite diagnosis reference standard (mixed bacterial-fungal infections included). The number of positive and negative test results are shown on the left. The sensitivity values are shown on the right.

Diagnostic method	**Composite diagnosis reference standard †**		**Totals ‡**		**Value (% CI)**	
	Positive	Negative				
Microscopy						
Positive	479	0	479	**Sensitivity %**	90.7	(87.9-93.1)
Negative	49	103	152			
Total	528	103	631			
IVCM						
Positive	476	0	476	**Sensitivity %**	89.8	(86.9-92.3)
Negative	54	108	162			
Total	530	108	638			
Culture						
Positive	395	0	395	**Sensitivity %**	75.7	(71.8-79.3)
Negative	127	100	227			
Total	522	100	622			

IVCM, *in vivo* confocal microscopy

† The composite diagnosis reference standard is where an individual tests positive for an organism group (*Acanthamoeba*, bacteria or fungus) in one or more of the three diagnostic investigations

‡ The total number of individuals in the composite diagnosis reference standard differs for each organism group and investigation as not every individual had all three investigations performed. When comparing the tests to the composite reference standard, only the total number of patients who had the particular test in question being performed are included. Please refer to Table 1 for the number of diagnostic tests performed for each organism group in question.

Table 3: Sensitivity values for detecting filamentous fungal keratitis (n=532) using different smear microscopy stains compared to a composite diagnosis reference standard (mixed infections included). The number of positive and negative test results are shown on the left. The sensitivity values are shown on the right.

Diagnostic method	Composite diagnosis reference standard†		Totals		Value (% CI)	
	Positive	Negative				
KOH						
Positive	413	0	413	**Sensitivity %**	85.3	(81.9-88.4)
Negative	71	95	166			
Total	484	95	579			
Gram stain						
Positive	417	0	417	**Sensitivity %**	83.2	(79.7-86.4)
Negative	84	59	143			
Total	501	59	560			
Calcofluor white						
Positive	417	0	417	**Sensitivity %**	79.1	(75.4-82.5)
Negative	110	101	211			
Total	527	101	628			

KOH= Potassium hydroxide; CI = Confidence interval.
† Composite diagnosis reference standard is defined as a positive result for at least 1 of the following: culture, smear microscopy or *in vivo* confocal microscopy.

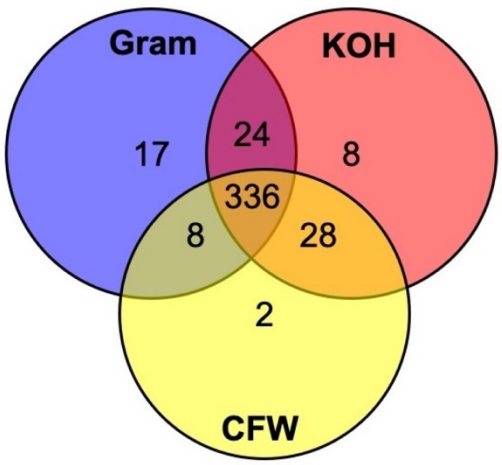

Figure 2: Venn diagram showing the number of cases that were positive for diagnosing filamentous fungal keratitis (including mixed bacterial-fungal cases) by smear microscopy (n=423) using different smear microscopy stains: Gram, potassium hydroxide (KOH), and calcofluor white (CFW). Cases that were positive for more than one stain are given within the overlapping areas.

4. Discussion

This study compares the relative sensitivity of three techniques in identifying fungal keratitis in a low-income setting with a high prevalence of fungal keratitis. We have shown in this study that the overall diagnostic yield was high at 88% (565/642) of patients with a clinical diagnosis of MK. Of these, we detected 468 cases of FK, 32 cases of BK and 65 mixed bacterial-fungal infections including one yeast. For FK, whilst all three investigations were positive in two-thirds of cases and all contributed to the overall yield, microscopy (both smear and IVCM) proved the most useful with the highest yield and sensitivities. All investigations contributed additional diagnoses that would been missed if only two of these three techniques were used. Due to the diagnostic criteria for bacterial keratitis, all cases of bacterial keratitis were diagnosed by culture. Whilst having all three diagnostic techniques increases the overall yield, in settings with limited resources and a high prevalence of fungal keratitis, these results suggest it is worth investing in smear microscopy performed by a suitably trained and experienced microbiologist. However, in these settings it is still important to remember that culture is required to accurately diagnose bacterial keratitis, and so should be performed as a minimum if no fungal hyphae are visible on microscopy or there is a significant presence of bacteria on Gram stain.

When comparing the three different smear microscopy staining techniques to a composite referent, we found all to fare well with sensitivities of over 79%; KOH performed best with sensitivities of 85.3%, although Gram staining identified the highest number of cases that were not diagnosed by other means (17 compared to 8 detected by KOH and 2 by CFW). Whilst increasing the number of stains performed increases the overall yield, in a resource limited setting our results suggest that KOH and Gram stain should be the minimum techniques employed. Gram stain has the further advantage of detecting bacterial organisms. Although we did not evaluate Giemsa staining in this study, it is also able to identify bacterial and fungal infections,[24] so could be considered if Gram staining is not available. Lactophenol cotton blue is another stain that has been shown to be useful in detecting fungal hyphae,[9,12] however this was primarily used for fungal identification from subcultured isolates in this laboratory, rather than for initial corneal smear examination, and so we chose not to report these results.

We have previously reported the unusual pattern of fungal organisms responsible in this region, with over half of cases of FK caused by dematiaceous fungi,[22] and, including mixed infections, fungal keratitis accounted for 82.9% of all MK cases. Nepal is a country with one of the highest reported incidences of FK globally,[25] and is the 29th poorest country in terms of GDP per capita.[26] Most secondary-level hospitals within Nepal have access to basic microbiology facilities, mainly in the form of smear microscopy, with culture facilities found only in tertiary clinical facilities which are mainly located in cities. The same scenario is commonly encountered throughout the LMICs in tropical latitudes, where diagnostic resources are limited or absent and the burden of fungal keratitis is the greatest.[3,25] As a result, cases of microbial keratitis are often treated empirically or based solely on clinical examination, which is known to be inaccurate.[27,28] Anecdotally, there is a reluctance to perform corneal scrapes and send specimens for smear microscopy due to the perceived costs involved and limited benefit in terms of diagnosing the organism responsible. We feel that this view should be challenged in settings with a high burden of FK: smear microscopy, particularly with KOH, is fast, low-cost, and accurate.

Our finding that smear microscopy gave a high yield with high sensitivities is in contrast to previous work from eastern Nepal conducted between 1998-2001, which found the yield to be low at 48%.[29] This may be due to subsequent improvements in techniques and that this earlier study was conducted in a general hospital using routine microbiology services, as opposed to a dedicated ophthalmic hospital laboratory as in our study. Smear microscopy with KOH gave the highest sensitivity of all smear microscopy techniques for diagnosing fungal keratitis. This finding agrees with results from most other studies from Asia, where the sensitivity of KOH has been demonstrated to range between 80-99.3% when compared to a non-clinical diagnosis-based gold standard (e.g. culture).[9,13,30]

The high sensitivity of smear microscopy for detecting FK in this region is likely in part attributable to the amount of smear material available for analysis; the yield from large ulcers (>2mm) has been shown to be higher than small ulcers.[9] Patients with FK in our study have large ulcers, with median ulcer size 2.55mm.[21] This is largely due to the late presentation of patients, and inappropriate initial treatment given by pharmacists and traditional healers. Conversely, the yield from smear microscopy in temperate, high-income countries, where patients present earlier in the course of the disease with small infiltrates and bacterial keratitis is far more commonly implicated, is considerably lower,[8,31,32] bringing into question its usefulness in the routine diagnosis of microbial keratitis in such settings.[33]

Fluorescent staining of fungal material using calcofluor white has previously been shown to be a highly accurate technique, with two studies reporting sensitivities as high as 97% and superior to KOH staining.[24,30] It is believed the high sensitivities are attributable to CFW fluorescence being easy to identify against the background of heterogenous corneal material,[34] although it does require a ultraviolet microscope to function, which not all laboratories may have access to. Given the fluorescent nature of the result, it may even be possible for technicians or non-microbiologists to use with limited smear microscopy experience. However, we found the sensitivities of CFW to be lower than previously reported and not as high as KOH. A possible reason for these false negatives may be that the corneal scrape did not contain enough material or there were no hyphae in the portion of the sample taken, or that our "expert" microbiologist was very competent in other staining techniques due to previous experience, meaning CFW, although easy, was not of much additional use. Further research comparing the accuracy of non-microbiologists in using CFW is warranted to see if there is a role for it in this setting. It is noteworthy that a previous study from Iran reported the sensitivity of CFW and KOH to be low, at 42% and 28.5% respectively,[35] although the "gold standard" referent in this study was a clinical diagnosis of FK, making comparisons limited.

There have been several previous studies assessing the diagnostic accuracy of IVCM for FK.[7,10,17,36] The two studies from south Asia reported similar sensitivities of 85.7% and 89.2% to what our present study found.[10,36] Of note, the sample size of our present study is larger (642) compared to these (239 and 148). Two studies from the UK, where FK is relatively rare and based on considerably smaller sample sizes (11 and 15), found the sensitivity of IVCM to range between 55.8% and 81.8%, and was dependent on the experience of the grader.[7,17] Our study confirms that IVCM is a very useful tool in detecting fungi, particularly in areas with a high prevalence of FK in the hands of experienced graders.

There were several strengths to our study. This was a large, prospective, consecutive-case study, conducted in an area with a high prevalence of FK, that followed a rigid, published protocol and standard operating procedures.[20,21] Most other studies assessing diagnostic accuracy for diagnosing fungal keratitis have used culture as the "gold standard" reference.[7,8,10,11,17-19] However, culture is known to have low sensitivity for fungal keratitis.[7] Our study used a composite reference standard rather than culture, which also included IVCM and smear microscopy to detect as many cases as possible.

Limitations to this study are also acknowledged. Firstly, we did not have the facilities for performing PCR on our participants and so we are unable to comment on how this investigation could fit into the overall diagnostic approach for infectious keratitis in our setting. However, the accuracy of PCR for fungal keratitis in a clinical setting has recently been found to be lower than expected,[7] whilst PCR remains too expensive for routine use in LMICs. Secondly, when using a composite reference as the "gold standard", it is not possible to calculate a useful specificity from this data. However, sensitivity could be argued to be of more clinical use in settings where atypical organisms can be encountered and treatment differs considerably between potential diagnoses (e.g. bacterial versus fungal keratitis). Thirdly, our diagnostic criteria for bacterial keratitis relied on positive culture, making any assessment of the accuracy of culture itself impossible, although it

should be noted that no diagnosis was available in only 12% of cases overall. Related to this, the number of patients with bacterial keratitis in this study was low. It is possible that bacterial keratitis is more successfully treated than fungal keratitis in secondary eye care settings, leading to more fungal cases presenting at this tertiary level. Furthermore, we did not detect any cases of *Acanthamoeba* and only one yeast (polymicrobial mixed bacterial-yeast infection). This may be due to the low prevalence in this setting, the limited diagnostic facilities available, or the lack of experience in detecting *Acanthamoeba* and/or yeasts on IVCM.[17] Finally, we did not have a complete data set for corneal smear slides examined with lactophenol cotton blue-stained smear microscopy because this technique was predominantly used for fungal identification, as is standard practice, and so we did not report the results from this staining technique.

Considering our findings, we propose the following diagnostic approach given in Table 4 for clinicians working in low-resourced settings in tropical and sub-tropical latitudes where fungal keratitis is more prevalent.

Table 4: Proposed diagnostic approach for clinicians working in low-resourced settings in tropical and sub-tropical latitudes where fungal keratitis is more prevalent

1. High index of suspicion. Fungal keratitis should be considered at first presentation, particularly if there are clinical features that are more commonly seen in fungal keratitis such as serrated / feathery margins, satellite lesions, raised slough and pigment.[25,37,38]
2. Perform confocal microscopy (if available). However, most settings will not have access to IVCM and therefore clinicians should proceed directly to step 3.
3. Perform corneal scrapes. Follow previously published techniques on corneal scraping including how to streak the material on slides and culture media.[2,37] The yield from corneal scrapes increases with increasing ulcer size. [9] Any infiltrates larger than 2mm must be scraped, and ideally infiltrates between 1 and 2mm should also be scraped. However, a negative result in a smaller ulcer may be false-negative and so should be interpreted with caution. Ideally corneal material should be smeared thinly and evenly onto each microscope slide; if the smear preparation is too thick this may affect the staining process and will be difficult to interpret. Corneal scaping itself may be of some therapeutic benefit by improving penetration of topical medications and reducing the infectious burden.[2,39] As a minimum, two slides should be sent (one for Gram stain and one for KOH) for smear microscopy.
4. Microbiology testing. We recommend the use of KOH and Gram stain on two separate specimen slides prior to inoculating culture media. Culture is also recommended, especially to diagnose bacterial cases, but facilities may not be available. The use of CFW is a helpful addition if a UV microscope is available as it allows for the hyphae to be clearly visible in relation to the septae. Smear microscopy should be performed as soon as possible after taking the specimen, to enable a diagnosis to be made and treatment to be started before the patient leaves the facility. A strong working partnership with the hospital laboratory is desirable.

5. Conclusion

In conclusion, we found that microscopy (both smear and confocal) were the most sensitive tools for identifying fungal keratitis. KOH-wet mount slides appeared to be the most sensitive of the different staining techniques we analysed. Culture remains a useful tool for diagnosing bacterial infections, although in settings where there is a high

prevalence of fungal keratitis, a diagnosis can be made based on the presence or absence of fungal hyphae.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. It was approved by the London School of Hygiene & Tropical Medicine Ethics Committee (Ref. 14841) and Nepal Health Research Council Ethical Review Board (Ref. 1937).

Informed Consent Statement: Written informed consent in Nepali was obtained from all subjects before enrolment in the study. If the patient was unable to read, the information was read to them, and they were asked to indicate their consent by application of their thumbprint, which was independently witnessed.

Data Availability Statement: The datasets generated during and/or analysed during the current study will be available upon request from M.J.B. (matthew.burton@lshtm.ac.uk). The full data set will be available with all patient identifiable details removed. Data will be available after formal reporting of the study findings in a peer-reviewed scientific publication. Datasets will only be available to bona fide scientific investigators. Requests should be made to the Chief Investigator in writing detailing the scientific investigators background and intended use for the data. Consideration will be given to all proposed analyses, with likely envisaged uses including investigators planning on conducting meta-analyses for example. Patient Information Sheets and consent forms specifically referenced making anonymised data available and this has been approved by the relevant ethic committees.

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Chapter 7: Topical chlorhexidine 0.2% versus topical natamycin 5% for fungal keratitis in Nepal: rationale and design of a randomised controlled non- inferiority trial

View of Mount Everest (Sagarmatha) from Lahan on an unusually clear day

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Student ID Number	324283	**Title**	DR
First Name(s)	JEREMY JOHN STANTON LUNN		
Surname/Family Name	HOFFMAN		
Thesis Title	Managing Fungal Keratitis in Nepal: A Randomised Controlled Trial Comparing Chlorhexidine 0.2% to Natamycin 5%		
Primary Supervisor	PROF. MATTHEW J. BURTON		

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Topical chlorhexidine 0.2% versus topical natamycin 5% for fungal keratitis in Nepal: rationale and design of a randomised controlled non-inferiority trial

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ABSTRACT

Introduction Fungal infections of the cornea, fungal keratitis (FK), are challenging to treat. Current topical antifungals are not always effective and are often unavailable, particularly in low-income and middle-income countries where most cases occur. Topical natamycin 5% is usually first-line treatment, however, even when treated intensively, infections may progress to perforation of the eye in around a quarter of cases. Alternative antifungal medications are needed to treat this blinding disease. Chlorhexidine is an antiseptic agent with antibacterial and antifungal properties. Previous pilot studies suggest that topical chlorhexidine 0.2% compares favourably with topical natamycin. Full-scale randomised controlled trials (RCTs) of topical chlorhexidine 0.2% are warranted to answer this question definitively.

Methods and analysis We will test the hypothesis that topical chlorhexidine 0.2% is non-inferior to topical natamycin 5% in a two-arm, single-masked RCT. Participants are adults with FK presenting to a tertiary ophthalmic hospital in Nepal. Baseline assessment includes history, examination, photography, in vivo confocal microscopy and cornea scrapes for microbiology. Participants will be randomised to alternative topical antifungal treatments (topical chlorhexidine 0.2% and topical natamycin 5%; 1:1 ratio, 2–6 random block size). Patients are reviewed at day 2, day 7 (with reculture), day 14, day 21, month 2 and month 3. The primary outcome is the best spectacle corrected visual acuity (BSCVA) at 3 months. Primary analysis (intention to treat) will be by linear regression, with treatment arm and baseline BSCVA prespecified covariates. Secondary outcomes include epithelial healing time, scar/infiltrate size, ulcer depth, hypopyon size, perforation and/or therapeutic penetrating keratoplasty (corneal transplant), positive reculture rate (day 7) and quality of life (EuroQol-5 dimensions, WHO/PBD-VF20, WHOQOL-BREF).

Ethics and dissemination The Nepal Health Research Council, the Nepal Department of Drug Administration and the London School of Hygiene and Tropical Medicine ethics committee have approved the trial. The results will be presented at local and international meetings and submitted to peer-reviewed journals for publication.

Trial registration number ISRCTN14332621; pre-results.

Strengths and limitations of this study

- First large-scale randomised controlled clinical trial comparing chlorhexidine 0.2% to natamycin 5% for the treatment of fungal keratitis.
- This study benefits from a pragmatic design: as a non-inferiority trial, if chlorhexidine is found to be within a predefined non-inferiority margin of 0.15 logMAR of natamycin at 3 months a recommendation to use chlorhexidine 0.2% can be made; this is a far cheaper, easy to formulate medication that could significantly increase access to antifungal treatment for the target population.
- Clinicians are masked to the treatment allocation, however, due to different physical appearance it is not possible to mask patients to their allocated treatment.
- This study will also assess the superiority of either medication as well as a number of key secondary outcome measures, analysed by arm.
- First randomised controlled trial investigating fungal keratitis to use in vivo confocal microscopy as a diagnostic tool for the detection of fungal elements.

INTRODUCTION

Fungal keratitis (FK) is a severe and potentially blinding corneal infection (figure 1).[1 2] The burden is greatest in tropical and subtropical countries, probably due to a combination of climate (higher temperatures and humidity) and frequent agriculture-related eye injuries.[3] It is one of the causes of microbial keratitis (MK) and accounts for between 20% and 60% of corneal infections diagnosed in

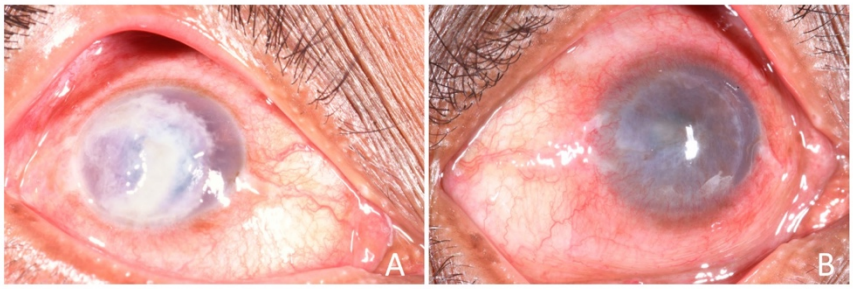

Figure 1 Fungal keratitis and corneal scarring. (A) Active fungal keratitis with signs of acute inflammation and corneal ulceration. Photograph taken at presentation to SCEH. (B) Corneal scar, the blinding sequela of a resolved episode of fungal keratitis. Photograph taken at 2 months following presentation (same patient as (A)). SCEH, Sagarmatha Choudhary Eye Hospital.

tropical regions.[4] It is often inadequately treated with significant barriers to receiving appropriate, timely intervention, compounded by indiscriminate and inappropriate use of conventional medicines such as topical corticosteroids or harmful traditional eye medicines.[1 2 5] Furthermore, when appropriate treatment is available, up to 30% of patients receiving current 'gold-standard' therapy progress to corneal perforation and/or eye-loss (figure 2).[1 6 7]

Treatment for FK almost always involves topical antifungal agents. Surgical intervention, usually in the form of therapeutic penetrating keratoplasty (TPK), is generally reserved for cases of corneal perforation or progressive infection refractory to medical therapy. Corneal transplantation is also performed for visual rehabilitation after the acute infection has resolved. There are a limited number of antifungals available for treating FK, which fall into four main groups: imidazoles, triazoles, polyenes and fluorinated pyrimidines. These may be available topically, orally or by intravenous injection. Subconjunctival, corneal stromal or intracameral injections may also be given. The treatment of yeasts (*Candida spp*) is often different to filamentous fungi, with the former being more common in temperate climates and the latter in hot and humid locations.[8 9]

There have been several clinical trials comparing treatment options for FK, which have been systematically reviewed.[10 11] Natamycin (NATA), which was approved in the 1960s by the U.S. Food and Drug Administration (FDA) for FK, has been compared with a number of newer agents, including voriconazole. Natamycin and voriconazole have been compared in four trials, with the meta-analysis favouring natamycin.[6 10 12–14]

As a result, first line management of filamentous FK is usually with topical natamycin 5% when this is available. This was added to the WHO Essential Medicines List in 2017 for this indication. However, even when intensive topical natamycin is initiated, infections frequently progress relentlessly to perforation and loss of the eye in about a quarter of cases, figure 2.[1 6 7] Moreover, in many countries, antifungal eye-drop treatments are simply not available. This includes most countries in sub-Saharan Africa, some Asian countries and some countries in Europe.[1 2] Natamycin is relatively expensive even if it is available. Therefore, additional alternative and more affordable drugs are clearly needed if the outcome of these infections is to improve.

Chlorhexidine (CHX) is an antiseptic agent, with both antibacterial and antifungal properties. It is a widely used broad-spectrum biocide, killing micro-organisms through cell membrane disruption.[15–17] CHX has been used in ophthalmology for over 30 years as an eye-drop preservative and for sterilising contact lenses, and has also been used to treat *Acanthamoeba* and FK.[9 18–22] In a study of potential antifungal treatments, CHX was effective both in vitro against FK isolates from India and Ghana, as well as in an Indian case series.[23] Subsequently, two pilot randomised controlled trials (RCTs) of CHX for FK were conducted. In the first, three CHX concentrations (0.05%, 0.1%, 0.2%) were compared with each other

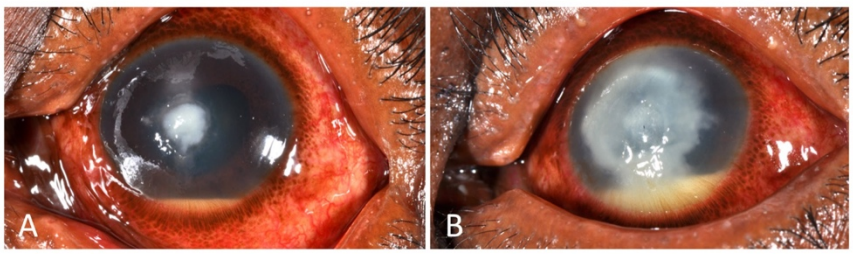

Figure 2 Progressive fungal keratitis. (A) Early filamentous fungal keratitis; started immediately on intensive topical antifungal treatment (Natamycin 5%). (B) The same case 1 week later, unresponsive to intense natamycin 5% treatment, with progression of the infection.

and natamycin 5%; this concluded CHX 0.2% had reliable antifungal action.[19] The same concentration is used in mouthwash to prevent oral candidiasis. In the second trial, CHX 0.2% was compared with topical natamycin 2.5% (half standard concentration). There was evidence CHX produced a more favourable response by 5 days (RR 0.23, 95% CI 0.09 to 0.63).[18] A systematic review found a trend favouring CHX over natamycin in 'curing' by 21 days (RR 0.70, 95% CI 0.45 to 1.09), suggesting CHX might prove superior in adequately powered trials.[10] CHX is safe and well tolerated at these concentrations.[18 19 22 24] Based on this, CHX is used for treating FK in several countries.[1 9 11] However, the combined size of these two pilot trials comparing CHX and natamycin is not sufficient to reach firm conclusions. Currently, the meta-analysis indicates equipoise in terms of which treatment is the best for treating FK.

Objectives

The primary objective of this study is to determine if topical CHX 0.2% is non-inferior to topical natamycin 5% for treating filamentous FK, in terms of vision at 3 months. The secondary objectives are: (1) to determine whether either treatment (CHX 0.2% or natamycin 5%) is superior to the other, in terms of vision at 3 months and (2) to determine whether there is a difference between CHX 0.2% and natamycin 5% in terms of secondary clinical outcomes including infiltrate/scar size, time to re-epithelialisation, reculture rates at 1 week and the effect of the alternative treatments on the Quality of Life of participants.

CHX is cheap, stable and easily prepared by aqueous dilution. If CHX is found to be non-inferior (or even superior) to natamycin this offers the potential of an effective, affordable and accessible treatment for FK, which could benefit millions of people each year who currently have no treatment options. This trial is a response to this expressed need from both clinicians and patients for a readily available and affordable medication for fungal infection.

METHODS AND ANALYSIS

Trial design

We are conducting a single-masked, non-inferiority RCT comparing CHX 0.2% to natamycin 5% for the treatment of FK. The non-inferiority design of this trial offers a clinically pragmatic way to address this important question: if CHX is found to be within the prespecified non-inferiority margin of 0.15 logMAR (about 1.5 Snellen lines) then CHX may prove to be a sustainable solution for this aspect of the complex problem of FK.

Trial summary

This RCT follows a two-stage recruitment process (figure 3). All patients presenting with acute MK are reviewed and enrolled into stage 1, following written, informed consent. This involves history, examination and investigations (corneal scrapes for microbiological assessment and in vivo confocal microscopy (IVCM)). If there is evidence of fungal hyphae on smear or confocal microscopy, patients then proceed to stage 2. A trial eligibility checklist is completed and stage 2 written informed consent is conducted. We will recruit 500 patients into

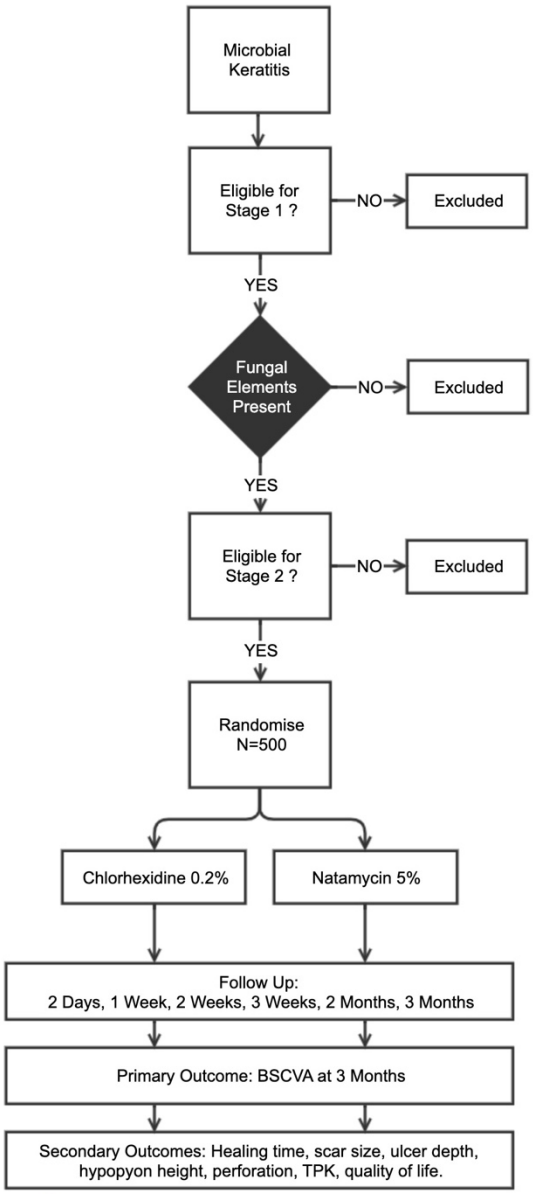

Figure 3 Overview of the clinical trial. Microbial keratitis is defined as presence of corneal epithelial ulceration (>1 mm in diameter), corneal stromal infiltrate and signs of acute inflammation (eg, conjunctival injection, anterior chamber inflammatory cells, hypopyon). Fungal elements to be detected by smear microscopy and/or confocal microscopy. Those eligible will be randomised 1:1 to CHX or NatA (n=500). BSCVA, best spectacle corrected visual acuity; CHX, chlorhexidine; TPK, therapeutic penetrating keratoplasty.

stage 2. Eligible FK patients are then randomised 1:1 to receive either natamycin 5% or CHX 0.2% topical treatments hourly for the first week, then 2 hourly for the subsequent 2 weeks. Ongoing treatment duration will then be tailored to clinical response. Study personnel are masked to the treatment allocation. Patients are usually initially admitted and followed up on day 2, day 7 (with reculture), day 14, day 21, month 2 and month 3. The primary outcome is the best spectacle corrected visual acuity (BSCVA) at 3 months.

Trial setting

Low-land Nepal, a region with a high burden of FK, provides a suitable location to conduct a clinical trial that requires a relatively large sample size. This trial will be conducted in Sagarmatha Choudhary Eye Hospital (SCEH), Lahan, Siraha District in south eastern Nepal. SCEH treats approximately 800 cases of keratitis per month, half of which is attributable to fungal infection.[1 25] SCEH is a tertiary-level ophthalmic hospital, with several satellite Eye Care Clinics (ECCs) that refer patients to SCEH directly as required. SCEH and its ECCs serve an estimated population of about 5 million people. Due to its proximity to the border, approximately 50% of the outpatients are Indian nationals. It is anticipated that the study participants will present to the hospital from multiple districts within the region. Potentially eligible individuals will be recruited from outpatient clinics or referred directly by the ECCs to the study team. Based on the numbers of patients attending, it should be possible to complete recruitment of 500 participants within 6–12 months. A second separate trial in East Africa (Tanzania and Uganda) will also be conducted to compare CHX 0.2% to natamycin 5% following a very similar protocol to the one described here. This will be registered as a separate trial. This will enable us to assess the generalisability of the findings in two geographically distinct regions with potentially different fungal aetiologies and susceptibility patterns.

Eligibility criteria

Potential participants need to meet all the inclusion criteria and have none of the exclusion criteria listed in table 1. In summary, they need to have an active FK defined as acute MK characterised by corneal epithelial ulceration (>1 mm in diameter), corneal stromal infiltrate and signs of acute inflammation (eg, conjunctival injection, anterior chamber inflammatory cells, hypopyon) in conjunction with evidence of a filamentous fungal infection on smear microscopy and/or IVCM. There is strong evidence supporting the use of IVCM for diagnosing filamentary FK. Studies have reported sensitivities of 85.7%–89.2% and specificities of between 81.4% and 92.7%, respectively.[26–28] As some patients will be enrolled on the basis of the results of IVCM which is unable to detect most bacteria reliably, some patients with microscopically confirmed fungal infection will subsequently also be found to have had mixed infection at the time of being recruited into the study, as bacterial cultures may become positive a few days after enrolment. Based on previous experience at SCEH this is likely to account for about 10% of cases. These patients are included in the study but excluded from the primary analysis of the primary outcome (see below). Secondary analyses will include mixed infections.

Consent procedures

There are two independent consent stages in this trial: stage 1, where all adult patients with MK are eligible; and stage 2, only for FK patients meeting the eligibility criteria. The two-stage process enables data collection on all potential patients at baseline before a diagnosis of FK is confirmed. All patients who are eligible to participate will be given a participant information sheet in Nepali and its contents read to them. There will be an opportunity to discuss any questions that they might have. If the patient would like to participate, they will be asked to read and sign or place a thumb print on the study consent form. The consent will be witnessed by the eye health worker, confirmed by a signature on the form. For patients who are unable to read the documentation a second witness who is unrelated to the study is required. Consent forms are given in online supplementary appendix 1.

Baseline assessment

The detailed baseline assessment is described in table 2. This includes clinical examination, corneal photography, IVCM and the collection of microbiology samples. Quality of life questionnaires will also be completed (EuroQol-5 dimensions (EQ-5D), World Health Organization Prevention of Blindness and Deafness 20-item Visual Functioning Questionnaire (WHO/PBD-VF20), World Health Organization Quality of Life: Brief Version (WHOQOL-BREF), details of the scoring for these are given in table 2.

Randomisation and masking

Sequence generation

A computer-generated randomisation list will be prepared by an independent statistician at London School of Hygiene and Tropical Medicine (LSHTM), who will hold the sequence, will not be masked, and will not be involved in any other aspect of the trial. The sequence is in a 1:1 allocation ratio of CHX to NATA, with a random block size (2, 4 or 6).

Allocation concealment and implementation

The randomisation sequences will be concealed in sequentially numbered, opaque envelopes. The envelopes will be prepared by a person independent of all other aspects of the trial. The randomisation administrator (nurse or pharmacist) will conduct the random allocation procedure. The investigational products will be stored in the trial coordination office in a locked and separate drug cabinet dedicated for this clinical trial only. The cabinet will only be used for drug storage and will only be accessed by the randomisation administrator. The randomisation administrator will work in a separate room

Table 1 Inclusion and exclusion criteria for enrolment in stage 1 (MK cases) and stage 2 (the randomised controlled trial)

Inclusion criteria (all must be met)	Exclusion criteria (any of the following)
Stage 1	
1. Acute MK characterised by:	1. Patients aged less than 18 years
► Corneal epithelial ulceration >1 mm diameter	2. Patients unable or unwilling to provide informed consent
► Corneal stromal infiltrate	3. Patients who do not have acute MK or where there is a more likely alternative diagnosis
► Acute inflammation: for example, conjunctival injection, anterior chamber inflammatory cells, hypopyon	
2. Adults (18 years and older)	
3. Able to provide informed consent	
Stage 2	
1. Acute MK characterised by:	1. Unwilling/unable to participate in trial and/or attend follow-up
► Corneal epithelial ulceration >1 mm diameter	2. Aged less than 18 years
► Corneal stromal infiltrate	3. Pregnancy: self-reported, or by urine pregnancy test if uncertain.
► Acute inflammation: for example, conjunctival injection, anterior chamber inflammatory cells, hypopyon	4. Breast feeding: self-reported
2. Filamentous fungal hyphae visualised on smear microscopy and/or IVCM	5. Prior topical antifungal treatment
3. Agree to be randomised to either treatment arm and are able to give informed consent	6. No light perception in the affected eye
4. Agree to be followed up at 2 days, 1 week, 2 weeks, 3 weeks, 2 months and 3 months	7. Fellow eye visual acuity <6/60
5. Adults (18 years and older)	8. Acanthamoebic infection visualised by smear microscopy or IVCM
	9. Clinical evidence of herpetic keratitis
	10. Known allergy to study medication (including preservatives)
	11. Previous keratoplasty in the affected eye
	12. Bilateral corneal ulcers
	13. Very severe ulcers warranting immediate evisceration or conjunctival flap
	14. Endophthalmitis

IVCM, in vivo confocal microscopy; MK, microbial keratitis.

in the clinic. After the patient has been recruited and assessed, they will be guided to this separate room where the randomisation allocation will be conducted.

Masking

The topical treatments being compared in this trial have a different appearance: the CHX 0.2% is a clear, colourless solution and the natamycin 5% is an opaque, white suspension. Therefore, it is not possible to mask the participants to this difference in visual appearance. However, the patients will not be told which treatment they have been allocated. Prior to any follow-up clinical examinations, a nurse, otherwise uninvolved in the study, will wipe away any white natamycin residue from the patient's eyes to avoid unmasking the clinical assessor. This procedure was used successfully in other trials.[6] All clinicians involved in the clinical assessment of the patients will be masked to the allocation. The statistician who will perform the primary analysis will be masked to the allocation and only receive the actual allocation code sequence from the independent statistician after the analysis code has been prepared and pre-tested with a test sequence. The primary outcome is visual acuity at 3 months assessed by an optometrist who will not have been involved in any other aspects of the trial and masked to the allocation. By 3 months, the treatment courses are likely to be completed. There will be masked grading of the photographs to independently confirm outcome measures and assess for any systematic bias on the part of the clinical examiners.

Unmasking

Unmasking is a serious action and should only be performed if necessary to ensure the safety of a study participant. Anyone unmasked to randomised treatment

Table 2 Baseline assessment

Assessment	Details
Visual acuity	Presenting, Pin-Hole and best spectacle corrected visual acuity) will be measured using an ETDRS Tumbling-E logMAR 3 m chart (Good-Lite Inc, USA) mounted on an ESC 2000 ETDRS LED Cabinet, (Good-Lite Inc, USA) by a trial-certified optometrist, for each eye separately
Contrast sensitivity	Measured using the Peek Contrast Sensitivity smartphone application running on Android OS with a Sony Xperia Z3 Compact smartphone (Sony, Japan).[26]
Clinical photographs	Photographs will be taken separately of both corneas using a Nikon D7500 camera with an AF-S Micro Nikkor 105 mm lens and lens mounted SB-200 flash units (Nikon, Japan). A standardised photography protocol is used to ensure images can be compared between time points. Standardised magnification will be used to allow epithelial defect and stromal infiltrate size measurements to be made.
Slit-lamp examination	Both eyes will be examined using a slit-lamp biomicroscope (standard ophthalmology examination) to assess the anterior segment of the eye. This examination will be performed by an ophthalmic clinician experienced in managing MK. Particular attention will be paid to the following features: 1. Eyelids: trichiasis, lagophthalmos, facial weakness, Bell's reflex 2. Suppuration 3. Conjunctival inflammation 4. Corneal sensation 5. Cornea epithelial defect (measuring the longest dimension and the longest perpendicular) and ulcer depth 6. Corneal inflammatory infiltrate depth, size, profile, colour, edge pattern, texture, satellites 7. Anterior chamber inflammatory cells, hypopyon, endothelial plaque 8. Relative afferent pupillary defect
In vivo confocal microscopy (IVCM)	The Heidelberg Retinal Tomograph 3 IVCM enables the clinician to examine the cornea down to the cellular level. It is able to detect the presence of fungal hyphae.[27 28] A sterile, single-use disposable cap covers the objective lens and is changed between patients. Volume scans will be performed which provide a series of 400×400 µm images over a depth range of 80 µm. The resolution of the corneal scanning module is 7.6 µm. IVCM images will be collected in a systematic way, starting at the centre of the ulcer, then at the superior, inferior, nasal and temporal borders of the ulcer. Volume scans will be performed in all of these locations, starting at the level of the corneal epithelium, and ending at the deepest affected aspect of the cornea assessed from IVCM images. Images will be assessed during the examination.
Ocular sample collection	The following samples will be collected from the corneal ulcer of each patient at the baseline assessment: 1. Corneal scrape specimens for microscopy and microbiological culture. A corneal scrape will be collected from the corneal ulcer after application of preservative free proxymetacaine local anaesthetic eye-drops (Minims). Sterile needles are used to take corneal scrape specimens and then place on to glass slides for immediate Gram stain, KOH and Calcofluor white. Samples will be directly inoculated onto blood, chocolate, Sabouraud agar and broths for culture. 2. Corneal specimen collection for PCR. Two sterile swabs will be gently swept over the surface of the corneal ulcer and placed into a 2 mL tube. The swabs will be for pathogen detection by PCR, fungal sequencing and assessment of point of care tests for fungal infections. Swabs will be stored dry at −80°C. If swab yields are found to be too low for analysis an additional corneal scrape will be collected for PCR. The analysis of the PCR samples will not form part of the RCT workup and report.
HIV testing	All individuals presenting with MK would be offered counselling and testing services. If this is found to be positive and the patient is unknown to the HIV care services an appropriate referral will be made. HIV testing is performed using HIV Tri-Dot rapid diagnostic test (J. Mitra & Co, India)
Random blood glucose	There is a suggestion that individuals with diabetes may be more susceptible to FK. Participants will be offered a random blood glucose test, on a finger prick sample, analysed using HumaLyzer Primus (HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Germany). If this is above 6.1 mmol/L they will be referred to the hospital physicians for assessment and formal diagnosis of impaired glucose tolerance or diabetes mellitus. This level is considered a suitable cut-off to detect individuals with diabetes and has been validated in a south-Asian population.[34]

Continued

Table 2 Continued

Assessment	Details
Quality of life questionnaires	For those with confirmed FK and who are enrolled in the trial, there will be several additional baseline assessments to evaluate the impact of FK on quality of life. Vision-related quality of life (VRQoL): will be assessed by a vision disease specific tool the WHO/PBD-VF20.[35] This tool measures the impact of visual impairment in the person's life including mental well-being, dependency and social functioning. These have been used in a number of other vision related studies to show a difference in QoL.[36 37] This instrument consists of 20 questions divided into three sub-scales: visual symptom, general functioning and psychosocial. It begins by asking the patient *'Overall, how would you rate your eyesight using both eyes?'*; and uses a five point scale answer option such as *'very good', 'good', 'moderate', 'bad', 'very bad'*. The test is scored out of 100, with higher scores reflecting a better VRQoL. General health-related quality of life: We will use the EQ-5D questionnaire and EQ-Visual Analogue Scale. The EQ-5D is a standardised tool to measure health outcomes.[38] Patients will also be assessed using the WHOQOL-BREF.[39] This has good applicability in low and middle-income countries as it was developed simultaneously from concept across 18 countries in Africa, Asia and Latin America. It measures 4 domains of health: Physical Health, Psychological Health, Social Relationships, and Environment. It asks respondents 26 questions how much (frequency) they have experienced and/or were able to do things (eg, feel safe, able to concentrate, enjoy life) in the past 4 weeks and how satisfied they are with certain aspects of their lives (eg, sleep, capacity for work). Each question is scored between one and five in a positive direction, with one being attributed for a very low or dissatisfied quality of life, and five being very good or very satisfied with their quality of life (ie, higher scores denote a higher quality of life). Each domain has its own score calculated by calculating the mean of the item scores within each domain. In addition, there are two items that are examined separately: question 1 asks about an individual's overall perception of quality of life and question 2 asks about an individual's overall perception of their health. These are scored on the same positive scale from one to five. The mean domain scores can then be multiplied by four in order to make domain scores comparable with the scores used in the WHOQOL-100.[39]

Assessment performed at baseline with details of how they are made.
AF-S, Autofocus Single; EQ-5D, EuroQol-5 dimensions; ETDRS, Early Treatment Diabetic Retinopathy Study; FK, fungal keratitis; KOH, Potassium Hydroxide; MK, microbial keratitis; RCT, randomised controlled trial.

for the purpose of creating analyses for independent data and safety monitoring committees will not be involved in any other aspects of the conduct or final analysis of the study. A list will be maintained of all unmasked members of staff and will be approved by the chief investigator. Such staff members must sign a document to indicate that they are aware of their responsibilities with respect to confidentiality. The processes used to provide access to unmasked treatment codes and reports relating to these codes shall be documented.

Intervention and treatment

The standard of care in this hospital is for cases of FK to be admitted. If willing, patients will be admitted for close observation and supervised treatment, until there are signs of improvement and the supervising clinician considers it safe to provide ongoing management on an outpatient basis.

Trial treatment arms

1. CHX 0.2% w/v eye-drops
   CHX 0.2% w/v solution, as eye-drops, will be applied to the surface of the infected eye (one drop per application). The CHX 0.2% w/v eye-drops used in these studies is produced by Mandeville Medicines, UK.
2. Natamycin 5% eye-drops
   Natamycin 5% w/v suspension, as eye-drops, will be applied to the surface of the infected eye (one drop per application). Topical natamycin 5% is produced by FDC Pharmaceuticals, India.

Dosing schedule

Both trial treatment arms will follow the same dosing schedule. Eye-drops will be given hourly day and night for 48 hours, then hourly while awake for 5 days, and then 2 hourly while awake for two further weeks. If the ulcer has healed (epithelial defect less than 1 mm, infiltrate resolved, with or without corneal scarring), then treatment is stopped. If resolving stromal infiltration and/or epithelial defect >1 mm but <5 mm, treatment is reduced to four times daily. If resolving but with epithelial defect >5 mm and/or stromal infiltration/hypopyon, treatment is reduced to six times daily. Treatment duration will be tailored to clinical response, with patients reviewed regularly while on treatment in addition to their scheduled study visits.

Topical treatments

Several other topical medicines are used in the standard care of people with corneal infections:

1. Fluorescein sodium ophthalmic strips (Contacare Ophthalmics and Diagnostics, India) to highlight the area of cornea epithelial defect.
2. Anaesthetic eye-drops to anaesthetise the cornea before procedures such as microbiology samples:

Proxymetacaine 0.5% eye-drop Minims (Bausch and Lomb, UK).
3. Antibiotic eye-drops may be used as per the judgement of the treating ophthalmologist if there is a suspicion of mixed bacterial and FK: Moxifloxacin 0.5% eye-drops (Centaur Pharmaceuticals, India).
4. Mydriatic eye-drops for pupil dilation to reduce discomfort from the infection: cyclopentolate 2% eye-drops (Aurolab, India), three times a day.
5. Ocular hypotensive eye-drops if the intraocular pressure is elevated >25 mm Hg. This will be at the discretion of the supervising clinician; usual first line treatment is timolol 0.5% eye-drops (Allergan, India).

Ancillary treatment in patients refractory to trial medication

In patients with progressive FK despite 7 days or more of trial medication, additional treatment options will be considered and offered. After repeating microbiological tests to rule out a mixed bacterial infection, deteriorating patients are started on topical voriconazole 1% hourly if the ulcer is superficial only. For ulcers that are deeper than 75% of the corneal thickness, oral ketoconazole 200 mg two times a day is added (with monitoring of liver function). The choice of ancillary treatment is due to local availability. If, following 7 days of additional treatment, despite these measures there is ongoing progression, surgical management such as a TPK can be considered.

Non-pharmacological treatment

It is sometimes necessary to perform surgical procedures during the management of corneal infections. In the context of the trial these will be performed by the supervising consultant ophthalmologist. These can include:

1. Insertion of a bandage contact lens for very small perforations.
2. Tissue glue and patch for small perforations.
3. Corneal transplant (TPK) for progressive fungal infections that are refractory to medical management or large perforations.
4. Conjunctival flaps to cover corneas in non-healing ulcers.

Outcome measures

Primary outcome measure

The primary outcome will be BSCVA in logMAR units at 3 months. This will be measured by a trial-certified optometrist, independent of all other aspects of the study and masked to allocation. This has been the primary outcome of several other trials and will therefore facilitate comparison. Three months is the time at which clinical experience suggests most corneal ulcers have usually healed. BSCVA is chosen as it is easy to measure and is of functional significance. We will measure the BSCVA using an LED-backlit, Tumbling-E LogMAR chart (Good-Lite, Illinois, USA) under controlled conditions. We will also measure vision using Peek Acuity, a validated smartphone application, in situations where the patient is unable to attend the hospital for follow-up and outcome data is only available through a domiciliary visit.[29] For patients who have counting fingers (CF) vision or less, predefined logMAR values will be assigned based on previous clinical trials.[30 31] Similarly, for patients who undergo a corneal transplant (TPK), a predefined visual acuity of 1.9 logMAR will be given, or last observation carried forward (whichever is the better vision), as per previous studies.[6]

Secondary outcome measures

We will be assessing a number of secondary outcome measures that relate to different measures of visual acuity such as Peek Acuity; clinical signs of healing such as reduction of epithelial defect; microbiological culture rates and other clinical outcome measures such as scar size or perforation rate. These secondary outcomes together with their analyses are outlined in the Analysis Plan section.

Outcome assessments

Participants will be reassessed at 2 days, 1 week, 2 weeks, 3 weeks, 2 months and 3 months following enrolment. Additional examinations, outside the trial protocol schedule may be conducted by the supervising clinician as indicated. The specific assessments to be carried out at each visit are indicated in table 3. These will be conducted in the same way described for the baseline assessment as described (table 2). At each follow-up assessment, the participant will be asked about adherence to treatment and symptoms, including side effects, if still receiving trial treatment. This will be recorded in the case record file. To monitor trial medication adherence, the study participants will be asked to bring their eye-drop bottles to the 1-week, 2-week and 3-week follow-ups. The amount of remaining medication will be measured by weighing the bottle and will be compared with the anticipated remaining amount provided the drops were being used as instructed, resulting in a ratio of actually remaining to what is anticipated. Participants will be resupplied with medication as needed. The presenting visual acuity will be measured on each visit. In addition, the BSCVA will also be measured at the 3-month follow-up (primary outcome measure). At the final 3-month follow-up, the three quality of life questionnaires will be repeated. On each occasion the eyes will be examined using a slit-lamp and the cornea photographed. The IVCM will be repeated at 1, 2 and 3 weeks to determine whether there is evidence of fungal hyphae resolution with the fragmentation of linear elements. At the 1-week follow-up if the ulcer has not healed it will be rescraped for repeat culture to determine whether the keratitis is now culture positive or culture negative. We will give the study participants appointment cards for the next follow-up and they will be reminded about the follow-up a week prior to the date. Public transport costs will be paid for participants who are outpatients.

Treatment review

At each follow-up visit, participants will be reviewed by an ophthalmic clinician experienced in the management of FK. Clinical responses to antifungal therapy tend to be relatively slow (compared with bacterial infections). Prolonged topical treatment courses of 4–6 weeks are usually needed.

Table 3 Baseline and follow-up assessment components

Assessment item	Baseline	Day 2	Day 7	Day 14	Day 21	Day 60	Day 90
History/baseline questionnaire	X						
Check treatment adherence		X	X	X	X	X	X
Check for side effects		X	X	X	X	X	X
Visual acuity—presenting	X	X	X	X	X	X	X
Visual acuity—BSCVA	X						X
Contrast sensitivity	X						X
Slit-lamp examination	X	X	X	X	X	X	X
Cornea photography	X	X	X	X	X	X	X
In vivo confocal microscopy	X		X	X	X		
Cornea samples (microbiology/PCR)	X		X				
Quality of life tools	X						X

BSCVA, best spectacle corrected visual acuity.

Therefore, a change in therapy is usually not made for at least the first week. Other interventions may be indicated such as application of glue to corneal perforations, conjunctival flaps or TPK (corneal transplant).

Stopping rules

If the study eye develops any serious adverse outcomes, the antifungal study medication may be discontinued if it is considered to be responsible for the adverse event. The patient will then be treated at the discretion of the supervising ophthalmologist, without breaking the randomisation code. If the medication is stopped, the patient will continue with the scheduled follow-up examinations.

Lost to follow-up

Lost to follow-up rates are expected to be low, based on clinical experience. Participants who do not present for their follow-up visit will be contacted by telephone. Reasons for the lost to follow-up will be identified. Patients will be counselled about the importance of attending for ongoing treatment and monitoring. If they are unwell or unable to attend the hospital for some specific reason the study team will arrange to visit them in their home. Reasons for lost to follow-up will be recorded and reported.

Data collection, management, confidentiality and access to data

Data will be collected using paper clinical record forms. These will be stored securely at SCEH and scanned electronic copies taken at the end of the day, stored on an encrypted drive with encrypted backups made daily both on and off-site. These data will be double entered into two separate MS Access databases. After double data entry has been completed data will be cleaned using EpiData V.3.1 software. Data entry is supervised by the local study coordinator on a daily basis, with data collection and data entry progress being reviewed by the study coordinator and chief investigator at LSHTM on a weekly basis. Data protection and confidentiality is maintained through restricted access to the database system and cupboard where the paper documents are kept. Only authorised users will have access to the locked filing cabinet. The database will be password protected, with each data entry staff member having their own password. Data exports for further analysis will be anonymised.

Data and safety monitoring board

The data and safety monitoring board (DSMB) for this trial includes independent experts in bioethics, biostatistics, epidemiology and ophthalmology appointed by the trial steering committee and approved by the national regulatory authorities, before the start of the study. The DSMB meets at least twice each year and organises teleconferences as needed for progress reporting. The study protocol and modifications are subject to review and approval by ethics committees in Nepal and LSHTM, and by the DSMB. The DSMB will monitor any severe or unexpected events and oversees the data collected. The DSMB will be responsible for reviewing the results of the interim analysis and determining whether or not the trial should continue, with or without modifications.

Monitoring for harm

Patients will be monitored at each visit for adverse events or reactions. We will follow standardised definitions for adverse events, adverse reactions, unexpected adverse reactions, serious adverse events or reactions, and suspected unexpected serious adverse reaction. These, along with the reporting scheme, are given in online supplementary appendix 2.

Biological specimens

The processing and analysis of biological specimens are detailed in online supplementary appendix 3.

Sample size considerations

The study is powered to test the hypothesis that CHX is non-inferior to NATA in terms of the primary outcome (BSCVA at 3 months) at a prespecified non-inferiority margin (Delta) of 0.15 logMAR units. It is possible that CHX is non-inferior to NATA, given pilot RCT data that showed no statistically

Table 4 Secondary outcome measures that will be investigated as part of the trial, together with analysis details

Secondary outcome measure	Details
Three-week BSCVA	We will analyse the secondary outcome of 3 weeks BSCVA in logMAR in the same manner as the primary analysis of the primary outcome described above. The 3 weeks BSCVA will include values taken between 18 days and 5 weeks, with the value closest to 3 weeks used.
Presenting VA by Peek	We will analyse the presenting VA by Peek Acuity with and without pinhole at 3 months as a secondary outcome. This will be of interest if we are unable to get reliable BSCVA measurements at 3 months (ie, if patients fail to attend and we need to attend their houses for visual acuity testing). This will be performed in the same way as the primary analysis of the primary outcome. We will also perform a sensitivity analysis including those lost to follow-up, by using the most recent observation of this variable.
Scar/infiltrate size at 1 week, 3 weeks and 3 months by slit lamp examination	The geometric mean of the two principle axes in mm of the scar or infiltrate at 1 week, 3 weeks and 3 months will be used as a secondary outcome variable. The slit-lamp scar size will be compared at each of these time points between treatment arm in the same manner as described above. This will be by linear regression, with treatment arm and baseline infiltrate/scar size as pre-specified covariates. This controls for the baseline infiltrate/scar size.
Time to full epithelial healing (slit lamp examination by ophthalmic clinician)	Time of re-epithelialisation will be defined as the midpoint between the last review where an epithelial defect (ED) was present and the subsequent review where there was no ED. An area of fluorescein staining of less than 0.5 mm will be considered as a resolved ED due to the difficulty in differentiating a smaller defect from a small amount of fluorescein pooling observed in a healed defect. Analysis of time to healing will use Cox proportional hazards regression with treatment group as the primary predictor and with predictors of baseline ED size (using the geometric mean in mm as outlined above). Survival curves will be plotted using Kaplan-Meier analysis for both treatment arms up to the final visit at 3 months. The proportional hazards assumption will be checked by stratifying on quartiles of the baseline ED size and if the assumption does not hold, the stratified results will be the ones reported. Additionally, treatment failure (defined as persisting epithelial defect greater than 0.5 mm at the 3 month review) will be compared between treatment groups using Fisher's exact test.
Rate of healing	We will assess how quickly the area of ulceration reduces over time. The rate will be calculated between the 1-week, 3-week and 3-month review by taking the difference in ED size between the two time points, in mm, and diving by the number of days to give a rate of mm/day. Analysis will be performed using Cox regression.
Microbiological cure	Patients who have a persisting corneal ulcer (as defined by the presence of an ED) at day 7 will undergo a repeat corneal scrape and microbiological investigations. Microbiological cure at 7 days will be defined as the absence of any micro-organisms as no significant growth on culture. The number of patients with microbiological cure at day 7 will be compared between the two treatment arms using logistic regression with treatment group and organism (*Aspergillus* spp, *Fusarium* spp, or other) as covariates.
Ulcer depth at 1 week and 3 weeks (slit lamp examination by ophthalmic clinician)	The depth of ulcer in terms of percentage of healthy cornea will be compared at 1 week and 3 weeks between treatment arms, adjusting for baseline depth in the same manner with analysis performed by linear regression
Hypopyon height at 1 and 3 weeks, (slit lamp examination by ophthalmic clinician)	The hypopyon height in mm will be compared at 1 week and 3 weeks between treatment arms, adjusting for baseline hypopyon height in the same manner with analysis performed in the same way (linear regression)
Perforation and/or TPK and/or conjunctival advancement by 3 months (slit lamp examination by ophthalmic clinician)	The number of patients who undergo perforation and/or require TPK and/or have undergone conjunctival advancement by 3 months will be reported using CIs and descriptive statistics. The study is not powered to detect a difference in perforation rate or TPK between treatment groups; not reporting a significant difference may be wrongly interpreted as there being no difference between groups. We will therefore perform an exploratory analysis to compare TPK or perforation rates between treatment groups. This will be by logistic regression to compute an OR by arm.
Loss of eye	The number of patients who have their eye surgically removed (evisceration or enucleation) during the 3 months follow-up period will be reported using CIs and descriptive statistics in the same way as for TPK/perforation rate above, along with exploratory analysis using logistic regression to find risk factors for eye loss and OR for this by arm.

Continued

Table 4 Continued

Secondary outcome measure	Details
Ocular adverse effects, slit lamp examination by ophthalmic clinician	The proportion of patients with one or more adverse events will be compared using Fisher's exact test. Additional analysis to compare the rate of adverse events during the 3 months follow-up will be by Poisson regression as this can take into account multiple instances within one participant.
QoL assessed using: EQ-5D, WHO/PBD-VF20, WHOQOL-BREF	QoL can be assessed quantitatively using different tools depending on what is of interest. For example, disease-related QoL can be assessed (eg, vision related QoL, VRQoL) or more general health-related issues irrespective of the disease can be investigated (health-related QoL, HRQoL).[40] We will use the WHO/PBD-VF20 (WHO/ Prevention of Blindness and Deafness—Visual Functioning 20-item questionnaire) VRQoL tool. This tool measures the impact of visual impairment in the person's life including mental well-being, dependency and social functioning. These have been used in a number of other visual related studies to show a difference in QoL.[36 37] For HRQoL, we will use the EQ-5D questionnaire, EQ-Visual Analogue Scale and the WHOQOL-BREF. The EQ-5D is a standardised tool to measure health outcomes.[38] The WHOQOL-BREF has good applicability in (LMIC as it was developed simultaneously from concept across 18 countries in Africa, Asia and Latin America. It measures four domains of health: Physical Health, Psychological Health, Social Relationships, and Environment. Details of this scoring are given in table 2.[39] Analysis will be by comparing the scores obtained for each QoL assessment for the two treatment arms to estimate the effect of CHX and NATA on patients' QoL. This will be similar to that performed by Habtamu *et al*.[40] Comparisons between the two medication groups will be adjusted for the matching variables: age and sex. The VRQoL analysis was also adjusted for socio-economic status and the HRQoL analysis adjusted for both socioeconomic status and presence of health problems during the previous 4 weeks, as these factors may confound the association between fungal keratitis and QoL. Logistic, linear and ordinal logistic regression methods will be used for binary, continuous and ordered categorical outcome variable analysis, respectively. Linear regression models and the t-test were employed to compare significant differences in QoL scores and to generate mean and mean differences between the two treatment arms in each QoL subscale and domain, respectively.
Cost-effectiveness analysis, using EQ-5D data from 3 months and direct cost data	Direct cost data will be collected at the 3 months follow-up. Economic costs to the patient can also be calculated from the EQ-5D questionnaire, which will be asked at baseline and at the 3 months follow-up. Mean direct costs incurred by patients will be compared between interventional arms using the t-test for significance. The difference from the baseline EQ-5D and the 3 months EQ-5D mean scores will also be compared in a similar fashion.
Drug adherence	The rate of drug adherence will be compared between the two treatment groups using descriptive statistics.

BSCVA, best spectacle corrected visual acuity; EQ-5D, EuroQol-5 dimensions; LMIC, low-income and middle-income countries; QoL, quality of life; TPK, therapeutic penetrating keratoplasty.

significant difference between CHX and NATA, with a Cochrane review finding a non-significant trend favouring CHX over NATA.[10 18 19] Despite this trend favouring CHX, we have chosen to carry out a non-inferiority trial rather than a superiority trial as this is a more clinically pragmatic approach. It would be counterproductive to conduct a superiority trial and find no statistically significant difference between the two treatments leading clinicians to potentially disregard CHX as a treatment, when in fact it may be 'non-inferior' to NATA, and be the only available or most cost-effective treatment.

The choice of delta: This clinically meaningful difference of 0.15 logMAR was chosen as a difference of 0.15 logMAR corresponds to approximately 1.5 lines on a Snellen chart; any difference greater than this is clinically significant, as a difference of less than 0.15 log MAR could potentially be accounted for by testing/retesting error.[29] Furthermore, it was used in the MUTT1 trial, providing methodological consistency between studies.[6] In addition, previous studies have suggested treated ulcers improve at a mean of four Snellen lines from baseline.

Sample size was calculated for 90% power and adjusted final alpha of 0.0492, taking account of a single interim analysis using O'Brien-Fleming approach to maintain type-1 error rate of 5%. A sample size of 452 is required to detect a non-inferiority margin of 0.15-logMAR in BSCVA 3 months after enrolment between arms, assuming 0.5 SD for 3 months BSCVA and 15% drop-out. However, given approximately 10% of infections are mixed and these will be excluded from the primary analysis, 500 patients will be recruited. This sample size provides 90% power to detect superiority in BSCVA at 3 months if there is ≥0.17 LogMAR units difference as a secondary analysis.

Analysis plan

The analysis will be by intention to treat (ITT). All patient data will be analysed according to their randomisation

Table 5 Registration data and protocol summary

Data category	Information
Primary registry and trial identifying no	ISRCTN Registry; ISRCTN14332621
Date of registration in primary registry	15 May 2019
Secondary identifying numbers	
Source(s) of monetary or material support	Wellcome Trust
Primary sponsor	London School of Hygiene and Tropical Medicine
Secondary sponsor(s)	
Contact for queries	Jeremy Hoffman FRCOphth (Jeremy.hoffman@lshtm.ac.uk)
Title	Chlorhexidine 0.2% vs Natamycin 5% for the treatment of fungal corneal infections
Countries of recruitment	Nepal
Health condition(s) or problem(s) studied	Fungal keratitis
Intervention(s)	Participants will be randomised to either topical chlorhexidine 0.2% or topical natamycin 5%
Key eligibility criteria	1. Acute MK characterised by: ► Corneal epithelial ulceration >1 mm diameter ► Corneal stromal infiltrate ► Acute inflammation: for example, conjunctival injection, anterior chamber inflammatory cells, hypopyon 2. Filamentous fungal hyphae visualised on smear microscopy and/or in vivo confocal microscopy 3. Agree to be randomised to either treatment arm and able to give informed consent 4. Agree to be followed up at 2 days, 1 week, 2 weeks, 3 weeks, 2 months and 3 months 5. Adults (18 years and older)
Study type	Randomised controlled trial
Date of first enrolment	1 June 2019
Target sample size	500
Recruitment status	Recruiting
Primary outcome(s)	Best Spectacle Corrected Visual Acuity at 3 months by a trial certified optometrist
Key secondary outcomes	1. Time to full epithelial healing (slit lamp examination by ophthalmic clinician) 2. Pin-hole visual acuity in logMAR at 3 months, trial-certified optometrist 3. Scar/infiltrate size at 1 week, 3 weeks and 3 months (slit-lamp examination by ophthalmic clinician) 4. Ulcer depth at 1 week and 3 weeks (slit-lamp examination by ophthalmic clinician). 5. Hypopyon height at 1 and 3 weeks, (slit-lamp examination by ophthalmic clinician). 6. Perforation and/or TPK by 3 months (slit-lamp examination by ophthalmic clinician). 7. Positive culture rate at 1 week 8. Ocular adverse effects at each follow-up visit (day 2, day 7, day 14, 3 weeks, 2 months, 3 months), slit-lamp examination by ophthalmic clinician 9. Quality of life (QoL) assessed using: EQ-5D, WHO/PBD-VF20, WHOQOL-BREF (comparison between baseline and QoL measures at 3 months) 10. Cost-effectiveness analysis, using EQ-5D data from 3 months and direct cost data 11. Drug adherence at each follow-up visit (day 2, day 7, day 14, 3 weeks, 2 months, 3 months) while the patient is using study medications

EQ-5D, EuroQol-5 Dimension; LMIC, low-income and middle-income countries; MK, microbial keratitis; TPK, therapeutic penetrating keratoplasty.

allocation irrespective of whether or not the patient received or adhered to the allocated treatment. Consolidated Standards of Reporting Trials guidelines for analysing/reporting non-inferiority RCTs will be followed. A flow chart showing cases assessed, recruited and followed up by arm will be prepared.[32] Baseline characteristics will be summarised by arm. The Standard Protocol Items: Recommendations for Interventional Trials checklist is given in online supplementary appendix 4.

Primary outcome analysis: unadjusted analysis

The primary analysis will be conducted using all available data, missing data due to lost to follow-up will be excluded. The primary analysis of the primary outcome (BSCVA at 3 months) will be by linear regression, with treatment arm and baseline BSCVA as prespecified covariates. This controls for the baseline BSCVA. The treatment group is the primary predictor. Primary analysis will exclude mixed fungal and bacterial infection (isolated in the baseline sample).

We will use our alpha of 0.0492 to test the null hypotheses at 0.0492 significance. The null hypothesis for non-inferiority is that the mean BSCVA at 3 months for CHX is greater than or equal to 0.15 logMAR worse than the BSCVA when natamycin is used. Significance will be assessed using a two-tailed test at 0.0492 level for assessing non-inferiority. CHX will be non-inferior if the upper one-sided 95% confidence level for this regression coefficient (ie, the effect of CHX controlling for baseline BSCVA) exceeds 0.15 logMAR.

Primary outcome analysis: adjusted analysis

In the event that there is a baseline imbalance between the treatment groups in a baseline covariate due to chance, we will perform an adjusted (sensitivity) analysis (see below). This is particularly important if CHX has a better outcome than NATA, as the adjusted treatment effects may account for this observed imbalance while the unadjusted analyses may not. Sensitivity analyses will allow us to show that any observed positive treatment effect is not solely explained by imbalances at baseline in any of the covariates.

Secondary analyses of the primary outcome

Per-protocol analysis

Repeat analysis of the primary outcome will be done as per the protocol, based on what the participants actually took. The per-protocol population will include all the individuals included in the primary ITT analysis, excluding individuals who showed poor compliance with the medications (defined as taking less than 50% through self-reporting or bottle weighing, whichever is the lower); individuals where there have been major protocol deviations; and non-fungal or mixed corneal infections (should these patients happened to have been randomised). All analyses that are performed as ITT will be repeated as per protocol and labelled as such.

Mixed infections

Secondary analysis of the primary outcome (by ITT) will include mixed infections and will be carried out in the same way as in primary analysis of the primary outcome above.

Sensitivity analyses

For the primary analysis those individuals with missing outcome data (ie, lost to follow-up) will be excluded. Sensitivity analyses will be performed by imputing a range of scenarios to demonstrate a range of potential results, where there is missing outcome data. In the case of substantial missing data in the trial, the primary analysis will be carried out as previously stated excluding missing observations. This, however, assumes data are missing completely at random. As a sensitivity analysis, we will then apply a multiple imputation approach to the missing data, if we consider the data are randomly missing conditional on other observed covariates.

In the case that there is a systematic (ie, non-random) reason for a difference in the follow-up rates between the two groups, we will explore models in which these missing outcome data are assumed to be non-random, that is, dependent on the outcome being regressed, BSCVA or treatment group.

We will also perform sensitivity analyses on patients whose vision is CF or worse, those patients who have undergone a TPK or those with corneal perforation, to see if there is any change in the effect size or conclusions drawn.

Analysis of other potential determinants for success

Logistic regression random-effects models will be used to analyse potential factors that may be associated with a poor primary outcome, BSCVA at 3 months, defined as >1.0 logMAR. Individual baseline characteristics will be used separately as an exposure variable with BSCVA at 3 months as the outcome, with the model adjusted for trial arm. A multivariate model will be built using parameters with a $p<0.2$ in the log likelihood ratio test. Variables will be removed one by one, by omitting the variable with the largest p value each time, until all predictors in the model have a $p<0.05$.

Secondary outcome analysis

The secondary outcomes outlined above and detailed in table 4 will be analysed by arm. Additional adjustment for factors imbalanced between arms at baseline will be introduced as appropriate. Continuous outcomes will be analysed using linear regression. Binary and ordinal outcomes will analysed using logistic regression. Details of these are given in table 4.

Interim analysis

An interim analysis will be conducted for the DSMB by an independent statistician after 1/3 of patients recruited have completed follow-up.

Patient and public involvement

Mixed methods descriptive cross-sectional studies with semistructured interviews and focus group discussions with patients and eye health providers were carried out in Sagarmatha zone, Eastern Nepal at various points in 2018.[33] These conversations highlighted the delayed presentation often seen with FK combined with the often prohibitively high costs of treatment. Furthermore, treatment is not always felt to be effective. Eye health workers were keen to receive further training and highlighted the need for greater government support in the provision of eye care services in the community.

ETHICS AND DISSEMINATION

Ethics committee and regulatory review and approval has been obtained from the Nepal Health Research Council (NHRC) Ethics Committee, Kathmandu, Nepal; the Department of Drug Administration (DDA), Kathmandu, Nepal; and the London School of Hygiene and Tropical Medicine Ethics Committee, UK. The trial is registered with the ISRCTN clinical trials registry. Protocol modifications are submitted to the relevant parties for review and/or approval. Table 5 summarises the study protocol and trial registration information. At the end of the study period, patients who still require treatment or follow-up will continue to be treated at SCEH as per routine clinical practice. The trial sponsor is the LSHTM. The results of this trial will be presented at local and international meetings and submitted to peer-reviewed journals for publication.

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Contributors Searched the literature: JJH and MJB. Drafted initial protocol: JJH. Contributed to protocol development and revision: JJH, RY, SDS, PC, AR, SKS, SA, EM, DM, HAW, AL, VH and MJB. Drafted this manuscript: JJH. Critically revised this manuscript: JJH, RY, SDS, PC, AR, SKS, SA, EM, DM, HAW, AL, VH and MJB. Conceptualisation: MJB. Funding acquisition: MJB.

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Chapter 8: Topical Chlorhexidine 0.2% versus Topical Natamycin 5% for the Treatment of Fungal Keratitis in Nepal. A Randomized Controlled Noninferiority Trial

Dr Sanjay Kumar Singh (Medical Director of SCEH), Dr Reena Yadav (Nepali Primary Investigator), and members of the Data Safety Monitoring Board (DSMB), chaired by Prof. Reeta Gurung (second from right) pose for a photograph following the initial DSMB meeting

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Surname/Family Name	HOFFMAN		
Thesis Title	Managing Fungal Keratitis in Nepal: A Randomised Controlled Trial Comparing Chlorhexidine 0.2% to Natamycin 5%		
Primary Supervisor	PROF. MATTHEW J. BURTON		

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SECTION E

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Topical Chlorhexidine 0.2% versus Topical Natamycin 5% for the Treatment of Fungal Keratitis in Nepal

A Randomized Controlled Noninferiority Trial

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Purpose: To investigate if topical chlorhexidine 0.2%, which is low cost and easy to formulate, is noninferior to topical natamycin 5% for the treatment of filamentous fungal keratitis.

Design: Randomized controlled, single-masked, noninferiority clinical trial.

Participants: Adults attending a tertiary-level ophthalmic hospital in Nepal with filamentous fungal infection confirmed on smear or confocal microscopy.

Methods: Participants were randomly allocated to receive topical chlorhexidine 0.2% or topical natamycin 5%. Primary analysis (intention-to-treat) was by linear regression, using baseline logarithm of the minimum angle of resolution (logMAR) best spectacle-corrected visual acuity (BSCVA) and treatment arm as prespecified covariates. Mixed fungal-bacterial infections were excluded from the primary analysis but included in secondary analyses and secondary safety-related outcomes. The noninferiority margin was 0.15 logMAR. This trial was registered with ISRCTN, number ISRCTN14332621.

Main Outcome Measures: The primary outcome measure was BSCVA at 3 months. Secondary outcome measures included perforation or therapeutic penetrating keratoplasty by 90 days.

Results: Between June 3, 2019, and November 9, 2020, 354 eligible participants were enrolled and randomly assigned: 178 to chlorhexidine and 176 to natamycin. Primary outcome data were available for 153 and 151 of the chlorhexidine and natamycin groups, respectively. Of these, mixed bacterial-fungal infections were found in 20 cases (12/153 chlorhexidine, 8/151 natamycin) and excluded from the primary analysis. Therefore, 284 patients were assessed for the primary outcome (141 chlorhexidine, 143 natamycin). We did not find evidence to suggest chlorhexidine was noninferior to natamycin and in fact found strong evidence to suggest that natamycin-treated participants had significantly better 3-month BSCVA than chlorhexidine-treated participants, after adjusting for baseline BSCVA (regression coefficient, −0.30; 95% confidence interval [CI], −0.42 to −0.18; $P < 0.001$). There were more perforations and emergency corneal grafts in the chlorhexidine arm (24/175, 13.7%) than in the natamycin arm (10/173, 5.8%; $P = 0.018$, mixed infections included), whereas natamycin-treated cases were less likely to perforate or require an emergency corneal graft, after adjusting for baseline ulcer depth (odds ratio, 0.34; 95% CI, 0.15–0.79; $P = 0.013$).

Conclusions: Treatment with natamycin is associated with significantly better visual acuity, with fewer adverse events, compared with treatment with chlorhexidine. Natamycin remains the preferred first-line monotherapy treatment for filamentous fungal keratitis. *Ophthalmology 2021;■:1–12 © 2021 by the American Academy of Ophthalmology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).*

Supplemental material available at www.aaojournal.org.

Microbial keratitis (MK) is a severe, frequently blinding corneal infection. In tropical regions, fungal keratitis (FK) accounts for more than half of MK, with an estimated global annual incidence of approximately 1 million cases.[1,2] The incidence is higher in low- and middle-income countries,[1] particularly among agricultural workers.[3] In temperate regions, although less common than bacterial keratitis, FK is increasing, with infection associated with contact lens use.[1-3]

Fungal keratitis is challenging to manage; there are significant barriers for patients accessing treatment, resulting in

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delays and poor outcomes.[4,5] This is exacerbated by indiscriminate use of harmful topical corticosteroids and traditional eye medicines.[6] The preferred treatment for FK is topical natamycin 5% based on the results of the Mycotic Ulcer Treatment Trials (MUTT), 3 additional clinical trials, and a systematic review favoring natamycin over voriconazole[7-11]; however, despite treatment with natamycin, some reports suggest approximately one quarter of patients continue to progress to corneal perforation and ultimately blindness or evisceration/enucleation.[4,7,12] Despite recently being designated as an essential medicine by the World Health Organization,[13] natamycin is not available in most countries in sub-Saharan Africa, as well as some countries in Asia and Europe.[4] It is relatively expensive and difficult to formulate. Additional alternative and affordable medications are needed.

Chlorhexidine is a broad-spectrum, antiseptic biocidal agent that kills microorganisms through cell membrane disruption.[14] It has been used in various forms in ophthalmology for >30 years, including as an eye drop preservative, for sterilizing contact lenses, and for treating fungal and *Acanthamoeba* keratitis.[15,16] In the early 1990s, chlorhexidine 0.2% was reported effective in vitro against fungal isolates in an Indian case series.[17] A systematic review of 2 subsequent small, underpowered randomized controlled trials found a nonsignificant trend favoring chlorhexidine over natamycin in "curing" by 21 days (relative risk, 0.70; 95% confidence interval [CI], 0.45−1.09).[11,18,19] Chlorhexidine has the additional advantages of being inexpensive, easy to formulate, and generally well tolerated. In view of the clinical equipoise surrounding using natamycin or chlorhexidine for FK, we hypothesized that chlorhexidine might be noninferior to natamycin for the treatment of FK.

Methods

Study Design

We performed a randomized controlled, single-masked, noninferiority clinical trial comparing outcomes in patients with FK receiving natamycin 5% or chlorhexidine 0.2%. The trial was conducted at Sagarmatha Choudhary Eye Hospital (SCEH), Lahan, Nepal. The SCEH is a large, tertiary-level referral eye hospital, with 14 satellite eye care clinics that refer patients to SCEH. It serves a population of approximately 5 million people in Nepal. Additionally, because SCEH is 17 km from the Indian border, approximately half of patients are Indian nationals.

Ethical and regulatory approval was obtained from the Nepal Health Research Council Ethics Committee, the Nepal Department of Drug Administration, and the London School of Hygiene and Tropical Medicine Ethics Committee, United Kingdom. The study adhered to the principles of the Declaration of Helsinki. The trial protocol has been published.[20] The trial was registered with ISRCTN, number ISRCTN14332621.

Participants

Eligible patients were adults with acute MK (corneal epithelial ulceration >1 mm in diameter, corneal stromal infiltrate, and signs of acute inflammation) with evidence of filamentous fungal infection on in vivo confocal microscopy (by visualization of fungal hyphae) or smear microscopy (by visualization of fungal elements on potassium hydroxide, calcofluor white, or Gram stain). Exclusion criteria are listed in full in Table S1 (available at www.aaojournal.org) and included prior topical antifungal use, no perception of light visual acuity in the affected eye, and very severe ulcers warranting immediate surgical intervention (e.g., impending perforations and perforated corneal ulcers).

Recruitment was in 2 stages to facilitate data collection on all potential participants at baseline before FK diagnosis was confirmed. All consenting adult patients with signs of acute MK were eligible for Stage 1 before microscopy (in vivo confocal microscopy and smear after corneal scrape) to confirm the type of infection. In vivo confocal microscopy diagnosis of FK was performed by experienced operators. Corneal scrapes were also performed for microbiological cultures for fungal organism identification and to diagnose any mixed bacterial-fungal infections. Only consenting people with filamentous fungal infection meeting the criteria in Table S1 were eligible for Stage 2 and inclusion in the trial. These participants then underwent masked intervention assignment.

Randomization and Masking

Participants were randomly assigned (1:1) to chlorhexidine 0.2% or natamycin 5%. A computer-generated randomization list was prepared by an independent statistician using Stata 16 (StataCorp LP) and random block sizes of 2, 4, or 6. Treatment allocation was concealed in sequentially numbered, opaque envelopes. The random allocation procedure was conducted by an independent randomization administrator with no other involvement in the trial.

Given that the topical treatments under investigation in this study have different appearances (chlorhexidine is a clear, colorless solution, whereas natamycin is an opaque, white suspension), it was not possible to mask participants; however, patients were not told which treatment they were allocated to, and bottle labels were replaced with standardized labels with "A" or "B" replacing the drug name. Clinicians were masked to treatment allocation, with a nurse not otherwise involved in the trial ensuring that any natamycin residue was removed before examination.[7] Compliance was checked through self-reporting and weighing of study medication bottles. The statistician performing the primary analysis was masked to allocation and only received the allocation sequence after the analysis code was prepared and pretested. Best spectacle-corrected visual acuity (BSCVA) at 3 months (primary outcome measure) was assessed by an optometrist masked to treatment allocation and not otherwise involved in any other aspects of the trial.

Procedures

Patients were randomized to chlorhexidine 0.2% weight/volume (w/v) (preservative-free, aqueous dilution including unspecified buffer, produced by Mandeville Medicines) or natamycin 5% w/v (preserved with benzalkonium chloride 0.01% and manufactured by FDC Pharmaceuticals Ltd). Dosing schedules were identical between arms and consisted of 1 drop applied to the affected eye every 1 hour per day and night for 48 hours, then hourly while awake for 5 days, then every 2 hours while awake until 3 weeks from enrollment. Further continuation and treatment duration of the masked medication depended on the clinical response. If the ulcer had healed (epithelial defect [ED] <1 mm, infiltrate resolved, with or without corneal scarring), then the treatment was stopped. If there was resolving stromal infiltration or ED >1 mm but <5 mm, treatment was reduced to 4 times daily. If the stromal infiltration or hypopyon was resolving, but the ED was still > 5 mm, treatment was reduced to 6 times daily. All antifungal medications were kept

in a dark place at <25 ° C. Topical medications were replenished as needed at follow-up. For ethical reasons, ophthalmologists added or changed any adjunctive medications if deemed clinically necessary, including ocular antihypertensives for raised intraocular pressure and topical mydriatics for cycloplegia and pain relief. Ophthalmologists prescribed topical antibiotics (moxifloxacin 0.5% w/v) if there was evidence of a mixed bacterial-fungal infection (based on microbiological culture results reported to the ophthalmologist from baseline or subsequent corneal scrapes) or if the ED was >5 mm, as per local protocols.

In patients with progressive FK despite 7 days or more of trial medication, ancillary treatment was considered. After repeating microbiological tests to rule out a mixed bacterial-fungal infection, deteriorating patients with superficial infiltration were started on topical voriconazole 1% hourly in addition to their trial medication. For infiltrates involving >75% of corneal thickness on slit-lamp examination, oral ketoconazole 200 mg twice daily was added (with monitoring of liver function). Ancillary treatment choices were limited by local availability. If, after 7 days of additional treatment, despite these measures, there was progression, surgical management such as therapeutic penetrating keratoplasty (TPK) was contemplated. The decision to perform TPK or not rested with the ophthalmologist, who considered factors including location and depth of the ulcer, limbal involvement, and size of the perforation.

Participants were counseled before enrollment and advised to return to SCEH in the event of any concerns or worsening symptoms rather than attend alternative facilities. Patients were directly asked whether they had visited other health care facilities (including pharmacists and traditional healers) at each follow-up.

Outcomes

Patients were assessed at baseline and at 2, 7, 14, 21, 60, and 90 days postenrollment. The BSCVA was measured at enrollment and at 90 days by a masked trial-certified optometrist. The BSCVA protocol followed was that used in the MUTT and the Steroids for Corneal Ulcers Trials studies,[7,12] which was adapted from the Age-Related Eye Disease Study using a 3 m, proportionally reduced version of the 4 m Early Treatment Diabetic Retinopathy Study tumbling "E" chart (Good-Lite) at 3 m,[21] with low-vision testing at 0.75 m. Presenting and pinhole visual acuity were also measured at each visit by trial-certified eye health workers using Peek Acuity, a validated smartphone application.[22]

The primary outcome measure was BSCVA at day 90. Secondary outcome measures included presenting (unaided) visual acuity at 21 and 90 days; scar/infiltrate size at 7, 21, and 90 days; time to full epithelial healing (defined as the midpoint between the preceding visit when an ED was present and the subsequent visit when the ED was absent or measured <0.5 mm); microbiological cure rate (baseline culture-positive patients who became culture-negative at day 7); ulcer depth at 7 and 21 days; hypopyon height at 7 and 21 days; perforation or TPK by 90 days; loss of eye by 90 days; and ocular adverse events.

A calibrated slit-lamp biomicroscope was used to assess infiltrate or scar size, ED, depth, hypopyon, and ocular adverse events at each follow-up. Measurements of infiltrate, scar, and ED involved measuring the longest dimension and longest perpendicular,[7,12] a protocol previously adapted from the Herpetic Eye Disease Study.[23] Reepithelialization was defined as the absence of an ED with the administration of fluorescein. Depth was assessed in 4 categories: >0% to 25%, >25% to 50%, >50% to 75%, and >75%. All grading ophthalmic clinicians were trial certified and masked to treatment allocation. Patients were monitored at each visit for adverse events or drug reactions. We followed standard definitions for adverse events, including drug toxicity.

Statistical Analysis

Analyses followed a predetermined plan.[20] The trial was powered to test the hypothesis that chlorhexidine is noninferior to natamycin with respect to the primary outcome at a prespecified noninferiority margin of 0.15 logarithm of the minimum angle of resolution (logMAR) units. A sample size of 500 patients (250 per arm) was fixed before enrollment and estimated to provide 90% power to detect a 0.15 logMAR difference in BSCVA at 3 months between arms, assuming ±0.5 standard deviation for 3 months BSCVA, a type I error rate of 5%, 10% mixed bacterial/fungal infections and 15% drop-out, and a single interim analysis.

Baseline characteristics were summarized by arm. Linear regression was used for primary analysis of the primary outcome, with treatment arm and baseline BSCVA as prespecified covariates, excluding mixed infections. Primary analysis was by intention-to-treat. Secondary analyses of the primary outcome included a per-protocol analysis and analysis of the primary outcome (by intention-to-treat) including mixed infections.

For secondary outcome analysis, the geometric mean of the longest diameter and the longest perpendicular was used to assess infiltrate or scar size and ED size.[7] Analysis was by linear regression for infiltrate or scar size, using treatment arm and infiltrate or scar size at baseline as covariates. Linear regression was used for ulcer depth and hypopyon height, with treatment arm and baseline ulcer depth as covariates. Time to full epithelial healing was analyzed using a Cox proportional hazards model, with treatment arm and baseline ED size as covariates. Adverse events between arms were compared by Fisher exact test. A logistic regression model with covariates for treatment arm and baseline infiltrate depth was used to assess the odds of corneal perforation or TPK.

For patients who underwent TPK, we assigned a 3-month logMAR of 1.9.[7] For infiltrate, scar, and ED size, we used the most recent value before surgery. Sensitivity analyses for patients lost to follow-up were conducted using linear mixed-effects regression, including all outcomes measured for each patient. All analyses were conducted using Stata 16 (StataCorp LLC).

A Data Safety and Monitoring Board (DSMB) was established before enrollment to oversee safety, data quality, and trial conduct. The DSMB met every 6 months during recruitment. One planned interim analysis was conducted after one third of all planned patients (167/500) had completed their 90-day follow-up and presented to the DSMB. After this initial interim analysis, recruitment was paused by the trial steering committee and endorsed by the DSMB, with advice to perform a second (unplanned) interim analysis that included all patients who had completed their 90-day follow-up to that date (319/500). After the second interim analysis, recruitment was stopped at 354 participants. The 35 patients under active management completed their allocated treatment and follow-up.

Results

Between June 3, 2019, and November 9, 2020, 890 patients with suspected MK were assessed. Of these, 643 were confirmed to have clinical features of acute MK and consented to undergo further investigations (Stage 1). Smear or confocal microscopy identified 525 cases of filamentous FK, of which 354 patients (67.2%) met eligibility criteria and consented for enrollment in the clinical trial (Stage 2). Reasons for exclusion are given in Figure 1. All 354 eligible patients were randomized; 178 were allocated to chlorhexidine, and 176 were allocated to natamycin.

Recruitment and follow-up were paused on March 24, 2020, and resumed on June 13, 2020, because of regulatory restrictions relating to the coronavirus disease 2019 pandemic. There were 46 patients who completed the study whose final review was originally scheduled during this period; 33 of 46 patients were delayed by >30 days because of the restrictions; however, there was no evidence of a difference between arms ($P = 0.3311$). Recruitment was stopped on November 9, 2020, after the interim analysis and guidance from the DSMB. Follow-up was completed on February 8, 2021. There were 25 and 23 patients for whom day 90 outcome data were not available in the chlorhexidine and natamycin arms, respectively. An additional 2 patients were seen at home in the natamycin group; therefore, 90-day BSCVA was unavailable. Mixed bacterial-fungal infections were present in 15 of 178 patients (8.4%) and 10 of 176 patients (5.7%) at baseline, with 90-day outcome data available for 12 of 178 patients and 8 of 176 patients randomized to chlorhexidine or natamycin, respectively. Therefore, we included 141 patients randomized to chlorhexidine and 143 patients randomized to natamycin in the primary analysis (Fig 1).

The mean age of enrolled participants was 46.7 years (standard deviation ± 13.3), and 219 of 354 (61.9%) were female. Baseline demographic and clinical characteristics were generally well balanced between groups (Table 1). Most clinical features, including infiltrate size, ulcer depth, and presence of hypopyon, were similar across the 2 groups; however, in terms of visual acuity, there were more "blind" eyes in the chlorhexidine group than in the natamycin group (40 vs. 27 with visual acuity worse than 3/60).

Fungal keratitis was diagnosed by in vivo confocal microscopy alone for 30 cases (8.5%), whereas microscopy only was positive in 27 cases (7.6%). The most commonly isolated organisms were *Curvularia* species (118/354, 33.3%), *Fusarium* species (47/354, 13.3%), and *Aspergillus* species (32/354, 9.0%). There was no growth in 47 of 354 (13.3%) microscopy positive cases, whereas there were 34 of 354 (9.6%) unidentified filamentous fungi due to nonsporulation in vitro or loss to contamination (Table S2 available at www.aaojournal.org).

Among the 284 participants included in the primary analysis, the baseline mean BSCVA was 0.61 logMAR (95% CI, 0.51−0.70) in the chlorhexidine group and 0.55 logMAR (95% CI, 0.46−0.64) in the natamycin group. By day 90, this had changed to 0.64 logMAR (95% CI, 0.51−0.77) in the chlorhexidine arm and 0.26 logMAR (95% CI, 0.18−0.35) in the natamycin arm, which was a change of 0.03 logMAR and −0.29 logMAR, respectively. After adjusting for baseline BSCVA, we estimate that BSCVA among patients treated with chlorhexidine is approximately 3 lines worse than those treated using natamycin (regression coefficient, −0.30; 95% CI, −0.42 to −0.18). This provides no evidence ($P = 1.00$) that chlorhexidine is noninferior to natamycin and provides strong evidence ($P < 0.001$) that natamycin is superior to chlorhexidine (Fig 2). Visual acuity measurements were unavailable for 50 participants at 90 days; however, there was no evidence to suggest that loss to follow-up was associated with baseline visual acuity, baseline infiltrate size, age, gender, or treatment assignment (Table S3, available at www.aaojournal.org). Furthermore, if we used the last observation carried forward for 90-day BSCVA, the results were similar to the primary analysis (regression coefficient. −0.32; 95% CI, −0.21 to −0.43; $P < 0.001$). No participants who completed the study reported visiting other health care facilities (including traditional healers) or using additional medication after enrollment.

At 21 days, correcting for baseline BSCVA, there was no evidence of a difference in mean BSCVA between those allocated to chlorhexidine and those allocated to natamycin (regression coefficient, −0.088 logMAR; 95% CI, −0.18 to 0.059; $P = 0.066$). The 3-week mean BSCVA was 0.36 logMAR (95% CI, 0.28−0.44) in the natamycin arm compared with 0.50 logMAR (95% CI, 0.40−0.60) in the chlorhexidine arm; however, there was evidence of a difference in infiltrate or scar size between the 2 treatment arms at each follow-up interval. At day 7, the estimated mean infiltrate size was 0.26 mm (95% CI, −0.49 to −0.04; $P = 0.022$) smaller in the natamycin arm than in the chlorhexidine arm, adjusting for baseline infiltrate size. At 21 days, estimated mean infiltrate size was 0.42 mm (95% CI, −0.73 to −0.10; $P = 0.009$) smaller in the natamycin arm than in the chlorhexidine arm, adjusting for baseline infiltrate size. By 90 days, there remained evidence of a difference in scar/infiltrate size between patients randomized to the 2 treatments (regression coefficient, −0.40 mm; 95% CI, −0.57 to −0.23; $P < 0.001$), adjusting for baseline infiltrate size.

We found evidence of a difference in time to reepithelialization by treatment arm after controlling for baseline ED size through Cox proportional hazards regression ($P < 0.001$). Patients treated with chlorhexidine healed 39% more slowly than those treated with natamycin (hazard ratio, 0.61; 95% CI, 0.47−0.79) (Fig 3 and Fig S4 [available at www.aaojournal.org]). With regard to treatment failure, as defined by a persistent ED at 90-day follow-up of >0.5 mm, there were no patients in the natamycin group who had not reepithelialized compared with 11 of 122 (9.0%, $P < 0.001$; excluding mixed infections) in the chlorhexidine group.

After excluding mixed infections, there was evidence of a difference in hypopyon height at 1 week but not at 3 weeks in the patients who presented with a hypopyon, after controlling for baseline hypopyon height between arms (1-week regression coefficient, 0.46 mm; 95% CI, 0.024−0.89, $P = 0.039$; n = 59; 3-week regression coefficient, 0.19 mm; 95% CI, −0.21 to 0.59; $P = 0.340$; n = 56). Likewise, there was no evidence of a difference in ulcer depth at 1 or 3 weeks between arms after controlling for baseline depth (1-week regression coefficient, −0.92% of corneal thickness; 95% CI, −3.58 to 1.73 $P = 0.495$; 3-week regression coefficient, −1.42% of corneal thickness; 95% CI, −4.80 to 1.93; $P = 0.405$).

A slightly higher proportion of patients randomized to chlorhexidine remained culture positive at day 7 (22/83, 26.5%; 95% CI, 0.09−28.2) compared with those randomized to natamycin (11/65, 16.9%; 95% CI, 17.4−37.3), although this was not statistically significant ($P = 0.232$).

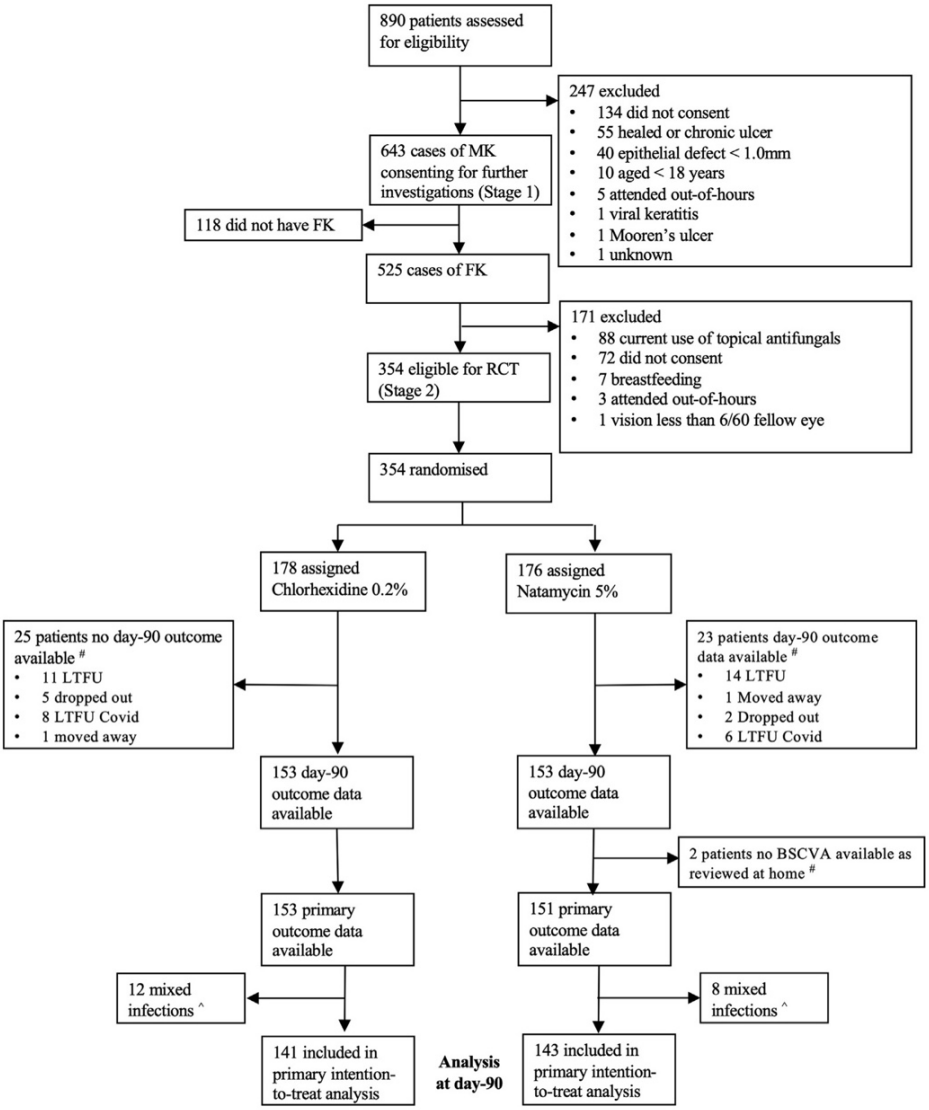

Figure 1. Trial profile. A total of 135 patients physically attended clinic for their 90-day follow-up in the chlorhexidine 0.2% arm, with additional visual acuity outcome data available in 18 patients (because they had undergone therapeutic penetrating keratoplasty or eye removal surgery), and 145 patients physically attended clinic for their 90-day follow-up in the natamycin 5% (NATA) arm, with additional visual acuity outcome data available in 8 patients (because they had undergone therapeutic penetrating keratoplasty or eye removal surgery or had no perception of light vision in the affected eye due to acute glaucoma). Ninety-day best spectacle-corrected visual acuity (BSCVA) outcome data were unavailable in 2 patients who attended in the NATA arm because these patients were reviewed at home. ^ Mixed fungal-bacterial infections are excluded from the primary analysis but included in the secondary analysis. There were 25 mixed infections in total (15 in the chlorhexidine 0.2% arm, 10 in the NATA arm) at baseline. At the day 90 follow-up, outcome data were available for 12 mixed infections in the chlorhexidine 0.2% arm and for 8 in the NATA arm. Mixed infections included the following: filamentous fungus plus any of gram-positive cocci (n = 9), gram positive bacilli (n = 6), gram negative cocci (n = 2), *Staphylococcus aureus* (n = 3), *Streptococcus pneumoniae* (n = 2), *Corynebacterium spp.* (n = 1), and *Streptococcus spp.* (n = 2). COVID = coronavirus; FK = fungal keratitis; LTFU = lost to follow-up; MK = microbial keratitis; RCT = randomized controlled trial. # Ninety-day best spectacle-corrected visual acuity (BSCVA) outcome data were unavailable in 2 patients who attended in the NATA arm because these patients were reviewed at home.

This remained the case after adjusting for the baseline causative organism by logistic regression (odds ratio [OR], 2.21; 95% CI, 0.84−5.81; $P = 0.107$). The cultured fungal organisms were different at baseline and day 7 in 8 patients (Table S4, available at www.aaojournal.org). Similar results were obtained if negative results were assumed in the 208 patients for whom repeat culture was not performed (data not shown).

Clinical outcome and adverse events are summarized in Table 2 for all patients who attended at least 1 follow-up

Table 1. Baseline Demographic and Clinical Characteristics for All Enrolled Patients (including Mixed Infections)

	Chlorhexidine (n = 178)		Natamycin (n = 176)		Total (N = 354)	
Demographic Features						
Age, yrs	46.1	(13.5)	48.2	(13.0)	46.9	(13.3)
Sex						
Male	73	(41.0%)	62	(35.2%)	135	(38.1%)
Female	105	(59.0%)	114	(64.8%)	219	(61.9%)
Literacy						
Illiterate	139	(78.1%)	138	(78.4%)	277	(78.3%)
Little Nepali (read/write)	16	(8.99%)	14	(7.95%)	30	(8.47%)
Nepali well (read/write)	9	(5.06%)	15	(8.52%)	24	(6.78%)
English and Nepali (read/write)	14	(7.87%)	9	(5.11%)	23	(6.50%)
Marital status						
Single	6	(3.37%)	3	(1.70%)	9	(2.54%)
Married	161	(90.5%)	164	(93.2%)	325	(91.8%)
Divorced	2	(1.12%)	3	(1.70%)	5	(1.41%)
Widowed	9	(5.06%)	6	(3.41%)	15	(4.24%)
Occupation						
Agriculture	91	(51.1%)	98	(55.7%)	189	(53.4%)
Nonagriculture*	87	(48.9%)	78	(44.3%)	165	(46.6%)
Trauma						
Vegetative matter/wood	73	(41.0%)	73	(41.5%)	146	(41.2%)
Other†	19	(10.7%)	18	(10.2%)	37	(10.5%)
Unknown object	2	(1.12%)	1	(0.06%)	3	(0.85%)
Contact lens	0	(0%)	0		0	
No history of trauma/unknown	84	(47.2%)	84	(47.7%)	168	(47.5%)
Previous treatment						
No	32	(18.0%)	28	(15.9%)	60	(17.0%)
Yes‡	146	(82.0%)	148	(84.1%)	294	(83.1%)
Previous topical steroids	33	(18.5%)	26	(14.8%)	59	(16.7%)
Previous TEM	2	(1.12%)	3	(1.7%)	5	(1.41%)
Previous antibiotics	120	(67.4%)	129	(73.3%)	249	(70.3%)
Previous other topical§	70	(39.3%)	60	(34.1%)	130	(36.7%)
Previous systemic medication	88	(49.4%)	81	(46.0%)	169	(47.7%)
Clinical Features						
Laterality						
Right	78	(43.8%)	89	(50.6%)	167	(47.2%)
Left	100	(56.2%)	87	(49.4%)	187	(52.8%)
BSCVA						
Mean (logMAR)	0.65	(0.62)	0.56	(0.57)	0.61	(0.60)
Median (logMAR) ^	0.45	(0.12–1.00)	0.38	(0.12–0.80)	0.40	(0.12–0.90)
6/5–6/12	61	(34.27%)	76	(43.18%)	137	(38.70%)
>6/12–6/18	40	(22.47%)	31	(17.61%)	71	(20.06%)
>6/18–6/60	34	(19.10%)	39	(22.16%)	73	(20.62%)
>6/60–3/60	3	(1.69%)	3	(1.70%)	6	(1.69%)
>3/60–1/60 (CF)	36	(20.22%)	22	(12.50%)	58	(16.38%)
>1/60 (CF) no light perception	4	(2.25%)	5	(2.84%)	9	(2.54%)
Contrast sensitivity ^	0.98	(0.45–1.20)	1.05	(0.75– 1.35)	1.05	(0.60–1.35)
Baseline infiltrate size (mm)						
Median ^	2.55	(1.75–3.70)	2.50	(1.68–3.70)	2.50	(1.75–3.70)
Median ^	2.55	(1.75–3.70)	2.50	(1.68–3.70)	2.50	(1.75–3.70)
≤0.5	0	(0%)	1	(0.57%)	1	(0.28%)
>0.5–1.5	30	(16.85%)	32	(18.18%)	62	(17.51%)
>1.5–2.5	58	(32.58%)	59	(33.52%)	117	(33.05%)
>2.5–3.5	36	(20.22%)	35	(19.89%)	71	(20.06%)
>3.5–4.5	26	(14.61%)	26	(14.77%)	52	(14.69%)
>4.5–5.5	13	(7.30%)	11	(6.25%)	24	(6.78%)
>5.5–6.5	5	(2.81%)	5	(2.84%)	10	(2.82%)
>6.5–7.5	5	(2.81%)	4	(2.27%)	9	(2.54%)
>7.5–8.5	1	(0.56%)	1	(0.57%)	2	(0.56%)
>8.5–9.5	2	(1.12%)	2	(1.14%)	4	(1.13%)
>9.5	2	(1.12%)	0	(0%)	2	(0.56%)
ED, mm ^	2.75	(2.05–3.90)	2.60	(1.90–3.78)	2.70	(2.00–3.80)
Ulcer depth						
1%–25%	117	(65.7%)	114	(64.8 %)	231	(65.3%)

Table 1. (Continued.)

	Chlorhexidine (n = 178)		Natamycin (n = 176)		Total (N = 354)	
26%–50%	54	(30.3%)	56	(31.8%)	110	(31.1%)
51%–75%	5	(2.81%)	6	(3.41%)	11	(3.11%)
76%–100%	2	(1.12%)	0		2	(0.56%)
Presence of hypopyon	37	(20.8%)	32	(18.2%)	69	(19.5%)
Hypopyon height, mm ^ ‖	0.5	(0.3–1.0)	0.6	(0.2–1.0)	0.5	(0.3–1.0)
Time from symptoms to presentation, days	8.13	(5.93)	8.36	(8.35)	8.25	(7.22)
Time from trauma to presentation, days	8.68	(6.14)	9.00	(8.09)	8.84	(7.16)
Ocular surface disease¶	3	(1.69%)	6	(3.41%)	9	(2.54%)
Dacryostenosis or dacryocystitis#	7/168	(4.17%)	3/168	(1.79%)	10/336	(2.98%)
Preexisting corneal abnormalities	0		0		0	
Preexisting eyelid or eyelash abnormalities**	3	(1.69%)	2	(1.14%)	5	(1.41%)
Diabetes mellitus††	1	(0.56%)	4	(2.27%)	5	(1.41%)

Data are n (%) or mean (standard deviation), other than where indicated with "^" when the data are median (interquartile range).
BSCVA = best spectacle-corrected visual acuity; CF = counting fingers; ED = epithelial defect; logMAR = logarithm of the minimum angle of resolution; TEM = traditional eye medicine.
*Includes unemployed, retired, and so forth.
†Includes soil, dust, insect, cow's tail, fingernail, chemicals, and clothes.
‡Some patients were receiving >1 medication at enrollment.
§Includes dilating eyedrops, lubricating eyedrops, topical antivirals, topical nonsteroidal anti-inflammatory drugs, and glaucoma medication.
‖If present.
¶Represents patients who had moderate to severe dry eye with significant punctate epithelial erosions, conjunctival scarring resulting from cicatrizing conjunctivitis or chemical burns, allergic eye disease, and so forth. It does not include patients with blepharitis alone.
#No enrolled patients had a history of dacrocystitis or had undergone a surgical procedure for dacryostenosis before enrollment. Patients were offered nasolacrimal duct syringing as part of their clinical examination. The numbers therefore represent patients who were incidentally found to have dacryostenosis during their baseline clinical examination. Some patients refused to undertake lacrimal syringing, or it was not possible due to coronavirus disease 2019 policy (10 in chlorhexidine arm and 8 in natamycin arm).
**Includes entropion, lagophthalmos, and trichiasis.
††Diabetes mellitus and human immunodeficiency virus infection were the only systemic diseases that were self-reported or investigated; there were no cases of human immunodeficiency reported or detected in study participants.

appointment (348/354), including those with mixed infections at baseline. A perforation developed or a TPK was required in 24 of 175 patients (13.7%) in the chlorhexidine arm compared with 10 of 173 patients (5.8%) in the natamycin arm (OR, 0.34; 95% CI, 0.15–0.79; $P = 0.013$, adjusting for baseline depth).

By including mixed infections in analysis of the primary outcome, we estimate that the mean BSCVA among patients

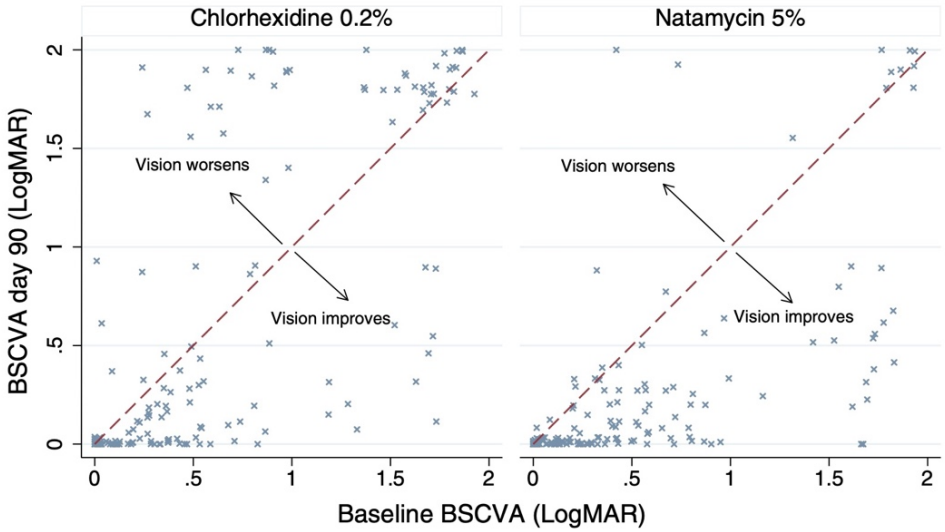

Figure 2. Ninety-day BSCVA versus baseline BSCVA for patients in each investigational arm (excluding mixed infections). Graph plotted with jitter added to prevent overlapping points. The red dashed line is where BSCVA at baseline and BSCVA at day 90 are the same. Note patients who have undergone a corneal graft (therapeutic penetrating keratoplasty) are allocated a BSCVA of 1.9 logarithm of the minimum angle resolution (logMAR) and those who have had their eye removed are allocated a BSCVA of 2.0 logMAR. BSCVA = best spectacle-corrected visual acuity; logMAR = logarithm of the minimum angle of resolution.

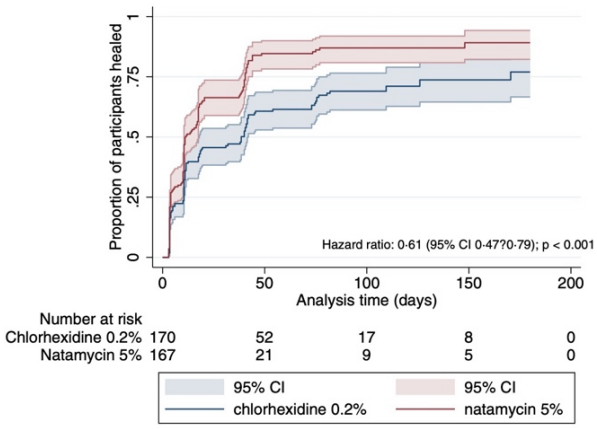

Figure 3. Kaplan–Meier survival curve plotting time to full epithelialization. Patients who had undergone a therapeutic penetrating keratoplasty or those who were eviscerated are included in this figure but are by definition "not healed." The time goes beyond the day 90 final follow-up because some patients were reviewed beyond this time because of delays resulting from the coronavirus disease 2019 pandemic. Data were missing for 8 patients in the chlorhexidine 0.2% arm and 9 patients in the natamycin 5% arm. CI = confidence interval.

randomized to chlorhexidine was approximately 3.2 lines worse than those randomized to natamycin (regression coefficient, −0.32; 95% CI, −0.43 to −0.20; $P < 0.001$) after correcting for baseline BSCVA. Given that there were more patients who had received topical steroids in the chlorhexidine arm than in the natamycin arm, we performed a sensitivity analysis excluding these patients, although these results were similar to the primary analysis results (regression coefficient, −0.283; 95% CI, −0.47 to −0.16; $P < 0.001$).

The odds of poor BSCVA (defined as worse than 1.0 logMAR at day 90 follow-up) was estimated to be 10 times higher in the chlorhexidine group than in the natamycin group (OR, 10.2; 95% CI, 3.6–28.5; $P < 0.001$) after adjusting for baseline BSCVA (OR, 6.2; 95% CI, 2.8–13.7; $P < 0.001$) and baseline mean infiltrate size (OR, 1.73; 95% CI, 1.3–2.3; $P < 0.001$). There was an extremely strong association between baseline BSCVA and a poor outcome, with almost all those who had good vision at baseline having a good outcome. In fact, none of the participants in the quartile with the best baseline BSCVA or the quartile with the smallest baseline infiltrate size had a poor visual outcome regardless of treatment allocation (Table S5, available at www.aaojournal.org). This made the overall OR estimation imprecise, which can be seen from the wide CIs. This could suggest that both chlorhexidine and natamycin are effective for mild disease, as evidenced by a good outcome for all participants within the quartile of patients with the best vision at baseline and 95% of patients within the quartile with the smallest infiltrate size at baseline (Table S5, available at www.aaojournal.org), the difference being found mostly for patients presenting with severe disease. Alternatively, mild disease may be self-limiting and heal regardless of treatment given. Other potential determinants of success (including age, sex, presence of hypopyon, ED size at baseline, clinical history or demographic features, genus and species of fungus, or presence of mixed infection) were not significant and therefore excluded.

Discussion

We tested the hypothesis that chlorhexidine 0.2% was noninferior to natamycin 5% for the treatment of FK; however, we found no evidence to support this. Visual acuity was significantly better at day 90 in participants randomized to natamycin than to chlorhexidine. Natamycin-treated cases were less likely to develop a perforation or need TPK. We also found evidence that natamycin was associated with faster reepithelialization and a slightly smaller scar or infiltrate size from day 7 onwards. There was no evidence of a difference between arms in clearing culture positivity at day 7.

Although chlorhexidine was less effective than natamycin, it is important to view these results within the broader global context, considering frequently limited availability of options and the evidence for their use. The aim of initial FK management is preservation of the eye. Previous studies have reported perforation and TPK rates of 11.1% to 43.8%.[1,4,7,12,24] In our study, both treatment arms fared generally better: 5.8% and 13.7% in the natamycin and chlorhexidine arms, respectively. In MUTT1, perforation and TPK rates for natamycin and voriconazole were 11.1% and 21.1%, respectively.[7]

If the eye has been saved, the aim is to achieve the best possible visual acuity. In MUTT1, the mean visual acuity at day 90 was 0.39 logMAR and 0.57 logMAR in the natamycin and voriconazole groups, respectively. This was an improvement of 0.27 logMAR and 0.07 logMAR from baseline for the natamycin and voriconazole groups, respectively.[7] This compares to 90-day BSCVA of 0.26 logMAR and 0.64 logMAR in the natamycin and chlorhexidine arms, respectively, in our trial, translating to an improvement of 0.29 logMAR in the natamycin arm from baseline and a worsening of 0.03 logMAR in the chlorhexidine arm. Although we cannot make direct comparisons between the 2 trials, these results suggest that chlorhexidine may have comparable effectiveness to topical voriconazole. Unlike MUTT1, which found natamycin more effective than voriconazole for *Fusarium spp.* cases,[7] the difference in BSCVA at day 90 did not vary with causative fungal organism in our trial. It is noteworthy that in clinical practice in many countries, voriconazole is often still used as a first-line or adjunctive agent.[24,25]

We found that, for patients presenting with mild disease (i.e., good baseline vision and small infiltrate size), there were no cases of poor visual outcome (BSCVA worse than 1.0 logMAR) in either treatment arm, suggesting that chlorhexidine may be an effective treatment for patients presenting early in the course of their disease. Alternatively, it is possible that in some cases, mild fungal infection may be self-limiting and improve regardless of the treatment given. This study was not explicitly designed to test this, and further work is warranted to investigate this further.

Table 2. Clinical Outcomes and Adverse Events by Treatment Group (including Mixed Infections)

	Chlorhexidine		Natamycin		Total		*P* Value
Clinical Outcomes							
Day 90 BSCVA (logMAR)							
Mean	0.64	(0.79)	0.26	(0.52)	0.45	(0.69)	<0.001*
Median ^	0.2	(0–1.7)	0.02	(0–0.26)	0.1	(0–0.58)	NA
6/5–6/12	86/178	(48.31%)	119/176	(67.61%)	205/354	(57.91%)	<0.001†
>6/12–6/18	9/178	(5.06%)	8/176	(4.55%)	17/354	(4.80%)	
>6/18–6/60	10/178	(5.62%)	13/176	(7.39%)	23/354	(6.50%)	
>6/60–3/60	0		0		0		
>3/60–1/60 (CF)	26/178	(14.61%)	3/176	(1.70%)	29/354	(8.19%)	
>1/60 (CF)–no light perception	47/178	(26.40%)	33/176	(18.75%)	80/354	(22.60%)	
Day 90 visual acuity (presenting, BSCVA) ^	0.2	(0–1.5)	0	(0–0.3)	0.1	(0–0.4)	
Day 90 scar size (mm)							
Median ^	2.3	(1.75–3.3)	2.25	(1.5–3.35)	2.25	(1.6–3.3)	0.837†
≤2	49/118	(41.5%)	62/145	(42.8%)	111/263	(42.2%)	
>2–4	50/118	(42.4%)	65/145	(44.8%)	115/263	(43.7%)	
>4–6	15/118	(12.7%)	15/145	(10.3%)	30/263	(11.4%)	
>6	4/118	(3.4%)	3/145	(2.1%)	7/263	(2.7%)	
Day 7 hypopyon	19/162	(11.7%)	31/158	(19.6%)	50/320	(15.6%)	0.064†
Day 7 hypopyon height, mm (median) ^‡	0.8	(0.2–1)	0.5	(0.2–1.5)	0.55	(0.2–1.5)	NA
Day 7 hypopyon height (mean)‡	0.81	(0.62)	0.93	(0.99)	0.88	(0.86)	0.636§
Day 21 hypopyon	11/144	(7.6%)	18/149	(12.1%)	29/293	(9.9%)	0.242†
Day 21 hypopyon height (median) ^‡	1.4	(0.3–3)	1.1	(0.5–1.5)	1.2	(0.5–1.8)	NA
Day 21 hypopyon height (mean)‡	1.52	(1.25)	1.14	(0.82)	1.28	(1.00)	0.3299§
Day 7 culture positive	22/83	(26.5%)	11/65	(16.9%)	33/148	(22.3%)	0.232†
Adverse Events							
Adverse Events–Serious							
Corneal perforation	13/175	(7.47%)	6/173	(3.46%)	19/348	(5.45%)	0.101†
TPK	11/175	(6.28%)	4/173	(2.31%)	15/348	(4.31%)	0.111†
Corneal perforation or TPK	24/175	(13.7%)	10/173	(5.79%)	34/348	(9.77%)	0.018*,†
Evisceration‖	8/175	(4.6%)	3/173	(1.73%)	11/348	(3.2%)	0.219†
Endophthalmitis	0		0		0		NA
Adverse Events–Nonserious							
Local allergic reaction							
None	155/175	(88.6%)	165/173	(95.4%)	320/348	(92.0%)	0.048*,†
Mild	18/175	(10.3%)	8/173	(4.6%)	26/348	(7.47%)	
Moderate	1/175	(0.57%)	0/173		1/348	(0.29%)	
Severe	1/175	(0.57%)	0/173		1/348	(0.29%)	
>2-mm increase in hypopyon	3/175	(1.71%)	1/173	(0.57%)	4/348	(1.15%)	0.623†
>50% increase in infiltrate size¶	13/175	(7.42%)	3/173	(1.73%)	16/348	(4.60%)	0.019*,†
Progressive corneal thinning to ≤50%¶	13/175	(7.42%)	5/173	(2.89%)	18/348	(5.17%)	0.088†
New cataract development	13/175	(7.42%)	9/173	(5.20%)	22/348	(6.32%)	0.510†
Persistent ED	15/175	(8.57%)	0/173		15/348	(4.31%)	<0.00*,†
Corneal edema	30/175	(17.1%)	11/173	(6.36%)	41/348	(11.8%)	0.002*,†
Secondary bacterial keratitis during study¶,#	49/175	(28.0%)	44/173	(25.4%)	93/348	(26.7%)	0.629†

Data are n (%) or mean (standard deviation), other than where indicated with "^" when the data are median (interquartile range). There were no systemic side effects reported in either arm, including death, need for nonelective surgery or hospitalization, or myocardial infarction or stroke, and are therefore not presented here. There were no cases of intraocular pressure ≥35 mmHg for 1 week despite therapy in either arm.

BSCVA = best spectacle-corrected visual acuity; CF = counting fingers; ED = epithelial defect; logMAR = logarithm of the minimum angle of resolution; NA = not available; TPK = therapeutic penetrating keratoplasty.

The denominator represents patients who attended for at least 1 follow-up during the study period or who attended the follow-up review in question; patients who did not attend after enrollment or who did not attend the specified follow-up review (e.g., day 21) are treated as missing data and excluded from this analysis.

**P* value calculated by linear regression after adjusting for baseline visual acuity.

†Calculated by Fisher exact test.

‡If present.

§Calculated by *t* test.

‖All patients who were eviscerated had already perforated.

¶From baseline.

#Secondary bacterial keratitis defined as a patient commencing a topical antibiotic during the study period because of clinical deterioration and clinical impression or as a patient who has microscopy from a corneal scrape during the study period with evidence of a bacterial infection.

There are some potentially relevant differences in eligibility criteria in our study compared with MUTT1. First, we excluded people who were already using antifungals; in MUTT1, 46% of participants had used a topical antifungal before recruitment.[7] We excluded 88 people from our trial because of prior topical antifungal use, the majority of whom were using natamycin and had been referred to the tertiary center because of deterioration while on this treatment. It is unknown how these would have fared had they been included. Second, MUTT1 recruited people with a visual acuity of between 0.3 logMAR (6/12 Snellen) and 1.3 logMAR (6/120 Snellen).[26] Our study only excluded people who had no light perception in the affected eye.

Despite natamycin being on the World Health Organization Essential Medicines List, it is still largely unavailable in most of sub-Saharan Africa.[4] Although our study clearly demonstrates the superiority of natamycin 5% for treatment of filamentous FK, it is important to recognize that in many settings, natamycin and other antifungal eye drops are unavailable. Although chlorhexidine should not be used first-line when natamycin is available, based on the results of this study, there may be situations where cautious use of chlorhexidine might be considered if alternative antifungal treatment is unavailable because without any treatment the eye will likely be lost.[27]

Our results contrast with the 2 earlier trials comparing chlorhexidine with natamycin.[18,19] Although these studies had limitations, including small sample sizes, being unmasked, and 1 using half-strength natamycin, they suggested that chlorhexidine could be superior to natamycin in terms of a favorable clinical response at day 5 or "curing" at day 21. Neither study looked at longer-term visual acuity outcomes. Our study, with adequate power, longer follow-up, and a primary outcome of visual acuity, addresses the clinical equipoise raised by these earlier studies and subsequent meta-analysis.[11]

The difference in vision at day 90 between arms likely results from slightly larger scars, more cases of corneal edema, and more persistent EDs in the chlorhexidine group. There was no difference in new cataract development. Prolonged treatment with higher concentrations of chlorhexidine sometimes can be toxic to the corneal epithelium and keratocytes, which may have contributed to these findings, alongside the primary infectious process.[28] The intensive treatment regimen that we followed for a microbiological cure, although the standard of care for FK, may be more intense than needed. The choice of chlorhexidine 0.2% w/v was based on an earlier pilot trial, which suggested greater efficacy than chlorhexidine 0.02% w/v.[18] This lower concentration is typically used to treat *Acanthamoeba* keratitis.[29] It is noteworthy that there were significantly more cases of persistent ED, corneal edema, and delayed reepithelialization in the chlorhexidine arm than the natamycin arm, which could be a result of corneal toxicity resulting from overtreatment with chlorhexidine (either too intensively or with too concentrated a formulation). This may have resulted in more visually significant corneal scarring in the chlorhexidine arm, contributing to the difference in 3-month BSCVA identified by this trial. Further research is necessary to evaluate if lower concentrations of chlorhexidine, or less frequent dosing, are sufficiently effective for treating FK, with the potential advantage of reduced corneal toxicity. It should also be noted that natamycin 5% contains benzalkonium chloride preservatives, whereas chlorhexidine 0.2% is preservative-free; benzalkonium chloride is known to be a broad-spectrum antimicrobial agent and therefore may increase the efficacy of topical natamycin compared with chlorhexidine.[30]

Study Limitations

Our study has several limitations. It was not possible to mask participants to treatment allocation; however, they were not told which medication they had received, and study-team members were masked to allocation. The primary outcome measure was assessed by an optometrist not otherwise involved in the study. Participants were enrolled in the lowland plains of Nepal, where the pattern of fungal isolates was dominated by dematiaceous fungi; this differs from studies elsewhere, where *Fusarium* and *Aspergillus* are more frequent.[7,12,18,19,24] The coronavirus disease 2019 pandemic caused significant disruption, with a 3-month period without participant contact because of restrictions. We reviewed participants as soon as possible after their scheduled follow-up date and included these patients in the primary analysis. This in part may account for the moderate loss to follow-up rate in this study (50/354, 14.1%), although this was less than what was accounted for in the sample size calculation. For adverse effects and clinical outcomes, we have presented multiple *P* values, and it is important to consider that when interpreting these results, one should remember that it is possible to get these results by chance alone; however, given the small size of many of these *P* values and the clear correlation with the conclusive results of the primary outcome, it is unlikely that any correction would alter the results significantly. The 2 treatment arms were generally well balanced in terms of baseline characteristics, although there was some evidence that the severity of disease was worse in the chlorhexidine arm: There were proportionally more patients in this arm who had a history of preceding topical steroid use, the baseline visual acuity was approximately 1 line worse, and there were more culture-positive cases than the natamycin arm; however, our primary analysis adjusted for baseline visual acuity and a sensitivity analysis excluding patients who had used topical steroids showed similar results to the primary analysis. Finally, this trial did not assess the potential role of chlorhexidine as an adjunctive agent to natamycin; this is currently being assessed by our group in East Africa.[31]

In conclusion, natamycin is superior to chlorhexidine for filamentous FK and remains the preferred first-line treatment. This study highlights the need to ensure that it is readily available in all countries where FK is a public health concern. Unfortunately, this is currently far from being the case. Further work is warranted to definitively answer whether lower concentrations of chlorhexidine or combination therapy with natamycin have a role to play in treating FK.

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Footnotes and Disclosures

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HUMAN SUBJECTS: Human subjects were included in this study. Ethical and regulatory approval was obtained from the Nepal Health Research Council Ethics Committee; the Nepal Department of Drug Administration; and the London School of Hygiene & Tropical Medicine Ethics Committee, UK. The study adhered to the principles of the Declaration of Helsinki. All participants provided informed consent.

No animal subjects were used in this study.

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Analysis and interpretation: Hoffman, Yadav, Sanyam, Chaudhary, Hu, Macleod, Leck, Burton

Obtained funding: Burton; Study was performed as part of regular employment duties at London School of Hygiene and Tropical Medicine and Sagarmatha Choudhary Eye Hospital.

Overall responsibility: Hoffman, Yadav, Sanyam, Chaudhary, Roshan, Singh, Singh, Mishra, Arunga, Hu, Macleod, Leck, Burton

Abbreviations and Acronyms:
BSCVA = best spectacle-corrected visual acuity; **CI** = confidence interval; **DSMB** = Data Safety and Monitoring Board; **ED** = epithelial defect; **FK** = fungal keratitis; **logMAR** = logarithm of the minimum angle of resolution; **MK** = microbial keratitis; **MUTT** = Mycotic Ulcer Treatment Trials; **NPL** = no perception of light; **OR** = odds ratio; **SCEH** = Sagarmatha Choudhary Eye Hospital; **TPK** = therapeutic penetrating keratoplasty; **w/v** = weight/volume.

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Chapter 9: Delay in accessing definitive care for patients with microbial keratitis in Nepal

A Nepali lady carries some of her day's harvest home in the Terai, Nepal

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Thesis Title	Managing Fungal Keratitis in Nepal: A Randomised Controlled Trial Comparing Chlorhexidine 0.2% to Natamycin 5%		
Primary Supervisor	PROF. MATTHEW J. BURTON		

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Delay in accessing definitive care for patients with microbial keratitis in Nepal

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Background: The aim of this study was to describe the health-seeking journey for patients with microbial keratitis (MK) in Nepal and identify factors associated with delay.

Methods: Prospective cohort study where MK patients attending a large, tertiary-referral eye hospital in south-eastern Nepal between June 2019 and November 2020 were recruited. We collected demographic details, clinical history, and examination findings. Care-seeking journey details were captured including places attended, number of journeys, time from symptom onset, and costs. We compared "direct" with "indirect" presenters, analyzing for predictors of delay.

Results: We enrolled 643 patients with MK. The majority (96%) self-referred. "Direct" attenders accounted for only 23.6% (152/643) of patients, the majority of "indirect" patients initially presented to a pharmacy (255/491). Over half (328/643) of all cases presented after at least 7 days. The total cost of care increased with increasing numbers of facilities visited ($p < 0.001$). Those living furthest away were least likely to present directly ($p < 0.001$). Factors independently associated with delayed presentation included distance >50 km from the eye hospital [aOR 5.760 (95% CI 1.829–18.14, $p = 0.003$)], previous antifungal use [aOR 4.706 (95% CI 3.139–5.360)], and two or more previous journeys [aOR 1.442 (95% CI 1.111–3.255)].

Conclusions: Most patients visited at least one facility prior to our institution, with time to presentation and costs increasing with the number of prior journeys. Distance to the eye hospital is a significant barrier to prompt, direct presentation. Based on these findings, improving access to eye care services, strengthening referral networks and encouraging early appropriate treatment are recommended to reduce delay, ultimately improving clinical outcomes.

KEYWORDS

microbial keratitis, fungal keratitis, epidemiology, health systems, Nepal, cornea

Introduction

Microbial keratitis (MK) is the leading cause of unilateral blindness after cataract in low- and middle-income countries (LMICs), previously estimated to be responsible for over 2 million cases of unilateral blindness annually in Asia and Africa (1). As blindness is usually defined as bilateral sight-loss, MK is often overlooked. However, MK leads to significant morbidity (2), reduced quality of life (3), and an associated high economic cost (4). There have been calls for MK to be recognized as a neglected tropical disease by the World Health Organization (5). The incidence of MK in Nepal is amongst the highest levels in the world, reported as 799/100,000/year (6).

MK can be caused by fungi (yeasts, molds and microsporidia), bacteria, viruses and protozoa (e.g., *Acanthamoeba spp.*), with filamentous fungi being more commonly implicated in tropical LMICs such as Nepal, where it accounts for up to 70% of cases, compared to bacterial keratitis which is more common in temperate latitudes (7, 8). It is an ophthalmic emergency, presenting with pain, photophobia, conjunctival hyperaemia, and corneal ulceration with a stromal inflammatory cell infiltrate. Effective treatment relies on promptly diagnosing and treating the patient with intensive antimicrobial agents. Any delay in presentation to appropriate eye care facilities allows the infection to become well-established, resulting in poor clinical outcomes (9, 10), with any treatment to improve this prognosis very challenging (11). Early application of topical antimicrobial agents following corneal abrasion prevents infection developing and allows for full recovery (12, 13).

A recent study from Nepal reported that patients referred to a tertiary level eye hospital in Kathmandu took on average 21.5 days to present from symptom onset, with 53% of cases directly presenting to a trained eye health worker or ophthalmologist at any clinical facility (including the tertiary hospital in Kathmandu) (14). However, this study did not investigate factors associated with delayed presentation. Our previous work from sub-Saharan Africa showed that delayed presentation is a key determinant of a poor outcome (9, 10, 15). There have been no published studies from Asia on associations with delay for MK patients.

Understanding the patient health-seeking journey can highlight gaps in the health system, helping direct resources into ensuring a rapid onward referral to appropriate care, with the goal of improving outcome for patients with MK before it is too late. The aim of this study was to describe the presentation journey of patients with MK to an eye hospital in south-eastern Nepal and to investigate factors associated with delayed presentation.

Methods

Ethical statement

This study followed the tenets of the Declaration of Helsinki. It was approved by the London School of Hygiene & Tropical Medicine Ethics Committee (Ref. 14841) and Nepal Health Research Council Ethical Review Board (Ref. 1937). Written informed consent in the local language was obtained before enrolment. If the patient was unable to read, the information was read to them, and they were asked to indicate their consent by application of their thumbprint, which was independently witnessed.

Study design and setting

We prospectively recruited patients at Sagarmatha Choudhary Eye Hospital (SCEH) in Lahan, Nepal between 3rd June 2019 and 9th November 2020. It formed part of the triaging assessment used to enroll eligible patients with fungal keratitis (FK) into a randomized controlled trial comparing natamycin 5% to chlorhexidine 0.2%. The full protocol for this study has been published (16). SCEH is a tertiary ophthalmic referral hospital within Province 2 of south-eastern Nepal that serves a population of ∼5 million people. It is located ∼18 km from the Indian border, with many patients treated in outpatients being Indian nationals. There are 22 satellite "Eye Care Centers" (ECCs) located within Province 2 that are operated by SCEH and provide routine eye examination and treatment, referring to SCEH for more complex cases and surgery.

Participants

Eligible patients were adults (>18 years) with acute MK, defined as having corneal epithelial ulceration >1 mm in diameter, corneal stromal infiltrate, and any/all signs of acute inflammation (conjunctival hyperaemia, anterior chamber inflammatory cells, hypopyon). All eligible patients who consented to participate in the study were included.

Data collection procedures

Detailed demographic information was recorded. Clinical history data collected included date of symptom onset, detailed history of any preceding trauma and any prior treatment received, including traditional eye medicine (TEM) and/or conventional medications. A comprehensive "journey" history was obtained, using similar methodology to our previous work in Uganda (9). In brief, this comprised information on the

number of journeys participants took prior to attending SCEH and their dates, the previous facilities visited, any previous medication used, and how much each step cost them in Nepali Rupees (transportation, consultation fees, medications). The complete patient "journey" ended when the patient presented to SCEH corneal clinic on the date of enrolment.

Global Positioning System (GPS) co-ordinates were generated for participants' residence using Google Maps and the closest patient-reported searchable landmark (e.g., village, school, health post). Straight-line distance from participants' home to SCEH were calculated from these co-ordinates using the haversine formula.

Clinical examination included best spectacle corrected visual acuity (BSCVA) in LogMAR and slit-lamp examination. The BSCVA protocol followed that used in the Steroids for Corneal Ulcers Trial (SCUT) (17), using a 3 m, proportionally-reduced version of the 4 m Early Treatment Diabetic Retinopathy Study tumbling "E" chart (Good-Lite, Illinois, USA) (18). Slit-lamp examination by a trial-certified ophthalmologist or ophthalmic assistant followed a structured approach: eyelid assessment, corneal ulcer features, anterior chamber characteristics (flare, cells, hypopyon shape, and size), and perforation status (16). Infiltrate and epithelial defect size was calculated as the mean of the maximum diameter of the infiltrate and the widest perpendicular diameter (19). *In vivo* confocal microscopy (IVCM) was performed prior to corneal sample collection. IVCM was performed by trained experienced operators using the HRT III/RCM confocal microscope (Heidelberg Engineering, Dossenheim, Germany) using a previously described technique (20, 21). All the images were reviewed during the procedure in real-time and classified into the various forms of keratitis, by one experienced observer. Corneal specimens were obtained for microbiological testing on site (16).

Analysis

Data were analyzed in Stata 17 (Stata Corp., USA). Similar to our previous work, we classified participants into either "direct" or "indirect" presenters, depending on whether they received their definitive diagnosis and treatment at SCEH Corneal Clinic as their first point of care (9). Patients attending SCEH but who were not referred to the Corneal Clinic and therefore did not receive definitive care at their first visit, necessitating a second visit to SCEH (and their first visit to SCEH Corneal Clinic) were classed as indirect presenters. Summary frequency tables of demographics and clinical features were created with statistical testing performed using Wilcoxon rank sum for continuous variables and χ^2 test for categorical variables. The geo-location of all known participants' home addresses were added to a custom map using the Google My Maps function (22). The patients' journeys from home to each facility, and the final journey from home to SCEH, was presented using median time intervals in days and interquartile ranges (IQRs). The cost of intermediate care was described by summarizing the total patient expenditure for each journey (consultation cost, travel cost and any medication cost, where applicable) and presented as median expenditure with IQRs in Nepal Rupees. The Cuzick non-parametric test for trend was used to test for an association between expenditure and the number of facilities visited.

Time from symptom onset until presentation at SCEH (presentation time) was divided into the following categories for analysis of "delay": "prompt" (0–3 days), "early" (4–7 days), "intermediate" (8–14 days), "late" (15–30 days), and "very late" (>30 days). Any visit other than "prompt" (i.e., four or more days after symptom onset) can be considered as delayed presentation (10). Ordered logistic regression was performed to determine the factors associated with these five categories of "delay," whilst pairwise associations between clinical or demographic factors and direct presentation were investigated using univariable logistic regression to estimate crude odds ratios (OR). We took a causal modeling approach to explore the association of different potential risk factors for delayed presentation or indirect attendance. The risk factors investigated are given in Supplementary Material 1. To help inform our modeling, we mapped out relationships between different variables to identify those to adjust for to determine the overall effect of the exposure on the outcome. A change in point estimate criteria was used to assess for confounding; if the log odds ratio changed by more than 10% we adjusted for that in the model. The model was further checked to identify any collinearity by reviewing uncentered variance inflation factors (VIF). If the VIF was >10, then it was deemed to suggest collinearity and therefore the confounding variable removed from the model. This process was repeated until all VIF values were acceptable. Adjusted OR were reported for the final model.

Results

Demographic features

We triaged 890 consecutive patients with suspected microbial keratitis, of which we enrolled 643 patients. We excluded 247 cases as follows: 144 did not consent or were children, 95 had a healed or chronic corneal ulcer, 5 attended out-of-hours, and 3 cases were not microbial keratitis. Only 152/643 (23.6%) of patients were direct presenters. The remaining 491/643 (76.4%) were indirect presenters, including 6 patients who initially visited SCEH but required a second journey and return visit to SCEH to receive a definitive diagnosis and treatment. Demographic and clinical features of direct vs. indirect presenters are shown in Table 1. Direct presenters lived

closer to SCEH (median distance 24.5 vs. 40.2 km, $p < 0.001$) and lived closer to a health center (median distance 1 vs. 2 km, $p < 0.001$). A higher proportion of direct presenters were Nepali (74.3%), compared to indirect presenters (53.2%, $p < 0.001$). A greater proportion of direct presenters were farmers (61.2%) compared to indirect presenters (48.7%, $p = 0.026$). The two groups were otherwise similar for the other variables investigated, including age, gender, education, marital status, and literacy. Most patients from India lived further away than patients from Nepal; 216/272 (79.4%) of Indian patients lived more than 50 km from SCEH, compared to 42/271 (15.5%) of Nepali patients.

There were some interesting differences between direct and indirect presenters in terms of clinical features, Table 2. Indirect presenters had a longer median presentation time from symptom onset (8 vs. 5 days, $p < 0.001$), worse vision (0.6 vs. 0.3 logMAR, $p < 0.001$) and larger corneal ulcers [median infiltrate and epithelial defect size 2.9 vs. 2.1 mm ($p < 0.001$) and 3.0 vs. 2.5 mm ($p < 0.001$), respectively], compared to direct presenters. The proportion of patients who had used treatment prior to presenting at SCEH was significantly higher for indirect presenters (98.4%) compared to direct presenters (44.1%, $p < 0.001$). This held true for all forms of conventional medication ($p < 0.001$). The numbers of patients who had used traditional eye medicines was low overall (12/643, 1.9%), with proportionally more in the indirect vs. direct group but not statistically significant ($p = 0.739$). There were proportionally more direct presenters with a diagnosis of bacterial keratitis (9.9%) compared to indirect presenters (3.5%), and conversely more indirect presenters with a diagnosis of fungal keratitis (77.4%) compared to direct presenters (67.6%, $p = 0.003$). There was no evidence of a difference in rates of trauma between direct and indirect presenters, although farmers were more likely to have a history of trauma compared to non-farmers ($p < 0.001$, Supplementary Table 1).

Factors associated with direct presentation

Distance from SCEH and residence in India were associated with reduced odds of direct presentation in the univariable analysis (Table 3), whilst being a farmer was associated with increased odds of direct presentation. Both these variables remained as significant independent associations in the multivariable model. Country of residence was removed as this was collinear with distance from SCEH.

Sensitivity analyses performed for patients living in India and Nepal separately found that for Nepali residents, distance >20 km was independently associated with reduced odds of direct presentation (Supplementary Table 2). However, there was no evidence of a similar association for Indian residents.

Care-seeking pathway

Figure 1 shows the locations of the participants' homes, SCEH and the satellite eye care centers. Most patients (75%) lived within 66 km of the eye hospital, clustered within Province 2 of Nepal and from neighboring Bihar state in Northern India (both low-land, sub-tropical plains areas). However, 15% of patients traveled more than 100 km to attend SCEH, mostly from the Indian states of Assam and West Bengal in north-eastern India.

Table 1 identifies the type of facility where patients first presented. Pharmacies were the most frequent, followed by direct presentation to SCEH, satellite ECC clinics and private clinics. Only one patient reported visiting a traditional healer first. Pharmacies were visited much less frequently on subsequent visits: only 6/491 (1.2%) of second journey destinations were pharmacies, and 1/103 (0.97%) of third journey destinations. There were no visits to pharmacies beyond this. Only 39/272 (14.3%) of Indian patients attended SCEH directly.

Figure 2 outlines the stages in the journey of patients from home to each intermediate facility, as well as their final journey for diagnosis and treatment at SCEH, including median times for each stage and cumulative median time from symptom onset. Nearly all patients (95.8%) returned home after visiting each facility and then made a subsequent journey; there were only 27 onward referrals between facilities (1 from a private hospital to a private clinic as an interim journey, and 26 from ECCs to SCEH as a final journey). The majority of patients (388/643, 60.3%) attended one facility (i.e., two journeys) before definitive treatment at SCEH, whilst 88/643 (13.7%), 9/643 (1.4%), 4/643 (0.6%), and 2/643 (0.3%) made three, four, five or six journeys, respectively. On average patients spent almost 1 week between visiting each facility.

Of the patients who had used steroids prior to attendance at SCEH, 64/105 (61%) had attended a pharmacy at one stage during their journey to SCEH, compared to 193/538 (35.9%) of patients who had no history of steroid use ($p < 0.001$).

Cost of care

Table 4 presents the cost of care in Nepali Rupees (NPR). The total cost of care increased with additional facility visits. This is supported by evidence to suggest an association between the total cost and number of visits made (Cuzick non-parametric test for trend $p < 0.0001$). Direct presenters spent the least overall [median NPR 760 (IQR 620–900)]. Most of the expenditure was on consultations, followed by transportation, with medicine costs accounting for the smallest component of expenditure.

TABLE 1 Baseline characteristics of direct vs. indirect presenters, $n = 643$.

		Total ($n = 643$)			Direct presenters ($N = 152$)			Indirect presenters ($N = 491$)			
Continuous variables		**Median**	**(IQR)**	**(Range)**	**Median**	**(IQR)**	**(Range)**	**Median**	**(IQR)**	**(Range)**	***P*-value**
Age		45.9	(35.7–57.7)	(18.1–100.1)	50.1	(35.8–60.6)	(18.9–75.8)	45.9	(35.6–55.9)	(18.1–100.1)	0.1450
Distance to SCEH, km[a]		37.3	(23.5–66.2)	(0.09–756.1)	24.5	(14.9–51.0)	(0.9–282.1)	40.2	(27.1–74.6)	(0.09–756.1)	<0.001
Distance to nearest health center, km[a]		2	(1–5)	(0–50)	1	(0–3)	(0–40)	2	(1–5)	(0–50)	<0.001
Categorical variables		**N**	**(%)**		**(N)**	**(%)**		**N**	**(%)**		***P* value**
Gender	Female	392	(61.0)		94	(61.8)		298	(60.7)		
	Male	251	(39.0)		58	(38.2)		193	(39.3)		0.849
Occupation	Agriculture	332	(51.6)		93	(61.2)		239	(48.7)		
	No job	263	(40.9)		49	(32.2)		214	(43.6)		
	Non-agriculture	48	(7.5)		10	(6.6)		38	(7.7)		0.026
Marital status	Married	577	(89.7)		140	(92.1)		437	(89.0)		
	Unmarried[b]	66	(10.3)		12	(7.9)		54	(11.0)		0.358
Literacy (read/write)	Illiterate	500	(77.8)		115	(75.7)		385	(78.4)		
	Little Nepali	51	(7.9)		16	(10.5)		35	(7.1)		
	Nepali well	48	(7.5)		12	(7.9)		36	(7.3)		
	English and Nepali	44	(6.8)		9	(5.9)		35	(7.1)		0.542
Country of residence	Nepal	371	(57.7)		113	(74.3)		258	(52.6)		
	India	272	(42.3)		39	(25.7)		233	(47.5)		<0.001
Education	None	494	(76.8)		114	(75.0)		380	(77.4)		
	Primary level	80	(12.4)		21	(13.8)		59	(12.0)		
	Secondary level	12	(1.9)		3	(2.0)		9	(1.8)		
	Tertiary level	57	(8.9)		14	(9.2)		43	(8.8)		0.897
Where first presented	Pharmacy	255	(39.7)		0	(0)		255	(51.9)		
	Health post	6	(0.9)		0	(0)		6	(1.2)		
	Private clinic	93	(14.5)		0	(0)		93	(18.9)		
	Government hospital	12	(1.9)		0	(0)		12	(2.4)		
	Private hospital	24	(3.7)		0	(0)		24	(4.9)		
	Traditional healer	1	(0.16)		0	(0)		1	(0.2)		
	Eye care clinic (ECC)	94	(14.6)		0	(0)		94	(14.6)		
	SCEH	158	(24.6)		152	(100)		6	(1.22)		<0.001

SCEH, Sagarmatha choudhary eye hospital. [a] Variables with some missing data: distance to SCEH [$n = 635$, (direct 154)], distance to nearest health center [$n = 641$, (direct 155)]. [b] Unmarried included single, divorced, and widowed.

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TABLE 2 Clinical history and clinical signs of direct vs. indirect presenters ($n = 643$).

		Total ($n = 643$)			Direct presenters ($N = 152$)			Indirect presenters ($N = 491$)			
Continuous variables		**Median**	**(IQR)**	**(Total range)**	**Median**	**(IQR)**	**(Total range)**	**Median**	**(IQR)**	**(Total range)**	***P*-value**
Presentation time, days[a]		8	(5–14)	(1–92)	5	(3–10)	(1–92)	8	(5–15)	(1–82)	<0.001
Presenting vision (LogMAR)		0.54	(0.2–1.4)	(0–1.9)	0.3	(0.08–0.89)	(0–1.9)	0.6	(0.24–1.5)	(0–1.9)	<0.001
Infiltrate size, mm[b]		2.75	(1.75–4)	(0.2–11.75)	2.1	(1.5–3.5)	(0.5–8.8)	2.9	(1.9–4.1)	(0.2–11.8)	<0.001
Epithelial defect size, mm[b]		2.9	(2–4.25)	(0–12)	2.5	(1.9–3.8)	(0.6–9)	3.0	(2.1–4.4)	(0–12)	<0.001
Categorical variables		**N**	**(%)**		**(N)**	**(%)**		**N**	**(%)**		***P* value**
Presenting time	Prompt (0–3 days)	86	(13.4)		42	(27.6)		44	(9.0)		
	Early (4–7 days)	229	(35.6)		54	(35.5)		175	(35.6)		
	Intermediate (8–14 days)	180	(28.0)		33	(21.7)		147	(29.9)		
	Late (15–30 days)	108	(16.8)		19	(12.5)		89	(18.1)		
	Very late (>30 days)	40	(6.2)		4	(2.6)		36	(7.3)		<0.001
History of trauma	None/unsure	326	(50.7)		72	(47.4)		254	(51.7)		
	Vegetative matter	226	(35.2)		64	(42.1)		162	(33.0)		
	Other	86	(13.4)		15	(9.9)		71	(14.5)		
	Unknown object	5	(0.8)		1	(0.7)		4	(0.8)		0.153
Previous treatment[c]	No	93	(14.5)		85	(55.9)		8	(1.6)		
	Yes	550	(85.5)		67	(44.1)		483	(98.4)		<0.001
	Steroids	105	(16.3)		12	(7.9)		93	(18.9)		<0.001
	Antibiotics	463	(72.0)		54	(35.5)		409	(83.3)		<0.001
	Antifungals	134	(20.8)		8	(5.8)		125	(25.7)		<0.001
	Other topical	260	(40.4)		18	(11.8)		242	(49.3)		<0.001
	Systemic medication	353	(54.9)		29	(19.1)		324	(66.0)		<0.001
	TEM	12	(1.9)		2	(1.3)		10	(2.0)		0.741
Most important symptom	Pain	471	(73.3)		115	(75.7)		356	(72.5)		
	Vision	57	(8.9)		13	(8.6)		44	(9.0)		
	Other	115	(17.9)		24	(15.8)		91	(18.5)		0.739
Hypopyon[d]	No	457	(71.2)		112	(74.2)		345	(70.3)		
	Yes	175	(27.3)		35	(23.2)		140	(28.5)		
	Unable to see	10	(1.6)		4	(2.7)		6	(1.2)		0.223
Perforation status[d]	No	634	(98.8)		151	(100)		483	(98.4)		
	Descemetocele	6	(0.9)		0	(0)		6	(1.2)		
	Perforated	2	(0.3)		0	(0)		2	(0.4)		0.483
Diagnosis[d]	Fungal keratitis	482	(75.1)		102	(67.6)		380	(77.4)		
	Bacterial keratitis	32	(5.0)		15	(9.9)		17	(3.5)		
	Mixed	51	(7.9)		18	(11.9)		33	(6.7)		
	Unknown	77	(12.0)		16	(10.6)		61	(12.4)		0.003

TEM, traditional eye medicine. [a]Presentation time was measured as duration in days it took to come to the eye hospital after onset of symptoms. [b]geometrical of the largest diameter and the diameter perpendicular to the largest diameter. [c]Previous treatment was often dispensed by local pharmacies without a prescription or clinician review; by definition this was the case for all "direct" attenders. [d]One patient was not able to be examined.

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TABLE 3 Univariable and multivariable logistic regression analysis of factors associated with direct presentation to the eye hospital, $n = 643$.

Variable	Univariable analysis			Multivariable analysis		
	cOR[c]	(95% CI)	*P*-value	aOR[c]	(95% CI)	*P*-value
Age	1.008	(0.995–1.022)	0.218			
Sex (being female)	1.050	(0.722–1.526)	0.800			
Marital status (being married)	1.442	(0.750–2.772)	0.273			
Farmer occupation	1.662	(1.147–2.408)	0.007	1.535	(1.024–2.30)	0.038
Distance to SCEH (km)						
0–5	–	–	–			
5–20	0.641	(0.240–1.715)	0.376	0.587	(0.215–1.601)	0.298
20–50	0.173	(0.066–0.453)	<0.001	0.167	(0.063–0.447)	<0.001
50–100	0.145	(0.053–0.397)	<0.001	0.137	(0.049–0.382)	<0.001
>100	0.116	(0.040–0.338)	<0.001	0.116	(0.039–0.349)	<0.001
Distance from nearest health center (km)						
0–12.5	–	–	–			
>12.5–25	0.750	(0.248–2.263)	0.609			
>25–37.5	0.354	(0.044–2.818)	0.327			
>37.5–50	1.593	(0.143–17.70)	0.705			
Positive history of trauma[a]	1.047	(0.710–1.543)	0.816			
Used TEM	0.641	(0.139–2.959)	0.569			
Education status						
None	–	–	–			
Primary level	1.186	(0.691–2.036)	0.535			
Secondary level	1.111	(0.295–4.173)	0.876			
Tertiary level	1.085	(0.575–2.055)	0.802			
Country of residence (India)[b]	0.382	(0.255–0.572)	<0.001			

SCEH, Sagarmatha choudhary eye hospital; TEM, traditional eye medicine; cOR, crude odds ratio; aOR, adjusted odds ratio. [a]Definite history of trauma only; patients who were unsure were included in the no history of trauma group for the purpose of this analysis. [b]Country of residence was removed from the multivariable regression model due to being collinear. [c]OR <1 means they were less likely to present directly to the eye hospital.

Factors associated with delay

The association between delay in presentation and multiple risk factors was modeled using univariable and multivariable ordered logistic regression (Table 5). We found evidence of independent associations between the following variables and presentation delay: distance from home to SCEH >50 km, visiting more than one facility prior to SCEH, prior treatment, previous use of antifungals, systemic medication and/or other topical medications, prior use of TEM, increasing number of journeys and residence in India. Distance from the nearest health center to home, prior use of topical antibiotics, and prior use of topical steroids, although significant on univariable analysis, were not associated with delay after adjustment. Conversely, there was evidence that higher educational achievement was associated with reduced odds of delay.

Sensitivity analyses performed for patients living in India and Nepal separately found that that for Nepali residents, distance >50 km was independently associated with increased odds of delayed presentation (Supplementary Table 3). However, there was no evidence of a similar association for Indian residents. However, Indian residency remained a risk factor for delayed presentation in a multivariable model that only included patients living more than 20 km away from the hospital (OR 2.535 95% CI 1.343–4.788 $p = 0.004$).

Discussion

This study describes the care-seeking journey of people with microbial keratitis in lowland Nepal and investigates factors associated with delayed presentation. This has highlighted several key issues, which are opportunities for intervention to improve care and outcomes.

We found that in our cohort of 643 patients, <1 quarter of patients (23.6%) attended the tertiary-level eye hospital directly, with the majority (60.3%) attending one facility beforehand. There were very few direct referrals from primary care providers; ECCs were responsible for the only onward referral of MK

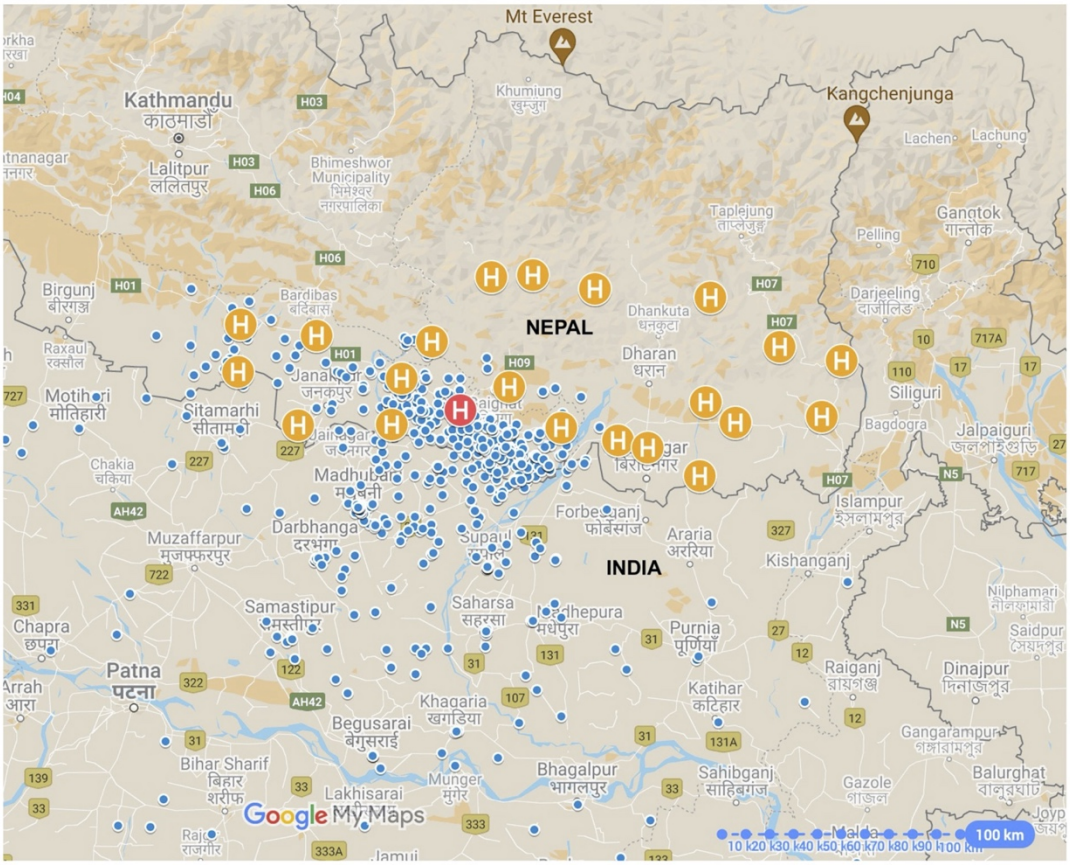

FIGURE 1
Map of eastern Nepal and north-eastern India showing patients' homes (blue pins) in relation to Sagarmatha Choudhary Eye Hospital (SCEH, red "H" pin) and Eye Care Centers (Orange "H" pin"). Not all positions are shown as some patients attended from outside of this map area. International borders shown as solid gray lines.

patients to SCEH in our study, with all other patients self-referring. Most patients (51%) presented to SCEH more than a week after symptom onset. As expected, patients living 20 km away or further were less likely to present directly ($p < 0.001$), whilst interestingly farmers were more likely to present directly ($p = 0.036$). Trauma was found to be more common in farmers than non-farmers, so a traumatic mechanism may have prompted farmers to attend sooner, although trauma itself was not associated with indirect presentation. This finding is contrary to work from Ghana that found farmers more likely to sustain trauma but less likely to make use of eye care facilities (23).

We found several variables to be independently associated with delayed presentation, with the greatest odds ratios for delay being distance from home to the eye hospital of 50 km or more, previous use of antifungals and four previous journeys. The further a person was from the point-of-care the greater the delay, as not only does travel become logistically more challenging, but also more expensive, meaning patients may not travel until they have exhausted easier options available locally. Furthermore, awareness of services offered by SCEH may be reduced the further someone is from the hospital. It is likely that the prior use of antifungals is associated with delayed presentation as it will have taken patients time and additional visits to other facilities to finally obtain this treatment. Conversely, we found higher than primary level education ($p = 0.015$) to be associated with prompt attendance, possibly due to improved health-awareness and health-seeking behavior as previously described in Nepal (24).

As expected, we found most patients to be clustered around SCEH on both sides of the Indian-Nepali border, with 75% living within 66 km of the eye hospital. However, 15% of

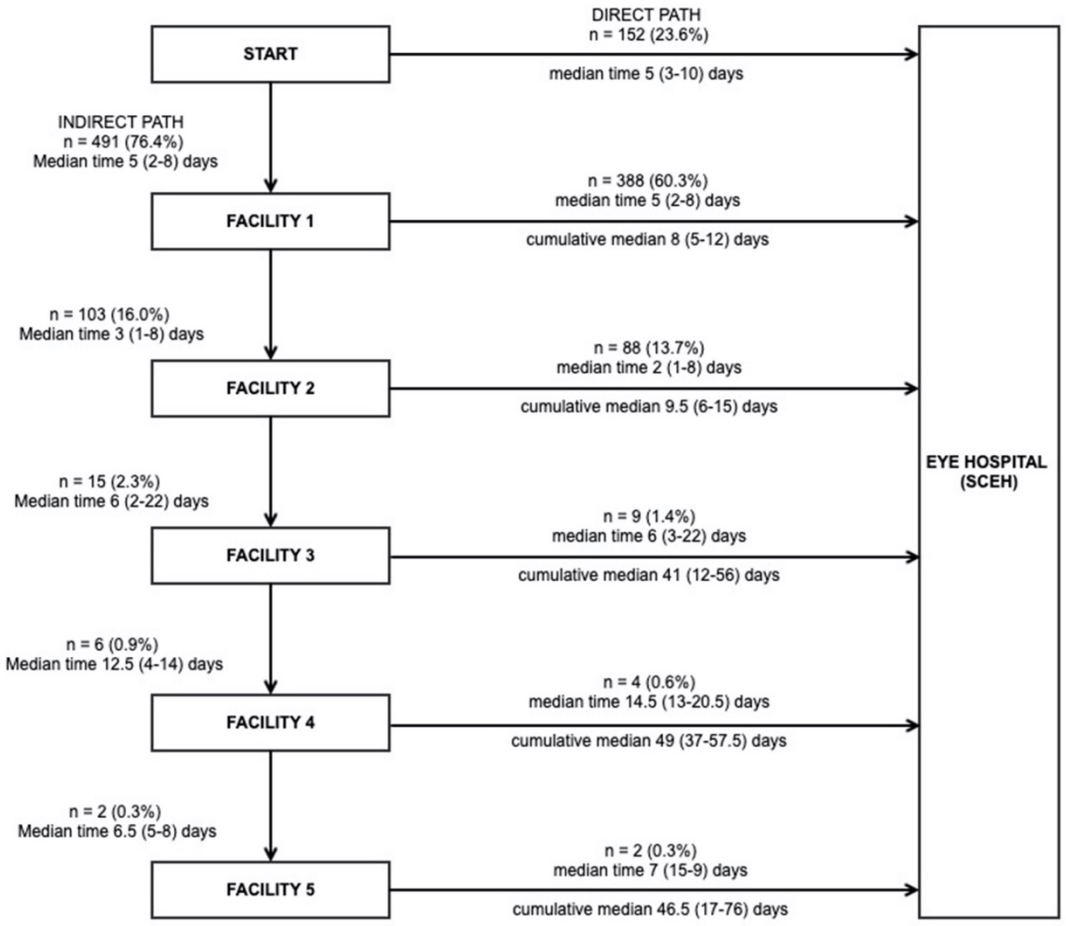

FIGURE 2
The care-seeking journey of patients with microbial keratitis, the time taken at each stage, and the cumulative time from onset of symptoms to presentation ($n = 643$). Note all patients returned home after attending each facility, from where they restarted any subsequent journeys, apart from the following exceptions: (1) Journey 2: one patient referred directly from a private clinic to a private hospital; seven patients referred directly from a satellite eye care clinic (ECC) to Sagarmatha Choudhary Eye Hospital (SCEH); (2) Journey 3: 19 patients referred directly from ECC to SCEH. Six patients attended SCEH as their first facility but did not receive a definitive diagnosis and/or treatment at that point and all had to make one additional journey to SCEH for final definitive treatment.

patients traveled more than 100 km to attend SCEH, with one patient traveling more than 750 km. Nearly all patients were from low-land plain areas, with only a handful coming from hilly or mountainous locations, which are sparsely populated with limited agricultural activity. Living 50 km or further from SCEH was associated both with delayed and indirect presentations, remaining true with Indian-resident patients excluded. Conversely, Indian residence remained a risk factor for delayed presentation when only including patients living more than 20 km away from SCEH. These findings suggest that distance, as well as the international border, are significant barriers to prompt, direct attendance. A significant proportion of patients were from India (42.3%), greater than previously reported between 2010 and 2014 (16%) (25). The nearest eye hospitals within Bihar are in the state capital Patna, 180 km from SCEH. The lack of ophthalmic care within the northern part of Bihar state is likely the main driving force for Indian patients to attend SCEH, with the vast majority (84.5%) attending one or more facilities prior to SCEH.

The median time from symptom onset to attending the first facility was 5 days for both indirect and direct presenters, with the interval between subsequent journeys about 1 week on average. This is similar to the mean symptom-presentation interval to the first facility of 5.6 days reported elsewhere in

TABLE 4 Money spent by patients per number of facilities visited before coming to the eye hospital.

			Median cost of care (IQR) in Nepali Rupees[a]							
Number of journeys	***n***	**(%)**	**Transportation**		**Consultation**		**Medicine**		**Total expenditure**	
1[b]	152	(23.6)	240	(120–400)	480	(480–480)	0	(0–20)	760	(620–900)
2	388	(60.3)	320	(200–600)	530	(480–960)	38	(20–220)	1,055	(764–1,502)
3	88	(13.7)	415	(275–710)	560	(560–980)	220	(20–301)	1,325	(980–2,120)
4	9	(1.4)	850	(700–1,000)	1,480	(1,060–2,480)	24	(20–284)	2,718	(1,740–3,440)
5	4	(0.6)	1,965	(895–2,875)	2,480	(2,480–2,730)	515.5	(453.5–593)	5,038	(3,881–6,146)
6	2	(0.3)	830	(450–1,210)	1,205	(880–1,530)	464	(428–500)	2,499	(1,758–3,240)
P-value for test of trend										<0.001

[a]All costs are quoted in Nepali Rupees. The US $ exchange rate on 10/09/2021 was US$ 1 = 118.12. [b]Patients with 1 journey were direct presenters to the hospital whose only consultation cost was the fixed cost for opening a file at the facility. As patients were seen as part of this study, most medicine costs were covered by the hospital.

Nepal (14), but a little slower than the 2-day interval reported in Uganda (9).

Previous work from Uganda found that TEM use and visiting one or more facilities prior to attendance were independently associated with delay to the eye hospital for patients with MK (9), similar to findings for other serious eye conditions in the African region (26–28). In contrast to this, we found TEM was used very infrequently (1.87%). Similarly, only one patient initially visited a traditional healer, in stark contrast to our experience in Uganda (9).

The total cost of care increased with increasing numbers of facilities visited ($p < 0.001$), consistent with previous studies (9, 29). Cost can be a major barrier to accessing eye health services (30). Consultation fees accounted for the largest component of overall expenditure, followed by transport and medications. This contrasts with Uganda, where the order was reversed due to government subsidies for health services (9). Medications are relatively cheap in Nepal, as most are generics manufactured in India and therefore easily imported at relatively affordable cost. Although there is currently limited literature concerning the economic burden of microbial keratitis in LMICs, it is likely that there are significant direct (cost of care, medicines, transport etc.) and indirect costs (lost earnings and assistance from carers). Studies from the USA and UK found increasing direct and indirect costs with increasing disease duration (31, 32), whilst in India patients who lived further away had a delayed presentation and spent more than those nearby (29). The additional costs incurred due to convoluted health-seeking journeys and the related delayed presentation add additional expenses to patients who are already under a significant financial pressure due to MK. By improving access to ophthalmic services and reducing the delay, these additional costs can be reduced.

This study highlights several areas for intervention to reduce delay to accessing eye care. A first opportunity is the initiation of appropriate early treatment and avoiding harmful treatment in community and primary care settings. Pharmacists were the first point of contact for many patients (39.7%). Pharmacists in Nepal are loosely regulated and can dispense most eye drops and oral antibiotics without a prescription. We found that they frequently dispensed steroid eye drops. Steroids can mask clinical signs and suppress the immune response, resulting in worse outcomes, particular for fungal infections (7). If pharmacists can be trained to avoid using steroids, dispense topical antibiotics alone, and refer urgently to an eye hospital, then delay might be reduced and outcomes improved. Pharmacists should be seen as an integral part of primary ophthalmic care and given the training and resources required to support this.

A second opportunity for intervention is improving access to primary eye care facilities. Given distance from the eye hospital is a significant risk factor for delayed presentation, such delays could potentially be mitigated by improved access to primary eye care services or through satellite hospital clinics (as in the case of ECCs for SCEH). There are currently no ECCs in the region of India where most of the Indian patients come from. Introducing such facilities, which could refer to SCEH or equivalent institutions in India, would be expected to reduce the presentation time for Indian patients. Increasing awareness through advertising or media campaigns amongst the northern Bihar population to attend SCEH in the event of symptoms suggestive of MK may further improve access. Co-operation on a regional scale between local governments in both Nepal and India may help reduce logistical barriers by improving transport links and streamlining border crossings.

The knowledge and skills amongst primary healthcare workers (PHCWs) in Eastern Nepal where our study was conducted have been shown to be inadequate to provide quality primary eye care services (33). For example, only 8.4% of 107 PHCWs surveyed across 35 different health posts in the region had received eye care training, with 72.9% of PHCWs unable to diagnose MK. At the same time as improving access to primary care, there needs to be significant investment in training primary healthcare workers in ophthalmology to prevent missed diagnoses of ophthalmic emergencies such as MK, and to start

TABLE 5 Univariable and multivariable ordinal logistic regression analysis of factors associated with delay among patients with microbial keratitis ($n = 643$).

Variable	Univariable analysis			Multivariable analysis		
	cOR	(95% CI)	*P*-value	aOR	(95% CI)	*P*-value
Age	1.002	(0.992–1.013)	0.631			
Sex (being female)	0.981	(0.730–1.320)	0.903			
Marital status (being married)	0.844	(0.536–1.333)	0.468			
Farmer occupation	0.870	(0.651–1.162)	0.346			
Distance to SCEH (km)						
0–5	–	–	–	–	–	–
>5–20	0.896	(0.300–2.675)	0.843	1.132	(0.343–3.733)	0.839
>20–50	2.172	(0.776–6.078)	0.140	2.152	(0.694–6.675)	0.184
>50–100	8.069	(2.835–22.97)	<0.001	5.760	(1.829–18.14)	0.003
>100	13.37	(4.567–39.17)	<0.001	9.665	(2.974–31.41)	<0.001
Distance from nearest health center (km)						
0–12.5	–	–	–			
>12.5–25	2.024	(0.934–4.387)	0.074	1.259	(0.555–2.856)	0.581
>25–37.5	5.675	(1.745–18.46)	0.004	1.991	(0.575–6.891)	0.277
>37.5–50	4.680	(0.626–35.00)	0.133	1.165	(0.140–9.681)	0.887
Positive history of trauma[a]	0.872	(0.637–1.194)	0.394			
Education status						
None	–	–	–	–	–	–
Primary level	0.942	(0.606–1.464)	0.790	0.743	(0.441–1.254)	0.266
Secondary level	0.478	(0.143–1.596)	0.230	0.180	(0.045–0.720)	0.015
Tertiary level	0.434	(0.247–0.763)	0.004	0.272	(0.135–0.549)	<0.001
Country of residence (India)	5.205	(3.800–7.131)	<0.001	3.406	(2.417–4.800)	<0.001
Previous treatment	2.936	(1.835–4.696)	<0.001	2.068	(1.101–3.882)	0.024
Previous steroids	1.987	(1.357–2.909)	0.0004	1.580	(0.910–2.752)	0.106
Previous antibiotics	1.452	(1.045–2.019)	0.026	1.017	(0.683–1.516)	0.933
Previous antifungals	6.799	(4.685–9.865)	<0.001	4.706	(3.139–5.360)	<0.001
Previous other topical medication	1.778	(1.322–2.391)	0.0001	1.455	(1.027–2.061)	0.035
Previous systemic medication	3.610	(2.650–4.919)	<0.001	1.972	(1.352–2.878)	<0.001
Used TEM	2.603	(0.951–7.128)	0.063	2.512	(1.746–3.615)	<0.001
Number of journeys						
1	–	–	–	–	–	–
2	1.797	(1.239–2.608)	0.002	1.442	(0.968–2.1491)	0.072
3	2.976	(1.798–4.928)	<0.001	1.902	(1.111–3.255)	0.019
4	27.83	(6.774–114.4)	<0.001	11.80	(2.844–48.93)	0.001
Visiting one or more facilities prior to SCEH	2.099	(1.462–3.015)	<0.001	1.617	(1.096–2.386)	0.016

Ordered categories of delay to presentation from symptom onset: prompt (0–3 days), early (4–7 days), intermediate (8–14 days), late (15–30 days) and very late (>30 days). [a]Definite history of trauma only; patients who were unsure were included in the no history of trauma group for the purpose of this analysis. SCEH, Sagarmatha choudhary eye hospital; TEM, traditional eye medicine; cOR, crude odds ratio; aOR, adjusted odds ratio.

appropriate treatment promptly (and/or refer as appropriate); a third opportunity for intervention. These measures should further help reduce the delay for MK patients accessing appropriate care. Our results, in agreement with previous studies, suggest that patients tend to seek help promptly after developing symptoms, likely due to the pain and poor vision (9). The immediate actions of who sees them first have a significant bearing on any delay to appropriate facilities and their overall prognosis. If patients attended a facility with appropriate MK diagnostic facilities (microscopy and culture as a minimum), experienced clinicians and available treatment (quinolone antibiotics and antifungals such as natamycin) within 1 week of symptom onset, the outcome is likely to be much better (11, 12, 15).

SCEH operates on a hub-and-spoke model, similar to other tertiary ophthalmic centers, with ECCs acting as a primary-care level facility with trained ophthalmic clinicians who can see and treat a set number of conditions, referring more complex cases to the SCEH hub, including ophthalmic emergencies such as MK. ECCs provide a very valuable service by improving access to ophthalmic care for patients. Although ECCs were the only primary-level provider to refer patients directly to SCEH, only 26/150 (17.3%) of ECC attendances were referred to SCEH directly, with the remainder self-referring later. Given the time-critical nature of MK and the need for additional diagnostic facilities not available at ECCs, these patients should have all been referred directly. Direct referral reduces any delay and can easily be arranged by hospital transport if necessary. Strengthening this referral pathway is a fourth opportunity for intervention. In addition, ECCs need to be fully integrated into the primary care system in the region, and expanded to areas where they are lacking, to help improve access further.

Strengths and limitations

We believe this is the first study from Asia to systematically investigate the care-seeking journey of patients with MK and examine factors that influence this. It has a large sample from a wide geographic area, which affords examination of these questions. It highlights that more severe disease is associated with delayed and complex presentation journeys, and the harms linked to these. It points to current gaps in the health system in Nepal and northern India, and how these can be addressed to potentially improve outcomes. Furthermore, it includes high quality microbiology in addition to *in vivo* confocal microscopy to identify the causative organism.

There are several limitations. First, there may be incomplete recall by patients of medications used and costs incurred; not every patient had medications or receipts with them at the time of presentation. Second, we did not conduct any formal qualitative research as part of this study, which may have provided insights into the journey and choice of facilities used. Although informal conversations with participants did highlight some possible reasons for delay these were not collected in a systematic manner and therefore not included in this analysis. We are currently conducting qualitative research into the knowledge and beliefs of pharmacists and traditional healers to explore their dispensing practices to identify approaches to influence their practice to improve patient care. Third, we did not analyse how final clinical outcomes may be related to delay as this study formed part of recruitment for a clinical trial that only included patients with fungal keratitis and randomized patients to two different treatments. Fourthly, there may have been some additional delays due to extrinsic factors such as COVID-19 and local flooding during the monsoon. However, there was no significant difference between the interval from symptom onset to presentation for patients attending before or after the start of COVID-19 restrictions in March 2020. Given that this was an observational study, causality of any relationships was precluded, so we are unable to establish in this study if delayed presentation led to worse clinical outcomes, although this has been previously reported in other settings (9, 10, 15). Finally, there is a degree of selection bias within this study as all sampling occurs at the tertiary level hospital, meaning that any cases of MK that were managed in the community and subsequently improved were not captured in this study. For a more accurate assessment, all health facilities that managed MK would need to be studied, but this would not be feasible given the large geographical area across two countries with many primary and secondary health facilities including pharmacies and traditional healers.

Conclusion

We found that most patients attending a tertiary eye hospital with microbial keratitis did not present directly. They often visited multiple health facilities, requiring many journeys, leading to increased costs, delays, and more advanced disease at presentation. This highlights a number of factors that are worthy of further, more detailed investigation, that if improved, could reduce delay and improve outcomes: appropriate early treatment with antibiotics and avoiding harmful treatment (e.g., steroids); appropriate early, direct referral to an eye hospital with appropriate diagnostic facilities and treatments whilst strengthening the referral to the main hospital from satellite clinics; improving access to eye care professionals; and, educating patients to attend an eye specialist directly. Reducing delay, combined with improved diagnostics and more effective treatment, will help improve outcomes for the millions of patients who develop microbial keratitis annually.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by London School of Hygiene & Tropical Medicine Ethics Committee (Ref. 14841) and Nepal Health Research Council Ethical Review Board (Ref. 1937). The patients/participants provided their written informed consent to participate in this study.

Author contributions

JH and MB: conceptualization. JH, SA, DM, and MB: methodology. JH and DM: formal analysis. JH, RY, SD, PC, and AL: investigation. AR and SS: resources. JH, SD, PC, and AL: data curation. JH: writing—original draft preparation. VH, AL, and MB: supervision. JH, SD, and AR: project administration. MB: funding acquisition. All co-authors writing: review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.915293/full#supplementary-material

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Chapter 10: Further Discussion

Public education poster at entrance to Sagarmatha Choudhary Eye Hospital depicting the common causes of ocular trauma and urgent management. Corneal trauma is a significant risk factor for developing fungal keratitis

Overview

This research project has made a significant contribution to the evidence base on the management of fungal keratitis, by definitively answering the question as to whether chlorhexidine 0.2% is non-inferior to natamycin 5% in terms of vision at three months: natamycin 5% remains the gold standard, first-line treatment. Furthermore, the ancillary studies have helped to increase the understanding of reasons for delayed presentation, what diagnostic tests are most appropriate in countries such as Nepal, and what clinical signs are predictive of fungal keratitis in this setting. Taken together, these studies should help both clinicians and policymakers develop strategies to improve the management of fungal keratitis, and ultimately improve the outcome for patients. This chapter summarises the key findings from each of the different studies and explores how the results of each study can be utilised to affect policy and practice change in the management of fungal and microbial keratitis in Nepal, and across lower- and middle-income countries.

Topical Chlorhexidine 0.2% versus Topical Natamycin 5% for the Treatment of Fungal Keratitis in Nepal

We designed a non-inferiority randomised controlled trial to test the hypothesis that chlorhexidine 0.2% was non-inferior to natamycin 5% for the treatment of filamentous fungal keratitis (FK).[1] In this trial, we recruited 354 patients with FK, of which 178 were randomly assigned to chlorhexidine and 176 to natamycin, with primary outcome data available for 153 and 151 of the chlorhexidine and natamycin groups, respectively. Our original sample size had been calculated to be 500 patients (250 in each arm);[1] however, the trial was halted early on the recommendation of the Data Safety Monitoring Board, as there were significantly more perforations in the chlorhexidine arm, compared to the natamycin arm (13.7% compared to 5.8%, P=0.018).[2] The results did not support our hypothesis: there was strong evidence that natamycin-treated patients had significantly better 3-month best spectacle corrected visual acuity (BSCVA) than chlorhexidine treated patients. Patients treated with natamycin were able to read approximately 3 lines on the logMAR chart (0.30 logMAR, 95% confidence interval 0.18-0.42, P<0.001) compared to chlorhexidine-treated participants, significantly outside our non-inferiority margin of 1.5 lines.

These findings were disappointing. Had chlorhexidine 0.2% been found non-inferior (or even superior) to natamycin 5%, then we would have had clear, robust evidence to recommend the use of chlorhexidine 0.2% as first-line treatment for fungal keratitis, particularly in locations were natamycin is frequently unavailable. However, it is important not to dismiss chlorhexidine outright in light of our findings and consider the current global context. I would argue that there

is still a role for chlorhexidine in certain situations as I will discuss below, as well as opportunities for further research into using chlorhexidine in FK. I will also explore potential reasons for it not performing as well as natamycin in terms of vision and perforation rate.

There is little published data on the natural course of fungal keratitis without treatment; it is necessary to review the literature for case reports of fungal keratitis prior to antifungal therapy. It is fascinating to note that prior to the 1960s, fungal keratitis was considered a very rare disease. A case series of fungal keratitis from 1956 highlights this point eloquently in its introduction:

"From the time of Theodore Leber's first report of aspergillosis of the cornea,[3] in 1879, mycotic keratitis has been the subject of an occasional case report, averaging little more than one case per year in the ophthalmic literature in the subsequent three-quarters of a century."[4]

The authors of this paper report three cases of FK, all of which progressed to deep infection and/or perforation, requiring eye removal. They postulated that this increase in cases could be due to the increased use of corticosteroids and antibiotics.[4] There then followed a significant increase in reports in the literature of FK, at a time when there were no effective antifungals.[5, 6] All of these with culture-proven fungal keratitis required surgical removal of the eye. In an animal model, *Aspergillus* was inoculated onto a rabbit's cornea leading perforation.[3] Given these reports, one can conclude that without treatment, FK often, if not invariably, progresses to severe infection, perforation, and ultimately loss of the eye.

In our trial, the majority of chlorhexidine-treated patients healed (151/175, 86.3%), although this was less than natamycin-treated cases (163/173, 94.2%; P = 0.018). Given the assumption that untreated fungal keratitis leads to adverse outcomes (including perforation and need for eye removal), and that the first aim of treatment in managing fungal keratitis is to preserve the eye, chlorhexidine should be considered as a therapeutic option if natamycin is unavailable or prohibitively expensive.

This argument is further supported when one considers the perforation and/or therapeutic penetrating keratoplasty (TPK) rate of patients treated with chlorhexidine in our trial and contrasts this to similar studies. Previous studies have demonstrated perforation or rates of between 11.1-43.8%, despite treatment.[7-11] In our trial, participants fared considerably better: 5.8% and 13.7% in the natamycin and chlorhexidine arms, respectively. In MUTT1, perforation and TPK rates for natamycin and voriconazole were 11.1% and 21.1%, respectively.[9] Overall,

patients in our study did considerably better than anticipated, particularly those in the natamycin arm.

Despite the considerable evidence for the superiority of natamycin over voriconazole following MUTT1 and subsequent meta-analyses,[9, 12, 13] voriconazole is still often used as a first-line or adjunctive agent in many countries.[11, 14] Although we did not directly compare chlorhexidine to voriconazole, chlorhexidine-treated patients in our trial had a 0.03 logMAR worsening in their 90-day BSCVA from baseline,[2] whilst patients treated with voriconazole in MUTT1 had a mean improvement of 0.07 logMAR from baseline.[9] This suggests that treatment with chlorhexidine may yield similar results to treatment with voriconazole.

Our trial found that there was no evidence of a difference in re-culture positivity between arms at day 7.[2] This supports findings from previous *in vitro* work that found chlorhexidine to be an effective fungicidal agent.[15, 16] This finding suggests that chlorhexidine is as effective as natamycin in the initial "sterilisation" phase of treatment, and that the difference in the two treatment arms in terms of adverse outcomes (perforation or dense scar formation leading to poor vision) is more likely attributable to delayed healing or immune-mediated tissue destruction rather than related to resistance of the micro-organisms. The difference in vision at 90-days between arms is due to higher rates of larger scars, corneal oedema, and persistent epithelial defects in the chlorhexidine group compared to the natamycin group. This may indicate that whilst chlorhexidine was effective at killing the fungi, it was also damaging the host corneal tissue. The dosing regimen we followed was based on the standard of care for managing fungal keratitis and previous trials,[9, 11] whilst the chosen concentration of chlorhexidine 0.2% as opposed to weaker formulations was due to earlier pilot study results.[17] In practice, this concentration and frequency may have been too prolonged, resulting in toxicity to the corneal epithelium and keratocytes.[18] Adding weight to this theory is that we found no evidence of a difference between chlorhexidine- and natamycin-treated patients for those with mild baseline disease (and no cases of poor visual outcome in either arm for these mild cases). Dosing frequency was typically rapidly reduced in these cases, and rarely continued for more than one month. Large, deep ulcers, may have been "over-treated" with chlorhexidine, with the potentially misguided belief that they required prolonged, intensive therapy, that actually led to corneal toxicity and worse outcomes. Further research is necessary to evaluate if lower concentrations of chlorhexidine, or less frequent dosing, remains effective whilst less toxic to the cornea, ultimately leading to better clinical outcomes.

We chose a pragmatic primary outcome measure: BSCVA at 90 days. This has been the primary outcome of several other trials and facilitates comparison. Three months is the time

at which clinical experience suggests most corneal ulcers have usually healed, whilst BSCVA is easy to measure and is of functional significance. The two earlier trials comparing chlorhexidine to natamycin used a favourable clinical response at day 5 or "curing" at day 21, and did not look at longer-term outcomes.[17, 19] In our trial, we found no evidence of a difference between the two groups for visual acuity at 21 days (highlighting the importance of prolonged follow-up for comparison), whilst re-epithelialisation and infiltrate size was consistently slower and larger in the chlorhexidine group at each follow-up, respectively. These findings could be explained due to corneal toxicity. Corneal scarring can diminish with time with previous work in bacterial keratitis showing ongoing improvement up to 12 months from starting treatment;[20] it would be interesting to compare the two treatment arms in terms of vision at 12 months or beyond. Should chlorhexidine be within the non-inferiority margin at this time, there would be clearer evidence to recommend the use of chlorhexidine.

As mentioned above, regardless of treatment arm, patients performed considerably better than expected, with natamycin-treated patients performing particularly well. Chlorhexidine essentially did as well as we had expected, but natamycin significantly outperformed our expectations that were largely based on the observed outcomes in MUTT1.[9] There are several possible reasons for this. Firstly, we excluded all patients attending who had used antifungals prior to attendance, whilst MUTT1 did not, with 46% of patients using a topical antifungal before enrolment. These patients may have already been not-responding to treatment, potentially introducing a degree of bias to MUTT1. Secondly, patients in MUTT1 continued on their allocated treatment regardless of response to therapy, compared to our trial where we had a protocol for patients not responding to treatment (Appendix 9), largely based on the TST Protocol.[11] This strategy appeared to have been successful, and supports the use of a similar treatment approach should patients not respond as anticipated. Thirdly, we only excluded patients with no light perception vision in the affected eye, compared to MUTT1, which only recruited patients with a vision between 0.3 and 1.3 logMAR. Patients with mild disease with good baseline vision performed well regardless of treatment allocation; MUTT1 did not include such patients. Conversely, we also enrolled severe ulcers which were likely to do badly regardless of treatment allocation, which MUTT1 did not include.

Natamycin is superior to chlorhexidine for the treatment of filamentous FK and remains the first-line treatment. Whilst every effort should be made to ensure that natamycin is widely available and affordable to those most in need, in the meantime chlorhexidine can be considered an alternative, "better than nothing" agent. Treatment algorithms that recommend differing strategies (topical, systemic or targeted therapy) depending on the size and depth of the corneal ulcer at baseline, and how it responds to initial therapy, may result in improved

outcomes and may explain our relatively low “failure” rate overall. Natamycin should be the first-line agent in such a protocol, with chlorhexidine substituted should natamycin not be available (**Figure 32**). Chlorhexidine may have a role as an adjunctive agent to natamycin, with work currently under way in East Africa to investigate this.[21]

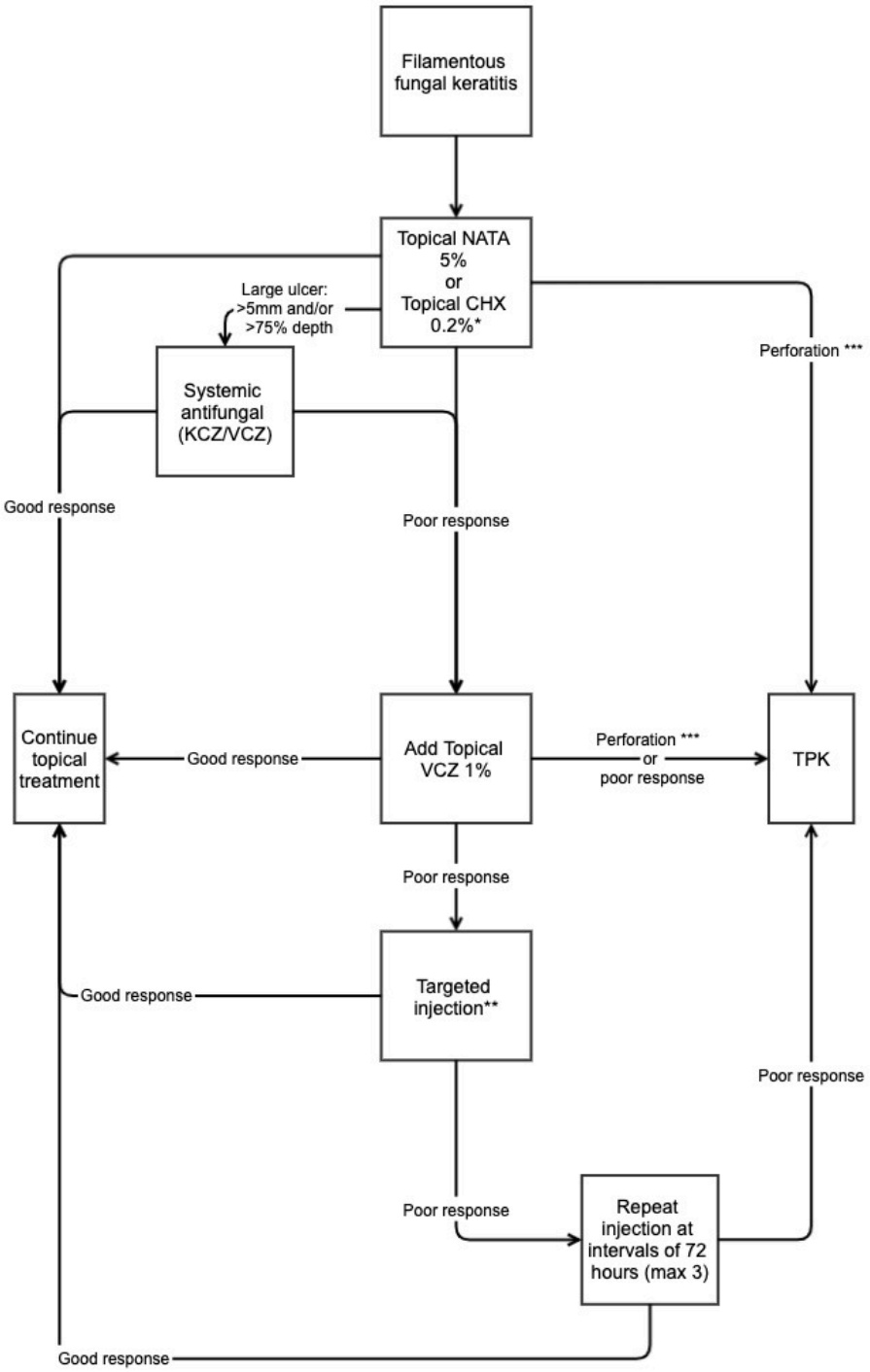

Figure 32: Suggested treatment protocol for filamentous fungal keratitis. * Topical Natamycin 5% is first-line but chlorhexidine 0.2% can be given if natamycin is unavailable. **Targeted injection refers to either intrastromal / intracameral voriconazole / amphotericin B. *** Perforation includes descemetocele, impending, and frank corneal perforations. Adapted from the TST Protocol.[11]

Microbial Keratitis in Nepal: Predicting the Microbial Aetiology from Clinical Features

As part of the two-stage consent process described in detail in Chapters 4 and 7,[1] we were able to conduct this nested cross-sectional study of all eligible, consecutive microbial keratitis (MK) patients presenting between June 2019 and November 2020 to Sagarmatha Choudhary Eye Hospital (SCEH). The aim of this study was to investigate the clinical and epidemiological features that can predict the microbial aetiology of MK in Nepal and to develop a clinical scoring tool that can help to make a predictive diagnosis of either bacterial or fungal keratitis in the absence of further investigations. We also presented the microbiological aetiology of MK in our cohort of patients.

There were several key findings from this study:

- **Fungal keratitis is the main cause of MK in this location, with some of the highest reported figures in the literature**

  Fungal organisms were found to be implicated in up to 82.9% of MK presenting to SCEH (including polymicrobial bacterial-fungal infections). To the best of our knowledge this is the highest reported proportion of MK to date, surpassing 81.5% of MK cases reported in Sri Lanka,[22] and previous work from Nepal, including 70% from nearby Biratnagar.[23]
- **Curvularia spp. was the most frequently isolated fungal genus**

  Curvularia spp. accounted for 42.8% of all fungal organisms. To the best of our knowledge this is the first study to report *Curvularia* spp. as the leading aetiological organism. Although dematiaceous fungi have been implicated in FK previously, typically *Fusarium* spp. is the most commonly isolated organism in similar climatic regions (for example in South India). Given both Fusarium and dematiaceous fungi are plant pathogens found in the soil in tropical and sub-tropical regions and therefore both usually associated with organic trauma, the reason behind the relatively higher proportion of dematiaceous fungi in this cohort is unclear. Further work is warranted to explore this further.
- **The proportion of patients with no microbiological diagnosis was low**

  In this study, there was no microbiological diagnosis (by culture or smear microscopy) in 17.3% (111/642) of cases. *In vivo* confocal microscopy assisted in diagnosing some of these “negative” cases by unequivocally detecting fungal hyphae, reducing the proportion of unknown organism to 77/642 (12%) of cases. This is low compared to other studies. In the recent review by Brown et al., the mean proportion of culture negative MK was found to be 40.8%, ranging from 5% to 74.4%.[24] Prior to our

collaboration with SCEH and our research project, there was no microbiology laboratory in this region. As part of our collaboration and capacity building, we recruited a microbiologist and installed a purpose-designed microbiology laboratory. The low culture negative rates reported by this study highlights that this setup is functioning well and can serve as a model to other tertiary units in the area looking at developing these facilities.

- **A relatively low proportion of patients reported a definite history of preceding trauma**

  In this study, only 33% of fungal keratitis gave a preceding history of trauma. This is considerably less than expected based on previous studies from a similar location in Nepal.[25] Fungal infection can only develop in the absence of an intact corneal epithelium, meaning that trauma, pre-existing ocular surface disease, contact lens use, or recent ocular surgery are the main risk factors. Given that there were no cases of contact lens wear or recent surgery in our cohort of patients, trauma and ocular surface disease (OSD) are likely to be the main contributing factors. There may have been an element of recall bias when collecting the clinical history, with minor trauma not being recalled, particularly for patients presenting late. Minor abrasions, potentially caused by dust of chaff, may not have been significant enough for most patients to recall. Regarding pre-existing OSD, there were no patients in our cohort with previously diagnosed ocular surface disease. Although we did examine the fellow eye at enrolment, fluorescein was not used, meaning we are unable to comment accurately on whether there was pre-existing undiagnosed OSD. As a result, we did not report OSD prevalence in this study. Although infrequently reported from LMICs,[7] OSD has been implicated in several studies for south Asia, where it has been reported to occur in between 1.5 and 19% of cases.[26, 27] The only previous study from Nepal identified OSD in 6% of cases.[25] Further work is required to identify the proportion of OSD in this or similar cohorts of patients.

- **Incidence was highest during the harvesting months, and not during the monsoon rains**

  The finding that there were more cases of MK (and in particular FK) presenting during the dry, harvesting months (October-January) supports the theory that direct trauma (during harvesting) is a key preceding factor. During this time, other activities such as winnowing and threshing take place, increasing occupational exposure to dust and fungal spores. Interestingly, there did not appear to be any correlation with rainfall. As the majority of our cases were found to be dematiaceous fungi, the relationship with climate and rainfall is likely to differ to other organisms. A study from North India with a similar climate reports a similar trend to ours for dematiaceous fungi.[28] Further work

is required to explore the relationship between different fungal organisms, climate, and fungal keratitis, particularly given a changing global climate.

- **Presence of a serrated or irregular margin is the most useful clinical sign in differentiating FK from BK**

  We found that the presence of a serrated margin, raised slough, patent nasolacrimal ducts, and a history of organic trauma were independently associated with fungal keratitis. Serrated margins are a particularly useful clinical sign, as in this study this was the only clinical sign where both the presence and absence were significantly independently associated with fungal or bacterial keratitis, respectively. A previous similar study found serrated margins, raised slough, colour other than yellow, and absence of fibrin to be discriminatory for fungal keratitis.[29] Putting this all together, although there are no clear pathognomic signs for FK, the presence of serrated margins and raised slough in a patient should be considered highly suggestive of fungal keratitis, and in the absence of further investigations empirical antifungal treatment should be given. Satellite lesions have previously been considered indicative of FK based on limited case reports, but this study, along with other cross-sectional studies,[29, 30] suggests their presence is not discriminatory.
- **Clinical features associated with dematiaceous fungi include raised slough, pigmentation, absence of satellite lesions, and absence of fibrin.**

  Although all pigmented corneal ulcers are likely to be caused by dematiaceous fungi, as was the case in our study, we found only 16% of dematiaceous cases were clinically pigmented on presentation. This is similar to work from India and Thailand.[28, 31] In addition to pigmentation and raised slough, which have previously been shown to be predictive of dematiaceous fungal infection,[32] we also found absence of satellite lesions and/or absence of fibrin to be independently associated with dematiaceous fungal infection. Due to the small sample size, we did not find any clinical features associated with either *Aspergillus* or *Fusarium* keratitis.

At present, the typical management for fungal keratitis is with natamycin 5% given empirically. However, different fungal organisms respond to treatment to differing degrees. For example, *Aspergillus* responds more favourably to voriconazole compared to *Fusarium.*[9] Fungal cultures are often unavailable in LMICs, and when they are available usually require incubation for several days. The ability to predict the causative fungal organism based on clinical signs, may help the clinician provide more tailored anti-fungal therapy from the outset and ultimately improve outcomes. Although there is currently no evidence for a preferred treatment regime for dematiaceous fungal keratitis, should this become clear, management

recommendations could potentially be made based on initial clinical signs, whilst culture results are awaited.

This study was conducted at a tertiary eye hospital, with 72% of patients having used topical antibiotics prior to attendance. The results should therefore be interpreted with a degree of caution, as the microbiological profile presented may not truly represent the responsible organisms at the community level, with bacterial keratitis cases being under-represented. We conducted this study at a tertiary centre as this was logistically much easier. Indeed, the majority of all studies reporting the microbial aetiology in LMICs have been conducted in secondary- or tertiary-level care settings,[7] making comparison with ours possible. However, further work at the primary-care level to deepen our understanding of the microbiological profile of MK, and how primary and secondary prevention measures can influence this, is required.

Burton and Leck created an algorithm to calculate the probability of fungal keratitis from clinical signs,[33] based on an earlier cross-sectional study.[29] This is a useful tool for a clinician without access to microbiology facilities to assist with clinical decision making. An updated version of this algorithm, based on the results from this study, is presented in **Figure 33**. We have removed vegetative trauma as an option, as although it was independently associated with fungal infection, when included in the algorithm it did not change the probabilities much and is subject to recall bias. Using this tool, the probability (in our population) of fungal infection in a MK patient with serrated margins, raised slough, and patent nasolacrimal ducts is 69.7%, compared to 10.8% in a patient with defined margins, flat profile, and nasolacrimal duct obstruction. We will be circulating a version of this algorithm in Nepali to the SCEH satellite clinics to assist the ophthalmic assistants with diagnosing and appropriately referring microbial keratitis.

Whilst this study provided some novel and clinically useful findings, one needs to build on this work to take it forward. For example, one of the limitations from the present study was we did not independently test the clinical scoring tool on a different cohort of patients. It would be useful to use our tool, and the algorithm presented in Figure 2, in a novel cohort of patients and see how accurate it fares. A deeper understanding of what organisms are being encountered further upstream is also warranted. A detailed discussion of future work is given in Chapter 11, including how unsupervised machine learning (“artificial intelligence”) could be used in predicting the microbiological aetiology from clinical features.

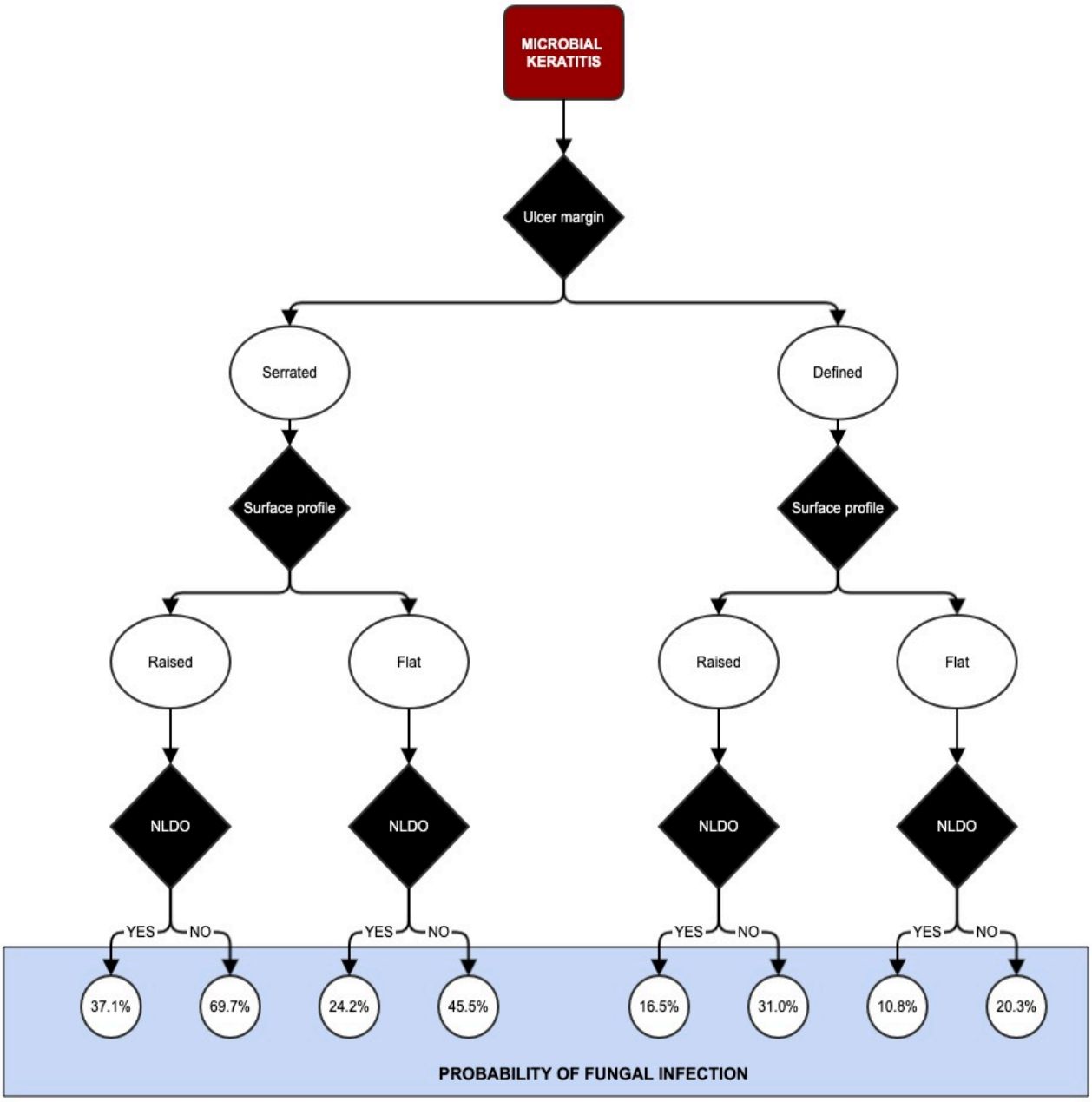

Figure 33: Algorithm for determining the probability of fungal keratitis. The black diamonds are decision points for the four clinical features: ulcer/ infiltrate margin, surface profile, history of vegetative trauma, presence of nasolacrimal duct obstruction (NLDO). NLDO, nasolacrimal duct obstruction; Y, Yes; N, No.

Diagnosis of fungal keratitis in low-income countries: evaluation of smear microscopy, culture, and in vivo confocal microscopy in Nepal

In this cross-sectional study nested within a prospective cohort study of 642 patients with microbial keratitis, we collected data including the patients' microbiological diagnosis and the results from smear microscopy (KOH, Gram Stain and/or calcofluor white), *in vivo* confocal microscopy, and culture. We then analysed this data to calculate the sensitivity of each investigation compared to a composite reference. We found that smear microscopy (sensitivity 90.7% [95% CI 87.9-93.1%]) and in vivo confocal microscopy (89.8% [95% CI 86.9-92.3%]) were the most sensitive tools for diagnosing fungal keratitis in our cohort, with KOH having the highest sensitivity of all the microbiological staining techniques (85.3% [95% CI 81.9-88.4%]). Placing the results of this study into the wider context, we recommend clinicians working in low-resource settings with a high burden of fungal keratitis perform corneal scrapes for microscopy using KOH and Gram staining as a minimum, with culture remaining a useful tool to diagnose bacterial infection.

In tropical and sub-tropical LMICs such as Nepal, where the burden of FK is the greatest, access to microbiological diagnosis beyond microscopy is very limited.[7, 24] Culture facilities typically only exist in secondary- or tertiary-level centres, and even when they are available, cost can be a significant barrier to performing them. Talking to colleagues in Nepal, there is a reluctance to perform corneal scrapes and send specimens for smear microscopy due to the perceived costs involved and limited benefit. This work challenges this preconception, providing evidence for the use of smear microscopy (and in particular KOH staining) in accurately diagnosing FK in these settings. Whilst we found IVCM to be a very useful and accurate tool in diagnosing FK, its high upfront and consumable costs (each patient-visit requires a new, sterile, single-use "Tonocap") make this investigation prohibitively expensive to such settings. However, there is certainly a role for it in select tertiary care centres where IVCM can play a key role in diagnosing referred "atypical" keratitis, particularly by identifying *Acanthamoeba* infection.

Given these findings, on a health system level my recommendation would be for secondary-level care settings where microscopy facilities already exist (in LMICs such as Nepal) to train their microbiologist or technician to accurately perform and interpret KOH and Gram-stained corneal scrapes. If the facility does not have a suitably trained eye care professional to perform corneal scrapes, then a partnership could be established with nearby eye care professionals who do not have access to microbiology facilities. In conjunction with this, stakeholders would need to receive further training and instruction on the usefulness of microbiological diagnosis.

Tertiary-level centres such as SCEH should perform culture, and where possible, sensitivity testing to further aid in the management of MK. IVCM, and potentially PCR, could be employed at a few select centres across the country to aid in diagnosing atypical keratitis. In Nepal, this could involve units in Pokhara in the west, Kathmandu in the centre, and Lahan (at SCEH) in the east.

On a clinician level, my recommendation is summarised in **Figure 34**. In settings where FK is endemic, FK should be considered until proven otherwise. Elsewhere, clinical features, as discussed above, may suggest fungal infection. If available, take anterior segment photographs (a modern smartphone image may be adequate) and perform IVCM. Corneal scrapes should then be taken, with a minimum of two microscopy slides (one for Gram staining and one for KOH). If culture facilities exist, then further samples should be taken, using a fresh needle (or flame-sterilised blade) for each medium and slide.

PCR is a potentially useful tool in diagnosing MK by detecting the presence of fungal DNA, including from dead organisms. Whilst early studies have suggested it may be more sensitive to culture,[34] contaminants can often lead to false positives, whilst a recent "real world" evaluation of PCR, IVCM and culture found PCR to be the least accurate.[35] PCR for fungal detection is not readily available in LMICs at present and previously considered prohibitively expensive, although following the COVID-19 pandemic and the rapid expansion of PCR facilities worldwide accessibility may increase.[36] This study did not assess the accuracy of PCR as these facilities were not available at SCEH. Samples for PCR and sequencing were taken from each participant that will be analysed in the future at the London School of Hygiene & Tropical Medicine. Once optimised primers have been developed, comparison of PCR can then be made with microscopy, culture and IVCM, against a new composite referent. Further planned molecular work is discussed in more detail in Chapter 11.

As the diagnostic criteria for bacterial keratitis in this study required positive cultures, it was not possible to assess the accuracy of culture for bacterial keratitis. Further work is therefore required to investigate this, potentially in conjunction with PCR as a composite reference standard, similar to recent work from Moorfields Eye Hospital in London.[35]

Whilst the main results of this study are of particular use in LMICs, they are also of relevance to microbiologists and ophthalmologists working in high-income settings. This study highlights the value of IVCM in diagnosing FK, as supported by numerous other recent studies.[35, 37-39] Furthermore, typically only Gram stains are performed on corneal scrapes unless additional slides are provided and the request form clearly suggests fungal keratitis as a potential

differential diagnosis. This should therefore be performed should the history or clinical features be suggestive of fungal keratitis. Microbiologists should be aware of the value of KOH in these scenarios.

In summary, microscopy – both smear and confocal – were the most sensitive tools in our study for detecting fungal keratitis, with KOH-wet mount slides the most sensitive of the different staining techniques assessed. Culture remains very valuable in diagnosing bacterial keratitis. These results have potential implications for allocating resources to improve the diagnosis of FK on a health system level, whilst providing clinicians with evidence for the routine use of corneal scrapes coupled with cheap, yet accurate, smear microscopy.

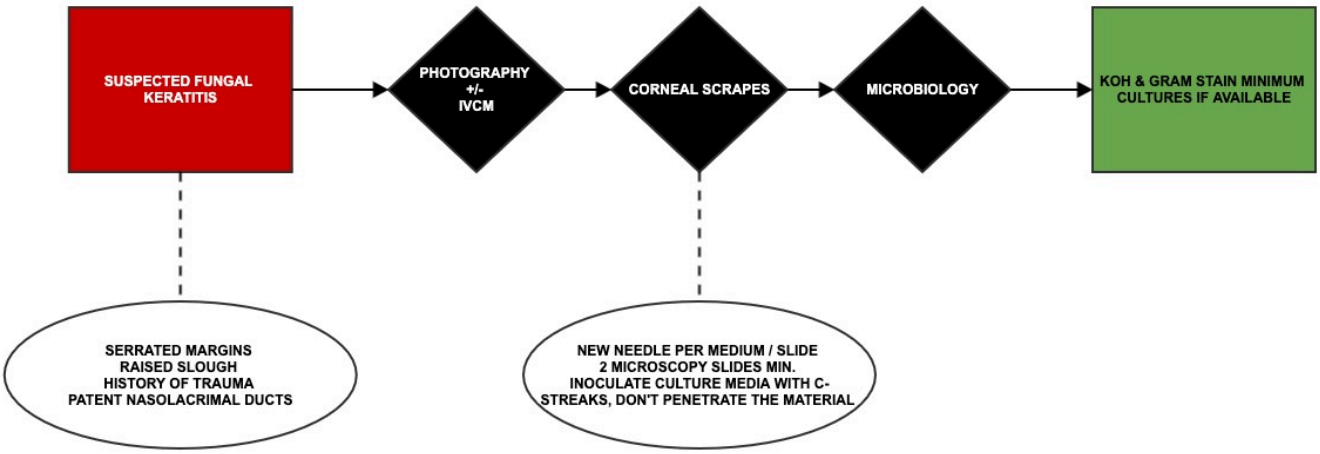

Figure 34: Proposed diagnostic approach for clinicians working in low-resourced settings in tropical and sub-tropical latitudes where fungal keratitis is more prevalent

Delay in accessing definitive care for patients with microbial keratitis in Nepal

In this cohort study, we collected data prospectively on 643 patients with microbial keratitis, including their demographic details, clinical history, and examination findings. Care-seeking journey details were captured including places attended, number of journeys, time from symptom onset, and costs. We then analysed this data to evaluate delay to presentation and reasons for this.

We found that more than three quarters of patients (76.4%) presented to another healthcare setting prior to our tertiary centre, with the majority (51.9%) attending a pharmacy, where frequently inappropriate initial treatment was given (including topical steroids). Over half (51%) of all patients presented after more than seven days from symptom onset. The total cost of care significantly increased with increasing numbers of journeys made ($p<0.001$), whilst we found that distance from the eye hospital of more than 50km (aOR 5.76 [95% CI 1.83-18.1, $p=0.003$]), previous treatment with antifungal medication (aOR 4.71 [95% CI 3.14-5.36]), and two or more previous journeys (aOR 1.44 [95% CI 1.11-3.26]) were factors independently associated with delayed presentation.

Placing the results of this study into the wider social context, we have suggested several opportunities to reduce this delay to improve the clinical outcome: appropriate early treatment and avoiding harmful treatment at the primary care level, improving access to eye care services, improved training in eye health to primary-level healthcare workers, and strengthening the referral network.

The results of this study together with these opportunities relate to different points along a previously described conceptual framework for access to healthcare,[40] with access defined as "the opportunity to have healthcare needs fulfilled". The concept of access being reliant on the interaction between the healthcare system (supply side) and patients' ability to seek and obtain care (demand side) was originally described by Levasque and has since been adapted by several authors;[40-43] it is this interaction between providers and patients that permits access. This model has been updated with the findings from this study and potential opportunities for improvement listed (**Figure 35**). These are discussed in more detail below.

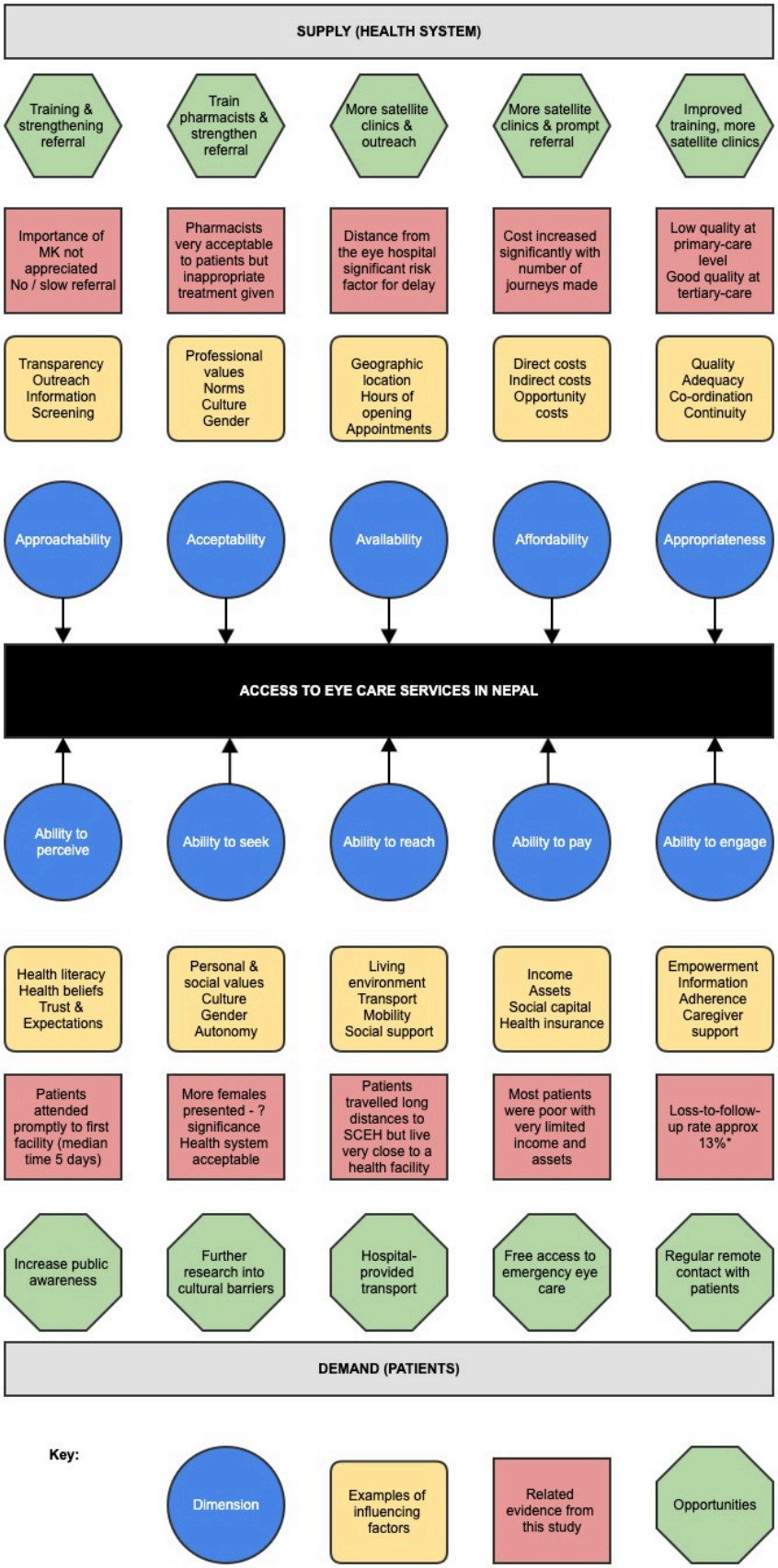

Figure 35: Adapted conceptual framework of access to health care with main results from this study and potential opportunities for improvement given. * Loss-to-follow-up data is what was reported in our trial. [2] Adapted from [40] and [44]

The first dimension in our updated framework that impacts on access to eye care services in Nepal is approachability, which refers to patients with MK being able to identify a reachable healthcare service that can have an impact on that patients' disease. This interacts with the patients' ability to perceive they have a problem. We found that whilst most patients were good at identifying MK as an issue (as evidenced by early attendance to a healthcare facility with a median time of 5 days for both direct and indirect attenders), when presenting to a primary-level facility (including pharmacists), the significance of their disease was not appreciated by the care provider resulting in inappropriate management (and not infrequently potentially harmful topical corticosteroids) and no referral. Early treatment is associated with improved clinical outcomes;[45, 46] with improved training of pharmacists and primary-level healthcare workers to deliver topical antibiotics and refer patients early, the clinical outcome should hopefully be improved. This can be further strengthened by increasing public awareness of the importance of corneal trauma and MK and highlighting the need for urgent treatment.

Acceptability refers to factors that impact on the patients' willingness to accept certain aspects of the health service. We did not find any evidence to suggest issues with acceptability of established eye care services in Nepal, although we did not specifically investigate this. This could be assessed through future focus group discussions and qualitative research. However, this study suggests that patients are very accepting of receiving care from pharmacists, with over 51% of all indirect attenders presenting initially to a pharmacist (and 39.7% of all patients). Unfortunately, pharmacists in Nepal are loosely regulated and we found patients who visited them had frequently been given topical steroids, which are known to lead to poor clinical outcomes.[7] This can be addressed through training pharmacists on eye care management, including dispensing topical antibiotics and early referral, in conjunction with legislation and enforcement against over-the-counter dispensing of steroids. Acceptability is influenced by the patients' ability to seek the health service, affected by factors such as gender inequality and culture. Interestingly, we found that the majority of patients presenting to SCEH with MK were female, in contrast to similar work from Uganda.[47] Whilst gender inequalities have been reported in other ophthalmic conditions including trachoma and cataract, women were typically disadvantaged compared to men. However, we are unable to draw any conclusions on this as we do not know the gender demographics of MK patients at the community level.

Availability refers to the health service being reached in a timely manner. This is a key dimension from our study, given that distance to the eye hospital is a significant risk factor for delay. Related to this is the patient's ability to reach the service. Patients lived a median distance of 37.3km (IQR 23.5-66.2) to SCEH, whilst only 2km (IQR 1-5km) from a primary-

level health centre. Access can be improved by increasing the number of satellite clinics with suitably trained ophthalmic assistants and eye care workers, thus bringing the service to the patient, whilst also offering hospital transport to patients to attend the tertiary-level hospital where appropriate. Although the number of ophthalmologists within Nepal is still relatively low, by "task-shifting" through training ophthalmic assistants, human resource shortage is less of a factor compared to sub-Saharan Africa.[48, 49] However, the location of personnel in relation to the patients remains a significant barrier to accessing eye care services in Nepal.

Affordability of the health system and the patients' ability to pay are clearly interlinked dimensions that affect access to eye care services, with cost an established major barrier.[50] Another key finding from this study was how cost significantly increases with increasing number of facilities visited, in keeping with earlier studies.[47, 51] The main direct cost for patients accessing eye care in this setting was for consultations, followed by transportation costs. We did not quantify the associated indirect costs through lost earnings, but given many patients were making multiple journeys over several days, these costs are likely to have been high for many patients. Unfortunately, most of the patients presenting with MK were poor individuals, from low-ranked castes, primarily involved in subsistence farming. The ability for these patients to pay for care is therefore low, further hindering access. By reducing the number of journeys made by patients (and therefore unnecessary medical consultations) through improved referral pathways, more satellite clinics and educating primary-level care workers, the direct costs will be reduced. This could be further reduced by offering free or heavily subsidised access to emergency eye care, with assistance from governmental and non-governmental organisations.

The appropriateness dimension refers to the fit between the services provided and the patients' needs.[40] It was clear that the primary-level facilities are currently providing inappropriate care, given this is largely being provided by untrained pharmacists or health workers who have received no formal ophthalmic training, with many patients having to attend multiple facilities before definitive treatment is given, as well as frequently receiving inappropriate medication. Linked to this is the patients' ability to engage with care, including their adherence to treatment. Whilst this study did not directly follow all patients within this cohort up through treatment, our randomised controlled trial of fungal keratitis patients (which formed a subset of 354 of these 643 patients) found a loss-to-follow-up rate of approximately 13%,[2] similar to previous trials. It is likely that follow-up rates are considerably lower in non-trial settings, given the efforts that our research team had to go to in order to achieve this. Potential opportunities to improve access within these dimensions include improved training

to primary-level staff, more satellite clinics, and close follow-up of treated patients, potentially using remote methods.

Whilst there are five distinct points on the pathway that affect access to eye care services in this setting, there are three relatively simple potential interventions that together would cover all five "supply" related dimensions: improved training, referral strengthening, and increasing the number of satellite clinics. A cluster-randomised controlled trial comparing standard care to an interventional package of enhanced care at the primary-care level (which includes referral strengthening and enhanced training of primary-level stakeholders) for patients with MK in Nepal is planned by our group to see if this will indeed improve access and ultimately clinical outcomes.[52] Increasing the number of satellite clinics will require considerable investment both in terms of personnel and infrastructure, which may be challenging in the current economic climate. Other secondary prevention strategies – such as giving topical antibiotics following corneal abrasions – have been shown to be affective,[53, 54] whilst a large community randomised trial that examines corneal ulcer prevention by trained village-level health workers is currently recruiting in Nepal.[55]

Another important aspect to consider is primary prevention. Given the strong association between trauma and developing microbial keratitis in LMICs,[56-58] ways to reduce the incidence of ocular trauma need to be investigated. There is surprisingly limited data on how eye protection can reduce the incidence of corneal trauma in LMICs,[59] although a recent RCT from India showed patients wearing protective eyewear had a 94% lower risk of ocular trauma compared to those without eye protection.[60] However, barriers remain to the acceptability of this intervention. More work is required to see how uptake can be improved and whether this could be a feasible measure.

The hope is that by implementing some of the suggested interventions from this study, access to eye care services in Nepal will be improved, ultimately improving clinical outcomes. Further studies investigating whether these strategies do in fact make a difference are currently underway.

Conclusion

The research conducted as part of this PhD has helped increase the evidence for the diagnosis and management of fungal keratitis in LMICs as well as increasing our understanding on patients' barriers to accessing care.

Bringing all these separate studies together, we are able to offer a diagnostic strategy, initially using clinical features and then supplemented with investigations. The clinical signs of raised slough and serrated margins are particularly helpful and should prompt the clinician into further investigations for FK, specifically IVCM if available and smear microscopy using KOH wet mount and Gram stain. If FK is confirmed, then treatment should be with topical natamycin 5% if available or chlorhexidine 0.2% if it is not. Improving access to eye care services, through improved training of pharmacists and primary-level health workers and strengthening the referral pathway is likely to reduce treatment delays and improve outcomes. This is currently under further investigation.

Whilst the lessons learned from this PhD should help clinicians and guide public health policy in managing fungal keratitis in LMICs, there is still a considerable amount of further work to be done to tackle the ongoing challenges resulting from this neglected tropical disease. Natamycin remains the gold standard treatment for FK and is on the WHO essential medicines list, yet access to it in many parts of the world is severely restricted. This must be addressed as a matter of urgency, with the use of chlorhexidine recommended in its absence. Making fungal keratitis an officially recognised neglected tropical disease by the WHO may help focus attention and resources to enable these challenges to be dealt with.

Ultimately further work is required to develop improved primary prevention strategies, improve public awareness and engagement, train primary-level health workers and pharmacists, and strengthen the capacity of the primary health system to diagnose, manage and refer patients with microbial and in particular fungal keratitis. Further planned research to help investigate these domains is detailed in Chapter 11.

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Chapter 11: Future work

Members of the study team outside a temple in the Terai region of Nepal we visited following a sensitisation visit to a satellite eye care clinic

There are several studies currently underway that we will conclude:

1. **Quality of life at 3 months:** we plan to investigate the effect of the alternative treatments (chlorhexidine 0.2% and natamycin 5%) on the quality of life of participants.

   History and examination alone give little to no insight into the effect that a disease can have on a patient's social well-being and their functioning. Quality of life (QoL) broadly refers to an "individual's perceptions of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns".[1] QoL can be assessed quantitatively using different tools depending on what is of interest. For example, disease-related QoL can be assessed (e.g. vision-related QoL, VRQoL) or more general health-related issues irrespective of the disease can be investigated (health-related QoL, HRQoL).[2]

   At baseline and at 3-month follow-up we conducted the WHO/PBD-VF20 (World Health Organisation/ Prevention of Blindness and Deafness—Visual Functioning 20 item questionnaire) VRQoL tool. This tool measures the impact of visual impairment in the person's life including mental wellbeing, dependency and social functioning. These have been used in a number of other visual related studies to show a difference in QoL.[3, 4]

   For HRQoL, we used the EQ-5D questionnaire, EQ-Visual Analogue Scale and the WHOQOL-BREF, again conducted at baseline and at 3-months. The EQ-5D is a standardised tool to measure health outcomes.[5] The WHOQOL-BREF has good applicability in (LMIC as it was developed simultaneously from concept across 18 countries in Africa, Asia and Latin America. It measures 4 domains of health: Physical Health, Psychological Health, Social Relationships, and Environment.

   Acute MK causes considerable ocular discomfort with tearing, photophobia, conjunctival hyperaemia and pain, in addition to blurred vision. These acute features of MK, together with vision loss as a sequelae of MK, can have a significant negative impact on people's quality of life.[6-9] MUTT1 assessed QoL as a secondary outcome measure and found patients randomised to natamycin found improvement in VRQoL compared to those taking voriconazole.[7] We plan to investigate how QoL may be affected for patients randomised to either CHX or NATA.

Analysis will be by comparing the scores obtained for each QoL assessment for the two treatment arms to estimate the effect of CHX and NATA on patients' QoL. This will be similar to that performed by Habtamu et al.[2] Comparisons between the two medication groups will be adjusted for the matching variables: age and sex. The VRQoL analysis will also be adjusted for socio-economic status and the HRQoL analysis adjusted for both socioeconomic status and presence of health problems during the previous 4 weeks, as these factors may confound the association between fungal keratitis and QoL. Logistic, linear and ordinal logistic regression methods will be used for binary, continuous and ordered categorical outcome variable analysis, respectively. Linear regression models and the t-test will be employed to compare significant differences in QoL scores and to generate mean and mean differences between the two treatment arms in each QoL subscale and domain, respectively.

2. Distinguishing bacterial and fungal corneal infections using unsupervised machine learning ("artificial intelligence")

A potential solution to help improve diagnostic accuracy, especially where the microbiology is negative or not available, is to develop automated, computer-assisted image analysis of good quality digital photographs of infected corneas. Ideally this algorithm would be developed to be capable of running as an application on a smartphone, utilising the built-in digital camera with or without hardware modification.

Algorithm-based image analysis is not new in ophthalmology: computer-aided automated diagnosis of diabetic retinopathy has been extensively investigated and is gaining significant momentum.[10-13] In particular, there have been some noticeable successes from using Unsupervised Machine Learning (UML) techniques. We will be making use of these advances in image analysis to develop an anterior segment image analysis algorithm, which has not previously been done. In addition, smartphones are becoming increasingly used in ophthalmology and offer novel, portable and low-cost imaging techniques for fieldwork.[14, 15] A key requirement of using UML for this purpose will be having access to a large number of corneal images with a known microbiological diagnosis to train the system.

The main advantage of UML is that the computer does not approach the problem with any preconceived ideas or previously learned pattern recognition, as is the case with clinicians. Studies assessing the diagnostic accuracy of corneal specialists when faced with clinical photographs of microbial keratitis with known aetiology showed correct

diagnosis in only 66% - 73% of cases.[16, 17] However, this rate was considerably lower at 38% when assessing fungal keratitis specifically.[17] It is hoped that by using UML the diagnostic accuracy would be as good as, if not better than these results. In addition, as geography influences the prevalence of bacterial and fungal keratitis, clinicians in different areas may not have equivalent clinical experience. UML would use images from a wide variety of locations, with the option to add geographic metadata to the images ("geo-tagging"), meaning that the probability of a given aetiology could be determined based on the geographical location of where the image was acquired.

We propose developing an anterior segment image analysis application to differentiate the broad causative organisms (bacterial, fungal, protozoal or viral) for microbial keratitis, providing a probability of a patient having a particular type of corneal infection. The ultimate goal is to have a portable, cheap and accurate diagnostic tool for use by clinicians in low- income countries to help correctly diagnose microbial keratitis and prevent the negative sequelae associated with any delay. We have already collected high quality corneal photographs during this study and are currently developing an image analysis algorithm.

3. **Antifungal and antibacterial susceptibility testing:** we plan to perform antifungal and antibacterial susceptibility testing of the micro-organisms isolated during this study.

   Unfortunately, skilled microbiologists and laboratory facilities are often lacking in the areas most in need; more too often treatment is empirical. Even when personnel and laboratories exist, this challenge is compounded by the lack of consensus on the role of susceptibility testing: there is a lack of data with regards to filamentous fungi susceptibility studies; most studies focus on yeast infections; and there is limited consensus on the minimum inhibitory concentration (MIC) clinical breakpoints (i.e. those that categorise the organism into being susceptible, intermediate or resistant).[18]

   MUTT1 yielded some very valuable data with regards to susceptibility testing for natamycin and voriconazole. *Fusarium* isolates were least susceptible to voriconazole, whilst *Aspergillus flavus* were least susceptible to natamycin.[19]

   Our current work with Radboud University testing in vitro susceptibility of MK fungal isolates from Tanzania indicates CHX has MICs that are substantially lower than the 0.2% concentration used for treatment.[20] Moreover, in contrast to other agents,

fungicidal concentrations are close to inhibitory concentration. This means that CHX may be more likely to kill the fungi, rather than just temporarily suppressing the infection. Although natamycin performed significantly better than chlorhexidine in our RCT in terms of clinical outcome measures, there was no difference in re-culture rates at seven days. Further microbiological testing will therefore help to understand these results better.

This further work will allow us to understand the sensitivity to antibiotics or antifungals within our study population, including susceptibility to chlorhexidine. Our data will include bacteria, fungi and amoeba. The results of the analysis of these data will be of great use to clinicians within the region when planning to treat empirically, and may help to form benchmark MIC clinical breakpoints.

Antibiotic susceptibility testing of bacteria culture isolates will be performed using standard disc diffusion techniques at SCEH. Antifungal susceptibility testing of fungal isolates will be performed at LSHTM. Our isolates from Nepal will be transferred to LSHTM, under a signed Material Transfer Agreement that is in place. They will be re-cultured and tested using a dilution series method for four drugs: Natamycin, Chlorhexidine, Voriconazole and Amphotericin-B. This technique has been developed at Radboud University, The Netherlands.

4. **Evaluating the utility of PCR testing for fungal keratitis and sequencing of isolated fungal specimens:** we will update our work on comparing the different diagnostic investigations for fungal keratitis with PCR. We will also conduct sequencing on isolated fungi that were unidentified, and also on those found to be PCR positive but that did not grow on culture.

   Molecular diagnostic techniques are increasingly used in MK. There is no widely agreed standard PCR methodology.[21-25] We currently use pan-fungal quantitative PCR in Tanzania (UK National Reference Laboratory protocol). Sequencing is increasingly important for fungal identification, providing greater specificity than classical microbiology.[26-28] There is growing consensus about the most useful fungal “DNA barcode” regions.[29, 30]

   PCR for microorganisms:
   DNA from the dry swab will be extracted using the QIAamp mini kit (Qiagen) with bead-beating. Samples will be tested for the presence of bacterial (16rRNA) and fungal

(ITS1) DNA by quantitative PCR. This PCR work will be performed at LSHTM. The performance of PCR as a diagnostic tool will be evaluated against a composite reference standard for positive cases. Cases will be considered positive if the pathogen is detected by either smear microscopy, IVCM and/or culture.

Fungal DNA sequencing:

It is not always possible to determine the species of a fungus from classical microbiological techniques. It is now common to use fungal DNA sequencing for species-level diagnosis for some genera, including *Fusarium spp.*, which is the commonest fungal genus reported for MK in the study settings. We will use sequencing of the fungal ITS and TEF1a regions for this.[31] DNA will be extracted from the cultured fungal isolate when available. If there no fungus is cultured but the PCR is positive, then this PCR product will be used for sequencing. The DNA will be extracted from the isolates at LSHTM, where sequencing will also be performed.

5. **Microbial Keratitis in eastern Nepal: prospective cohort study:** we will build on the initial cohort study conducted as part of this PhD to further evaluate the presenting features, patient demographics, microbiological diagnosis and clinical outcomes of patients presenting with microbial keratitis over a full year. This prospective cohort study will enable us to gain a more detailed knowledge and understanding of the presenting features of MK, results of investigations, and clinical course of MK at SCEH. We will be able to compare the results of this cohort study to ours, which had been affected by the COVID-19 pandemic and did not review the clinical course of patients that were not enrolled in the RCT, and see if there are any differences.

6. **Chlorhexidine 0.2% vs. Natamycin 5% for fungal keratitis: long-term clinical outcomes:** we plan to determine whether there is any difference in long-term clinical outcomes for patients treated with chlorhexidine 0.2% or natamycin 5%.

   We will attempt to contact all patients who enrolled in the RCT and examine them for best spectacle visual acuity (BSCVA) and scar size at 2 years following enrolment. Based on our previous analysis, we will use similar regression models with adjustments for baseline status.

In addition to the work directly related to my PhD described above, I will continue to work with my supervisor Prof. Matthew Burton on a 5-year Wellcome Trust funded programme related to severe corneal infections in low- and middle-income countries listed below:

7. **Comparison of two treatment regimes for the treatment of fungal eye infections in East Africa:** we are conducting a large clinical trial to find out whether chlorhexidine 0.2% eye drops in combination with natamycin 5% are as effective as or more effective than natamycin 5% eye drops alone for treating fungal corneal infections.[32] Recruitment is currently underway.
8. **Comparing a comprehensive package of primary care to the standard of care to reduce blindness caused by severe corneal infections in Nepal and Uganda:** we will investigate, in a cluster RCT, whether an early primary-care intervention package, including topical chlorhexidine, can reduce blindness from microbial keratitis. We are will be starting recruitment in the second half of 2022, in two parallel, single-masked cluster RCTs, to be conducted in Nepal and Uganda.[33]
9. **Point of care tests for fungal keratitis:** point-of-care tests for fungal keratitis would be very helpful. Molecular tests are generally more sensitive than antigen-based assays. Isothermal nucleic amplification techniques offer the sensitivity of PCR amplification without the need for specialized equipment. A result may be obtained within 30 minutes. Promising molecular approaches include isothermal amplification (Loop-mediated isothermal amplification (LAMP), Recombinase polymerase amplification (RPA)), associated with lateral flow assays for amplicon detection. Several assays have been developed that detect fungi, however, these have not been evaluated in fungal keratitis. The second ulcer swab will be used to test and develop isothermal molecular assays in comparison with the standard microbiological approaches described above. Results will be evaluated against a composite reference standard for fungal diagnosis as described above.

In addition to the above work, further research is warranted in the following areas, which I hope to develop as part of a post-doctoral grant application:

10. **Primary prevention:** given that the majority of fungal keratitis cases in LMICs are preceded by a history of corneal trauma – often with vegetative matter whilst carrying out agricultural activities – preventing corneal injury as a primary prevention strategy may reduce the incidence of fungal keratitis. A previous small RCT from India has shown that eye protection can help reduce the incidence of corneal abrasions in agricultural labourers, although there were challenges with acceptability which the authors concluded may limit widespread adoption.[34] I plan on carrying out qualitative research into how acceptability could be improved, testing out different designs of eye protection. I would then conduct a large-scale randomised controlled trial comparing

individuals given eye protection to those without, with outcome measures the number of corneal abrasions and microbial keratitis in each arm.

11. **Climate change and fungal keratitis:** we have shown that changes in fungal keratitis incidence at the tertiary healthcare level may be seasonal. Further work to understand this in more detail is required. In addition, with global warming and climate change, the incidence of FK may increase globally. Further work and modelling is required to understand this further.

12. **Qualitative research and public engagement:** a better understanding of the public's perception of microbial keratitis and their perceived barriers to accessing care will be helpful in improving access to eye care services and ultimately improving outcomes. Engaging the public may help as part of a strategy of primary prevention.

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Appendices

Appendix 1: Patient information and consent forms

Appendix 2: Case Report Forms

Appendix 3: Best Spectacle Corrected Visual Acuity Testing Form

Appendix 4: Journey History Completion Form

Appendix 5: Microbiology request form

Appendix 6: LSHTM Ethical Approval

Appendix 7: Nepal Health Research Council (NHRC) Ethical Approval

Appendix 8: Nepal Department of Drug Administration (DDA) Approval

Appendix 9: Deterioration whilst on treatment and treatment failure standard operating procedure (SOP)

Appendix 10: Sample size calculation for the randomised controlled trial of topical chlorhexidine 0.2% vs. topical natamycin 5% for the treatment of fungal keratitis in Nepal

Appendix 11: Method for preparing chlorhexidine 0.2% eye drops

Study Title: Randomised controlled trial of topical chlorhexidine 0.2% versus topical natamycin 5% for fungal keratitis in Nepal

Participant Information Sheet and Consent Form - No 1: For baseline assessment and investigations

Introduction
You are being invited to take part in a medical research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read or listen to the following information carefully and to talk to others about the study, if you wish. Ask us if there is anything that is not clear or if you would like more information. Do not sign the consent form unless you are satisfied with the answers to your questions and decide that you want to be part of this study. Take time to decide whether or not you wish to take part.

Why have I been invited?
You have been invited to take part because you have a corneal infection. To guide the treatment of this it is helpful to do tests to find out the cause, to guide the choice of eye drop treatment. We are asking for your permission to carry out some tests that are listed below to find out what is causing the infection and to decide if you may be suitable for a trial comparing treatments for fungal eye infections.

What is this study about?
Infection of the clear part of the front of the eye (the cornea) is a corneal ulcer. It is an important cause of blindness in Nepal. A scratch in the cornea allows infection to enter and an ulcer to begin. These infections can be very serious with some people losing the sight in the affected eye.

Different types of infectious organisms can cause corneal ulcers. These include bacteria and fungi which need to be treated with different types of eye drop medicines. To find out what type of infection is present it is necessary to collect a small sample from the ulcer and perform various laboratory tests. The test results guide the treatment. In Nepal about half of the infections are caused by fungus.

Fungal infections can be difficult to diagnose. No single test can reliably detect it. It may need several different tests to find it. Currently, in many countries where fungal ulcers are common laboratory services are limited or not available. Tests are expensive and may not detect all of the infections. There is a need to understand which of the current test options give the most useful information.

The first purpose of this study is to carefully evaluate your eye infection using several different diagnostic tests to try to find out what type of infection you have, and to learn which tests are most useful in this process.

There is a need for alternative, affordable and more easily available eye drop treatments for fungal infections. We are also conducting a clinical trial to compare two eye drop treatments for fungal infection, to try to find out which is better.

Therefore, the second purpose of this initial Assessment and Investigation study is to identify people who have a fungal corneal infection who may be eligible and willing to enrol in the clinical trial. If we find out that you do have a fungal corneal infection, we will then ask if you would be interested in hearing about the clinical trial. If you are interested, then at that stage we will explain about the treatment trial in detail, provide you with a second information sheet and go throw a second consent process.

Do I have to take part?
No. Your involvement is entirely voluntary. If you agree to take part, we will then ask you to sign a consent form. If you decide to join and change your mind, you are free to withdraw at any time without giving a reason. This will not affect the standard of care you receive.

Why have I been invited?
You have been invited to take part because you have a corneal infection. To guide the treatment of this it is helpful to do tests to find out the cause, to guide the choice of eye drop treatment. We are asking for your permission to carry out some tests that are listed below to find out what is causing the infection and to decide if you may be suitable for a trial comparing treatments for fungal eye infections.

What will happen to me if I take part?
If you agree to be part of this study, the following will happen:

- We will ask you a series of questions. This will include basic demographic information, the history of your current eye problem, and treatment you have had before arriving at the hospital.

- We will then carefully examine both eyes using a special microscope.

- Your eyes will be photographed with a camera. This is additional to standard care, and will help us to monitor the infection and the response to treatment.

- We will use a special microscope to look at the cornea to see if we can find a fungal infection. This involves putting anaesthetic drops on the eye. A soft plastic device then gently touches the eye so to see if you have a fungal infection. This is additional to standard care, and will help us to find out the type of infection you have more rapidly so that we can offer you the most appropriate treatment.

- We will collect samples form the corneal ulcer to test in the laboratory to try to identify what is causing the infection. Anaesthetic eye drops will be used numb the eye so you will not feel anything while we collect the sample by brushing the surface.

- We will check your blood sugar level for diabetes. This is done using a finger-prick blood sample. If this is raised, we will refer you to a separate group of doctors to help you with this.

- You will be offered a test for HIV infection. This will be done through the hospitals counselling and testing services. If you choose to accept this, then you will be separately counselled about the test and the implications of the results. This would involve the collection of a blood test sample from your arm. If the HIV test is positive, then you will be referred to the appropriate team for ongoing care. The result of this test will be shared with the us (cornea infection study team), with your consent, as it is potentially relevant to the treatment of your cornea infection.

- We will collect a sample of the cells from the inside of your cheek by gently rubbing a swab for a few seconds. This is additional to the standard of care. The purpose is to try to understand how the body fights the infection and why some people develop this eye problem and others do not.

- You will be asked to complete three short questionnaires to assess your quality of life and vision function. This is additional to standard care, and will help us to better understand the impact corneal ulcers can have on people's lives.

If we find evidence of fungus infecting your eye, then we will discuss the details of the trial with you further. At that point we will give you a second information sheet to read (or read to you), and if you meet all the eligibility criteria for the trial, to then go through a second consent process with you.

If you do not have a fungal infection, for example if the infection is caused by bacteria, then your will receive standard ongoing treatment for this by the same team at the hospital.

You may be withdrawn from the study without your consent if the researchers believe it is in your best interest or if you fail to follow study procedures.

What are the side effects or risks of taking part?

- The anaesthetic eye drops usually sting for a few seconds and then your eye should feel more comfortable. There is a very low risk of allergic reaction from the anaesthetic eye drops.
- The procedures including the history taking, examination, photography, in vivo confocal microscopy and sample collection are part of the standard of care for assessing a corneal infection. The risk associated with these are very low and no different from routine care. To minimise discomfort, topical anaesthetic will be given before examinations and sample collection.
- You will experience a little pain when a blood sample is collected. The people collecting the blood sample will ensure that this is done with as little discomfort as possible.
- The questions, tests and examinations will add about 15 minutes to your hospital visit above what we would usually expect in assessing a patient with this problem.

What are the benefits of taking part?

- The study will involve tests for the type of infection. This helps the doctor looking after you to choose the best type of treatment for your eyes
- The costs for the clinical assessment and the tests will be paid for by the study.
- By participating in this study, you will be helping to further research into this field so more informed decisions can be made when treating people with corneal infection.

What will happen to the clinical records, photographs and test results?

Your records will remain strictly confidential at all times. The information will be held in a secure office at your treating hospital. Only the people organizing or supervising the trial and regulatory authority auditors will have access to it. These include officials delegated by the Sponsor (London School of Hygiene and Tropical Medicine), the local National Ethics Committee, The local National Drug Regulatory Authority and trial Data Safety Monitoring Body (DSMB).

Your name will not be passed to anyone else outside the research team, unless we have your direct instruction to do so, for example to make a medical referral.

The photographs of the eye and the result of the laboratory test for infection will be shared with computer engineers to help develop a programme that can automatically analyse the image to see if this can provide some indication of the cause of the infection. Images of corneal infection may also be used for educational and teaching purposes, including in publications. All personal identifying information will be removed before sharing images.

What tests will we do on the sample?

The samples collected from the surface of your eye will be tested in several different ways to determine what is causing the infection. This work will be done in the hospital microbiology laboratory, where you are being treated. A portion of the infection sample will be transferred for additional special tests at KCMC Hospital Biotechnology (Tanzania), the London School of Hygiene and Tropical Medicine (UK) and Radboud University Nijmegen Medical Center (The Netherlands), as some of the tests will require additional special equipment that is not available at all sites.

- We will look for the type of infection using a microscope and by growing the organisms in the laboratory. We will test the organisms that grow to see which medicines work best to kill the infection, which is helpful in guiding the choice of treatment to be used.

- The swab samples from the ulcer will be used to test for infection using molecular diagnostic tests and to evaluate new tests that may be used to find the cause rapidly in the clinic.

- We will use study genetic material of the organism causing the infection to find out the exact type of infection and its ability to resist treatments. Samples from the ulcer may also be used to investigate your immune response to the infection, so that we can better understand what causes corneal scarring

- We will store a sample of the infection causing organism indefinitely for additional testing.

- The genetic material from the cells from your cheek will be sent to the UK or to KCMC Hospital in Tanzania. We would like to store it until a later time when a sufficiently large number of samples has been collected to conduct an analysis of this genetic material. The purpose would be to try to better understand how the human immune system fights the infection and why some people develop this eye problem and others do not. By doing these tests we hope that it will help us to develop approaches that will help to prevent or improve outcomes from this condition.

- As part of this consent we are asking that you give us permission to store this material to be able to test it at a later date as mentioned above. We do not know exactly for how long we shall store the genetic material before we have assembled a sufficiently large collection during the course of several planned studies to be able to proceed to the sample analysis, however, we anticipate a period of at least five years.

What will happen to the results of the research study?
The results of the study will be available after it finishes and will be included in peer reviewed medical and scientific journals and may be presented at medical meetings. Results will also be published on a publicly accessible trials database. The data will be anonymous and none of the patients involved in the trial will be identified in any report or publication. Should you wish to see the results, or the publication, please ask your study doctor.

Who is funding the research?
The research is being funded as part of a grant from the Wellcome Trust, UK.

Who is organising the research?
It is being organised through a research partnership between the London School of Hygiene and Tropical Medicine and Sagarmatha Choudhary Eye Hospital in Nepal, Mbarara University of Science and Technology in Uganda and Kilimanjaro Christian Medical Centre in Tanzania.

What if relevant new information becomes available?
It is not anticipated that new information will become available during the course of this short study. The information from the microbiology tests will be available to the doctors to help select the most appropriate treatment for you.

What if something goes wrong?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the head of the hospital eye department or the named person on the following page. The London

School of Hygiene and Tropical Medicine holds insurance policies which apply to this study. If you experience harm or injury as a result of taking part in this study, you may be eligible to claim compensation.

Who has reviewed the study?
Prospective research such as this is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and approved by (1) the London School of Hygiene and Tropical Medicine Research Ethics Committee; (2) Nepal Health Research Council.

What will happen if I don't want to carry on with the study?
Your participation in this study is entirely voluntary. You may refuse to participate or may withdraw from this study at any time without penalty or loss of any rights or benefits to which you are otherwise entitled. The study doctor may also stop your participation in the study at any time for safety reasons. If you decide to withdraw from the study you should contact a member of the study team immediately. You do not have to give a reason when stopping, however for safety reasons, it is suggested that you tell the study doctor if you decide to stop because of an unwanted side effect. If you withdraw from the study, we will only use data collected before this decision, unless you request this to also be withdrawn. If you withdraw from the study, researchers, authorized persons from the Sponsor and the regulatory authorities will still require access to your medical notes to verify the data collected up to the date of your withdrawal.

Contact Details
Nepal Study Site: Dr Lila Puri, Sagarmatha Choudhary Eye Hospital, Lahan. T: +977 9841460623

Study Coordinator: Dr Jeremy Hoffman: T: +44 7742502530, email: Jeremy.hoffman@lshtm.ac.uk
Chief Investigator: Prof. Matthew Burton: T: +44 2076368636, email: matthew.burton@lshtm.ac.uk

You will be given a copy of the information sheet and a signed consent form to keep.
Thank you for considering taking the time to read this sheet.

Randomised controlled trial of topical chlorhexidine 0.2% versus topical natamycin 5% for fungal keratitis

Consent Form No 1: Baseline assessment and investigations

Participant Name_____ **Study ID Number:** _____

	Please initial box
1. I confirm that I have read and understand the participant information sheet dated ..... (version .....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered fully.	
2. I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
3. I understand that sections of my medical notes and data collected during the study may be looked at by responsible individuals from the London School of Hygiene & Tropical Medicine, from national regulatory authorities or from this hospital, where it is relevant to my taking part in this research. I give permission for these individuals to access my records.	
4. I agree to take part in the above study.	
5. I agree to the collection, laboratory tests and storage for future analysis of the samples from the surface my eye infection to understand the disease as described above.	
6. I agree to the collection and storage for future analysis of the samples from the inside of my mouth to investigate how human genes affect this disease.	
7. I agree to having a blood sample collected to measure sugar levels	
8. I agree to being referred for a HIV pre-test counselling, testing and for the results to be shared with the research team.	
9. I agree for the photographs of the front of my eye to be used in the publication or report released on the study, and for teaching purposes, including on the internet.	
10. I understand that data about/from me/the participant may be shared via a public data repository or by sharing directly with other researchers, and that I will not be identifiable from this information.	

Name of Participant *(printed)*	Signature/Thumbprint	Date
Name of Person taking consent	Signature	Date

The participant is unable to sign. As a witness, I confirm that all the information about the study was given and the participant consented to taking part.

Name of Impartial Witness *(if required)*	Signature	Date

1 copy for participant; 1 copy for Principal Investigator; 1 copy to be kept with hospital notes

Study Title: Randomised controlled trial of topical chlorhexidine 0.2% versus topical natamycin 5% for fungal keratitis in Nepal

Participant Information Sheet and Consent Form – No 2: For enrolment into the clinical trial

Introduction

Thank you for earlier participating in the first stage of the study. You are now being invited to take part in the clinical treatment trial part of this medical research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read or listen to the following information carefully and to talk to others about the study, if you wish. Ask us if there is anything that is not clear or if you would like more information. Do not sign the consent form unless you are satisfied with the answers to your questions and decide that you want to be part of this study. Take time to decide whether or not you wish to take part.

Why have I been invited?

You have been invited to take part because you have a corneal infection which we have found out from tests is caused by fungus.

What is this study about?

Infection of the clear part of the front of the eye (the cornea) is a corneal ulcer. It is an important cause of blindness in Nepal. A scratch in the cornea allows infection to enter and an ulcer to begin. These infections can be very serious with some people losing the sight in the affected eye.

Different types of infectious organisms can cause corneal ulcers. These include bacteria and fungi. In tropical regions about half of all corneal ulcers are caused by fungi. Bacteria and fungi need to be treated with different types of eye drop medicines.

Treatments for fungal eye infections are frequently not very effective, in addition access to these treatments in many countries is very limited and can be expensive. In some countries they are simply not available. Currently the most commonly used treatment for fungal corneal ulcers is an eye drop called Natamycin. There is a need for additional, alternative, affordable and more easily available eye drop treatments for fungal infections.

There is an antiseptic solution called Chlorhexidine. This is very effective at killing bacteria, fungi and other types of infectious organisms. It is used in medical care worldwide in several different ways. For example, it is used to clean skin before surgical operations, in antiseptic creams for skin cuts and as a mouth wash to prevent and treat mouth infections. It has been used in eye care for more than thirty years as an eye-drop preservative, for sterilizing contact lenses, for pre-operative topical antiseptic and for treating *Acanthamoeba* and fungal corneal infections.

About twenty years ago chlorhexidine eye drops were tested in two small clinical trials conducted in India and Bangladesh for the treatment of fungal corneal infections. The results of these studies suggested that chlorhexidine was as good as and possibly better than natamycin at controlling the infection. Neither eye drop had any serious side effect. However, the studies were not large enough to be certain.

Chlorhexidine is currently used in several countries for the treatment of fungal corneal infections when natamycin or alternative treatment is not working or is not available. We would like to conduct a large clinical to find out whether chlorhexidine 0.2% eye drops are as effective as or more effective than natamycin 5% eye drops for treating fungal corneal infections

Do I have to take part?
No. Your involvement is entirely voluntary. If you agree to take part, we will then ask you to sign a consent form. If you decide to join and change your mind, you are free to withdraw at any time without giving a reason. This will not affect the standard of care you receive. If you decide not to participate in the study, then you will be offered the standard treatment for fungal keratitis using natamycin.

What will happen to me if I take part?
If you agree to be part of this study, the following will happen:

1) Baseline Assessment:
As part of the initial assessment that has already taken place we have carefully examined yours eyes, taken photographs, performed a scan for infection and collected samples. The tests have found that you have a fungal infection in your cornea. The risks to an unborn or breast-fed baby from antifungal eye drops use are unknown. Therefore, pregnant and breastfeeding women are excluded from participating in this study. Pregnancy testing will be offered to potential female participants to confirm pregnancy status.

2) Randomisation:
We will randomly allocate you to one of the two treatment option: either chlorhexidine eye drops or natamycin eye drops. Sometimes we don't know which way of treating patients is best. To find out, we need to make comparisons between the different treatments. We put people into groups and give each group one of the two alternative treatments; the results are compared after some time to see if one is better. To try to make sure the groups are the same to start with, each patient is put into a group by chance (randomly). You have an equal chance of being put into the chlorhexidine or natamycin treatment group. Neither you nor the people examining your eyes will be told which treatment group you are in. It is important that neither you nor we know which of the two you are given. This information would be in our files, but we would not look at these files until after the research is finished. This is the best way we have for testing without being influenced by what we think might happen. We would then compare which of the two treatments has the best results.

3) Treatment:
Once you are allocated to one of the treatment groups, you will receive clear instruction on how to take the eye drops. For the first week we will ask you to take one drop every hour. For the second and the third weeks the frequency of the eye drops will be reduced to every other hour (2-hourly). After that the frequency and duration of treatment will depend on the severity of the infection and how it is responding. You will be given clear guidance on this by the eye doctor who is looking after you.

4) Follow-up Assessment:
Initially, most people with corneal infections stay in hospital for several days so that the clinical team can monitor the response of the infection to the treatment. For the purposes of the study, we would like to review the response to treatment and document the clinical findings at the following times after you start treatment: two days, 1 week, 2 weeks, 3 weeks, 2 months and 3 months.

On each occasion we will ask you a few questions about your eye and the treatment. We will measure your eye sight. We will examine the eye with a microscope and photograph it with a camera.

At 1 week, 2 weeks and 3 weeks we will repeat the *in vivo* confocal microscopy test that was done at your first assessment. This is done to see how the infection is responding to treatment. This involves putting anaesthetic drops on the eye so that you do not feel any discomfort. A soft plastic device then gently touches the surface of the eye so that we can take special photographs of the front of your eye ("scan")

At 1 week if you still have an open ulcer on the cornea we will repeat the sample collection to test for the ongoing presence of the infection. This involves first putting anaesthetic eye drops on the surface of the eye. Then gently scraping the surface of the corneal ulcer and testing for the presence of fungus and bacteria in

the microbiology laboratory.

Sometimes fungal infections do not respond to the treatment. In such cases it may be necessary to alter the treatment or perform an operation. The eye doctors who will be looking after you will monitor your progress closely and advise you about further treatment might be needed.

At the three month review you will be asked to complete three short questionnaires to assess your quality of life and vision function. These are the same standardised questions used in the baseline assessment to try to understand the impact that different medical problems can have on people's lives.

You may be withdrawn from the study without your consent if the researchers believe it is in your best interest or if you fail to follow study procedures.

What are the side effects or risks of taking part?

1. **Random Allocation and Treatment Failure:** You will be randomly allocated to a treatment. The treatment you are allocated to may prove to be less effective or to have more side effects than the other study treatment or other available treatments.

   It is important to recognise that corneal infection is a serious, sight threatening condition. Many patients, whatever the treatment used, have reduced vision in the affected eye after it has resolved. In some people the affected eye will become blind. Sometimes the infection, despite lots of treatment, can progress to cause a hole to develop in the cornea (Perforation) and sometimes it is so severe it is necessary to perform an operation to remove the eye content.

2. **Local Irritation:** As with most eye drops, there is the risk of local irritation or stinging from either chlorhexidine or natamycin. This usually only lasts for a short time.

3. **Allergic Response:** Very rarely, either chlorhexidine or natamycin eye drops can provoke a local allergic reaction on the surface of the eye or the eyelids.

4. **Pregnancy and Breast Feeding:** The risks to an unborn or breast-fed baby from antifungal eye drops use are unknown. Therefore, pregnant and breastfeeding women are excluded from participating in this study.

5. **Natamycin 5% eye drops:** Natamycin is an approved antifungal medication that is currently being used for the treatment of fungal corneal ulcer. It is on the World Health Organisation Essential Medicines List for the treatment of fungal corneal infections. There are no known serious side effects with this medication. It may cause mild irritation and very rarely a local allergic response.

6. **Chlorhexidine 0.2% eye drops:** Chlorhexidine eye drops are used on the surface of the eye as an antiseptic before procedures and also in the treatment of fungal and other eye infections. It has not been associated we any serious side effects. It may cause mild irritation and very rarely a local allergic response. This concentration of chlorhexidine is approved to be used in much larger volumes as a mouth wash. It is considered to be safe and is not associated with any systemic side effects.

7. **Procedures:** including examinations, confocal microscopy, corneal sample collection and checking for the best glasses or contact lenses carry the same very small risk whether they are performed as part of this study or of usual care outside the study. To minimise discomfort, topical anaesthetic will be given before examinations and sample collection.

8. **Unknown Risks:** The treatments in this study may have rare side effects that are currently not known.

If during the course of the study new information becomes available, the researchers will share this with.

What are the possible benefits of taking part?

- The study will involve tests for the type of infection. This helps the doctor looking after you to choose the best type of treatment for your eyes
- The costs for your clinical assessment, tests, treatment and transport will be paid for by the study.
- By participating in this study, you will be helping to answer the question about whether or not chlorhexidine is a suitable alternative treatment for fungal corneal infections.

What will happen to the clinical records, photographs and test results?

Your records will remain strictly confidential at all times. The information will be held in a secure office at your treating hospital. Only the people organizing or supervising the trial and regulatory authority auditors will have access to it. These include officials delegated by the Sponsor (London School of Hygiene and Tropical Medicine), the local National Ethics Committee, The local National Drug Regulatory Authority and trial Data Safety Monitoring Body (DSMB).

A study number rather than your name will be used on study records or the database wherever possible. Your name and other facts that might identify you will not appear when we present this study or publish its results.

Your name will not be passed to anyone else outside the research team, unless we have your direct instruction to do so, for example to make a medical referral.

Images of corneal infection may be used for educational and teaching purposes, including in publications. All personal identifying information will be removed before sharing images.

What tests will we do on the sample?

The samples collected from the surface of your eye will be tested in several different ways to determine what is causing the infection. This work will be done in the hospital microbiology laboratory, where you are being treated. A portion of the infection sample will be transferred for additional special tests at KCMC Hospital Biotechnology (Tanzania), the London School of Hygiene and Tropical Medicine (UK) and Radboud University Nijmegen Medical Center (The Netherlands).

We will look for the type of infection using a microscope and by growing the organisms in the laboratory. We will test the organisms that grow to see which medicines work best to kill the infection, which is helpful in guiding the choice of treatment to be used. The swab samples from the ulcer will be used to test for infection using molecular diagnostic tests and to evaluate new tests that may be used to find the cause rapidly in the clinic. We will use the genetic material of the organism causing the infection to sequence its genetic code, which helps us to find out the exact type of infection and its ability to resist treatments. We will store a sample of the infection causing organism indefinitely for additional testing.

What will happen to the results of the research study?

The results of the study will be available after it finishes and will be included in peer reviewed medical and scientific journals and may be presented at medical meetings. Results will also be published on a publicly accessible trials database. The data will be anonymous and none of the patients involved in the trial will be identified in any report or publication. Should you wish to see the results, or the publication, please ask your study doctor.

Who is funding the research?

The research is being funded as part of a grant from the Wellcome Trust, UK.

Who is organising the research?

It is being organised through a research partnership between the London School of Hygiene and Tropical Medicine and Sagarmatha Choudhary Eye Hospital in Nepal, Mbarara University of Science and Technology in Uganda and Kilimanjaro Christian Medical Centre in Tanzania.

What if relevant new information becomes available?
It is not anticipated that new information will become available during the course of this study. However, if it does, this will be shared with you by the researchers in case this affects whether you wish to continue in the study.

What if something goes wrong?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the head of the hospital eye department or the named person on the following page. The London School of Hygiene and Tropical Medicine holds insurance policies which apply to this study. If you experience harm or injury as a result of taking part in this study, you may be eligible to claim compensation.

Who has reviewed the study?
Prospective research such as this is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and approved by (1) the London School of Hygiene and Tropical Medicine Research Ethics Committee; (2) Nepal Health Research Council.

What will happen if I don't want to carry on with the study?
Your participation in this study is entirely voluntary. You may refuse to participate or may withdraw from this study at any time without penalty or loss of any rights or benefits to which you are otherwise entitled. The study doctor may also stop your participation in the study at any time for safety reasons. If you decide to withdraw from the study you should contact a member of the study team immediately. You do not have to give a reason when stopping, however for safety reasons, it is suggested that you tell the study doctor if you decide to stop because of an unwanted side effect. If you withdraw from the study, we will only use data collected before this decision, unless you request this to also be withdrawn. If you withdraw from the study, researchers, authorized persons from the Sponsor and the regulatory authorities will still require access to your medical notes to verify the data collected up to the date of your withdrawal.

Contact Details
Nepal Study Site: Dr Lila Puri, Sagarmatha Choudhary Eye Hospital, Lahan. T: +977 9841460623

Study Coordinator: Dr Jeremy Hoffman: T: +44 7742502530, email: Jeremy.hoffman@lshtm.ac.uk
Chief Investigator: Prof. Matthew Burton: T: +44 2076368636, email: matthew.burton@lshtm.ac.uk

You will be given a copy of the information sheet and a signed consent form to keep.
Thank you for considering taking the time to read this sheet.

Randomised controlled trial of topical chlorhexidine 0.2% versus topical natamycin 5% for fungal keratitis

Consent Form No 2: Enrolment into the clinical trial

Participant Name_____ **Study ID Number:** _____

	Please initial box
1. I confirm that I have read and understand the participant information sheet dated ..... (version .....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered fully.	
2. I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
3. I understand that sections of my medical notes and data collected during the study may be looked at by responsible individuals from the London School of Hygiene & Tropical Medicine, from national regulatory authorities or from this hospital, where it is relevant to my taking part in this research. I give permission for these individuals to access my records.	
4. I agree to take part in this clinical treatment trial.	
5. I agree to the collection, laboratory tests and storage for future analysis of the samples from the surface my eye infection to understand the disease as described above.	
6. I agree for the photographs of the front of my eye to be used in the publication or report released on the study, and for teaching purposes, including on the internet.	
7. I understand that data about/from me/the participant may be shared via a public data repository or by sharing directly with other researchers, and that I will not be identifiable from this information.	

Name of Participant *(printed)*	Signature/Thumbprint	Date
Name of Person taking consent	Signature	Date

The participant is unable to sign. As a witness, I confirm that all the information about the study was given and the participant consented to taking part.

Name of Impartial Witness *(if required)*	Signature	Date

1 copy for participant; 1 copy for Principal Investigator; 1 copy to be kept with hospital notes

Appendix 2: Case Report Forms

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

RANDOMISED CONTROLLED NON-INFERIORITY TRIAL OF TOPICAL CHLORHEXIDINE 0.2% VERSUS TOPICAL NATAMYCIN 5% FOR FUNGAL KERATITIS IN NEPAL

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

NEPAL NETRA JYOTI SANGH
EASTERN REGIONAL EYE CARE PROGRAMME
SAGARMATHA CHOUDHARY EYE HOSPITAL, LAHAN
BIRATNAGAR EYE HOSPITAL, BIRATNAGAR
EREC-P

CASE REPORT FORM
BASELINE

Chief Investigator: Prof Matthew Burton

Primary Investigators: Dr Reena Yadav; Dr Sanjay Singh; Dr Jeremy Hoffman

Name of site: Sagarmatha Choudhary Eye Hospital, Lahan

CRF Version Number: V1.1

Patient Initials		Triage No.	
Enrolment date _ _ / _ _ _ / _ _		SRN	

CRF Completion Instructions

General

Complete the CRF using a **black ballpoint pen** and that all entries are complete and legible.
Avoid the use of abbreviations and acronyms.
The CRF should be completed during, or if not possible, as soon as possible after the scheduled visit.
Ensure that the header information (i.e. subject's initials and ID number) is completed consistently throughout the CRF. Missing initials should be recorded with a dash (i.e. D-L).
If a subject prematurely withdraws from the trial a single line must be drawn across each uncompleted page.

Ensure that all fields are completed on each page

- If a test was Not Done record **ND** in the relevant box(es)
- Where information is Not Known write **NK** in relevant box(es)
- Where information is not applicable write **NA** in the relevant box(es)

The Principal Investigator is responsible for the accuracy of the data reported on the CRF. The PI must sign and date the Principal Investigator's Sign Off page to certify accuracy, completeness and legibility of the data reported in the CRF.
Medications should be recorded using the generic name whenever possible excepting combination products which will be recorded using the established trade name.

Verbatim Adverse Event terms should be recorded as the final diagnosis whenever possible.

Complete all **dates** as day, month, year i.e. 13/NOV/2008. Partial dates should be recorded as NK/NOV/2008
All **times** are to be recorded in 24-hour format without punctuation always using 4-digits; i.e. 0200 or 2130. Midnight is recorded as 0000.
Weights should be recorded to the nearest 0.1 kg.

CRF Footer section

The 'completed by' Name must be legible and **CRFs should only be completed by individuals on the Site Delegation log, signed by the PI.**
Each CRF should be signed and dated by the person completing the form.

Serious Adverse Events (SAEs)

A SAE form should be completed and faxed to the study coordination centre for all SAEs within 24 hours. However, relapse and death due to a known pre-existing condition and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs. All Adverse Event reporting should be carried out as per SOP 19: Adverse Event Reporting. The Reporting Form is attached at the end of this CRF collection.

Corrections to entries

If an error is made draw a single line through the item, then write the correct entry on an appropriate blank space near the original data point on the CRF and initial and date the change.

Do NOT

- Obscure the original entry by scribbling it out
- Try to correct/ modify the original entry
- Use Tippex or correction fluid

Storage

The CRF documents should be stored in a locked, secure area when not in use where confidentiality can be maintained. Ensure that they are separate to any other documents that might reveal the identity of the subject.

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

QUALIFICATION PROCEDURES
STAGE 1 – ALL MICROBIAL KERATITIS CASES

ENROLMENT CHECKLIST:

Prior to enrolment into the study, confirm that the following has taken place:

- ☐ Explanation and presentation to patient about the study (counselling)
- ☐ Eligibility for **STAGE 1** confirmed (below)
- ☐ Consent taken from patient for **STAGE 1**
- ☐ The patient has read (or been read) and expressed an understanding of the informed consent,
- ☐ All the patient's questions were answered,
- ☐ Patient has signed and dated the Informed Consent form prior to any study-related procedures,
- ☐ Patient was given a copy of the signed consent
- ☐ Triaging logbook completed FULLY
- ☐ Triaging card given to patient
- ☐ Stage 1 checklist eligibility

Please tick each box above as it is completed

Inclusion criteria for STAGE 1 recruitment:

	The following criteria MUST be answered YES for participant to be included in the trial (except where NA is appropriate):	**Yes**	**No**
1.	Acute microbial keratitis (MK) defined as: o Corneal epithelial ulceration >1mm diameter o Corneal stromal infiltrate o Acute inflammation: e.g. conjunctival injection, anterior chamber inflammatory cells, hypopyon.	☐	☐
2.	Age ≥ 18 years.	☐	☐
3.	Able to provide informed consent	☐	☐

Exclusion criteria for Stage 1 recruitment

	The following criteria MUST be answered NO for participant to be included in the trial:	**Yes**	**No**
1.	Patients who do not have acute microbial keratitis or where there is a more likely alternative diagnosis	☐	☐
2.	Patients unable or unwilling to provide informed consent	☐	☐
3.	Patients aged less than 18 years	☐	☐

Consented ☐ **Yes**	**Date participant signed written consent form:**	_ _ / _ _ _ / _ _ _ _ (DD / MMM / YYYY)	**Version and version date of signed Informed Consent Form** Version ___.___ _ _ / _ _ _ / _ _ _ _ (DD / MMM / YYYY)

Name of person taking informed consent: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

BASELINE DEMOGRAPHIC DATA ENTRY FORM

1. Triage Number		
2. Hospital Number		
3. Surname		
4. First name		
5. DOB	Write in this format DD/MMM/YYYY. If they don't remember, use the year together with 01/ JAN / YYY	
6. Age (years)		
7. Sex	1 = Male 2 = Female	
8. Nationality	1 = Nepali 2 = Indian 3 = Other (specify)	
	If Q9 is **3= Other**, specify here	
9. Phone number – 1		
10. Whose phone number?	Please state Name and Relationship of the owner of the phone number mentioned above.	
11. Phone number – 2		
12. Whose phone number	Please state Name and Relationship of the owner of the phone number mentioned above.	
13. Occupation	0 = No job 1 = Mainly farmer 2 = Mainly employed (manual) 3 = Mainly employed (non-manual) 4 = Mainly self-employed (own business, merchant) 5 = Civil servant 6 = Retired 7 = Student 8= Taxi driver 9= Other	
14. Other Occupation	If Q13 is **9= Other**, specify here	
15. Education level	0 = No formal education 1 = Lower Primary (Grade 1-4) 2 = Upper Primary (5-7) 3 = Lower secondary (1-4) 4 = Upper secondary (5-6) 5 = Certificate 6 = Diploma 7 = Degree and above	
16. Literacy level: *Ask, do you know how to read?*	0 = Illiterate 1 = Able to read Nepali a little 2 = Able to read Nepali well 3 = Able to read English and Nepali	
17. Ethnic group / Caste group	1 = Dalit 2 = Disadvantaged Janajatis 3 = Disadvantaged non-Dalit Terai caste group 4 = Relatively advantaged Janajatis 5 = Religious minority 6 = Upper caste group	
18. Caste	1 = Tharu 2 = Mahato 3 = Shah 4 = Yadav 5 = Dalit 6 = Pahari 7 = Other	
	If Q18 is **7 = Other**, specify here	
19. Religion	1 = Hindu 2 = Muslim 3 = Christian	

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

	4 = Buddhist 5 = Jain 6: Other	
20. Marital status	0 = Single 1 = Married/cohabiting 2 = Divorced/ Separated 3 = Widowed	
For Q. 21 Address, fill in the relevant section for Nepali or Indian patients.		
21. Address	VDC / Local Level Body – Nepali Patients only – leave blank for Indian	
	District – Nepali / Indian	
	Province (Nepali) / State (Indian) 1 = Province 1 2 = Province 2 3 = Province 3 4 = Province 4 / Gandaki 5 = Province 5 6 = Province 6 / Karnali 7 = Province 7 / Sudurpashchim 8 = Bihar 9 = Uttar Pradesh 10 = West Bengal 11 = Sikkim 12 = Uttarakhand 13 = Other (Specify in geographic location below)	
	Country 1 = Nepal 2 = India 3 = Other (Specify)	
	Specify Other Country:	
22. Approximate geographic location (use Google Maps – free text)		
23. Directions to their home from the hospital (free text)		
24. Household Head's Name		
25. Name of the chairman		
26. Phone number of the Chairman		
27. Total number of household members? Write number		
28. Members of the household under 16 years of age? Write number		
29. Members of the household between 16 and 60 years of age? Write number		
30. Members of the household above 60 years of age? Write number		
31. How many under 16 year's members of the household went to school? Write number		
32. How many adult members of the household (≥16 years of age) are "literate"? Write number		
33. The highest level of education achieved in the household	0 = No formal education 1 = Lower Primary (Grade 1-4) 2 = Upper Primary (5-7) 3 = Lower secondary (1-4) 4 = Upper secondary (5-6) 5 = Certificate 6 = Diploma 7 = Degree and above	

34. What is the highest status occupation with in the household	0 = No job 1 = Mainly farmer 2 = Mainly employed (manual) 3 = Mainly employed (non-manual) 4 = Mainly self-employed (own business, merchant) 5 = Civil servant 6 = Retired 7 = Student 8= Taxi driver 9= Other	
35. Other Occupation	If Q34 is **9= Other**, specify here	
36. Are you the household head?	0 = No, 1=Yes	
If Q. 36 is YES, write 99 on Q. 37-39		
37. If No, what is the literacy level of the household head?	0 = Illiterate 1 = Able to read Nepali a little 2 = Able to read Nepali well 3 = Able to read English and Nepali	
38. What is educational level of the household head?	0 = No formal education 1 = Lower Primary (Grade 1-4) 2 = Upper Primary (5-7) 3 = Lower secondary (1-4) 4 = Upper secondary (5-6) 5 = Certificate 6 = Diploma 7 = Degree and above	
39. Occupation of the household head?	0 = No job 1 = Mainly farmer 2 = Mainly employed (manual) 3 = Mainly employed (non-manual) 4 = Mainly self-employed (own business, merchant) 5 = Civil servant 6 = Retired 7 = Student 8= Taxi driver 9= Other	
40. Other Occupation	If Q39 is **9= Other**, specify here	
41. Distance to the nearest Health Centre in KM		
42. Name of the nearest Health Centre		
43. Geo location of nearest Health Centre	Write the trading centre or village if it's a small clinic with no name	
44. Level of nearest health centre	1 = Primary Healthcare Centre 2 = District Hospital 3 = Zonal Hospital 4 = Tertiary Care Centre 5 = Don't know	
45. Nearest source of water in KM		
46. Type of main water source	0 = none 1 = Well 2 = Piped 3 = Roof collected tank 4 = Borehole 5 = Protected spring 6 = other (specify)	
	Specify other water source:	

Investigator's Signature: _____ **Date**: _ _ / _ _ _ / _ _ _ _

Investigator's Name: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

BASELINE CLINICAL HISTORY DATA ENTRY FORM

History of the presenting Complaint		
1. Eye affected	1 = Right 2 = Left 3 = Both	
2. Study eye	1 = Right 2 = Left	
Symptoms *(Ask the patient what has brought them to hospital and write **1=Yes, 0=No**)*		
3. Do you have eye pain?		
4. Do you have Reduced/loss of vision in the affected eye?		
5. Is there tearing?		
6. Is there eye discharge?		
7. Is there photophobia?		
8. Is there foreign body sensation?		
9. Is there any other complaint? other (specify, leave blank if none)		
10. Out of all those symptoms, which one is the most important to you?	1 = pain 2 = vision 3 = tearing 4 = discharge 5 = photophobia 6 = FB sensation 7 = other (specify below)	
	Other:	
11. When did the symptoms begin?	Encourage the patient to pinpoint the calendar date. Write in this format: DD/MMM/YYYY	
12. Is there history of trauma or something falling into the eye before onset of symptoms?	1 = Yes 0 = No 99 = don't know	
If Q 12 above is YES, ask Q 13 - 15, If Q 12 is NO, write 99 on Qs 14-15		
13. When did the traumatic event occur?	Leave blank if **NO** trauma and DD/MMM/YYYY	
14. What was the traumatising object?	1 = vegetative matter 2 = stick/wood 3 = soil/sand/dust 4 = insect 5 = metal 6 = Other (specify below) 7 = don't know 99 = Not applicable	
	Other:	
15. What happened to the traumatising object?	1 = did not "enter" the eye 2 = "entered" the eye and was removed 3 = "entered" the eye, was **NOT** removed 99 = Not applicable	
Current medical history		
16. Have you used any treatment up to now?	1 = Yes 0 = No ***(If NO then move to question 18)***	
17. Which treatment have you used to-date? Write ***1=Yes, 0=No***		
	Tablets	
	Traditional Eye Medicine	
	Antibiotic	
	Steroid	

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

Ask the patient to show you the medicine they have been using **1 = Yes** **0 = No**	Antibiotic-steroid	
	Anti-viral	
	Anti-fungal	
	Unknown name	
If the patient can remember the specific name or shows you a bottle, enter the name **1 = Yes** **0 = No**	Chloramphenicol	
	Ciprofloxacin	
	Tetracycline	
	Gentamycin	
	Iodine	
	antibiotic-steroid (write)	
	Acyclovir	
	clotrimazole	
	econazole	
	Natamycin	
	other (specify)	
	Not able to ascertain (Unclear)	
	Other: (specify here, leave blank if N/A)	
18. Have you used any traditional eye medicines (TEM)	1 = Yes 0 = No *If NO then go to Q26*	
19. What were the main contents?	1 = Sap 2 = Fresh leaves 3 = Dry leaves 4 = roots 5 = Powder 6 = Animal waste 7 = Other (specify below)	
	Other:	
20. How was it prepared	1 = Squeezed fresh 2 = Boiled 3 = Baked 4 = Chewed 5 = diluted in water 6 = other (specify below)	
	Other:	
21. Where did you get it from?	1 = Home-made 2 = Relative/Friend 3 = Community expert 4 = Traditional Healer	
22. How long did you use it?	(*write number of days)*	
23. If you experienced any complications, what was the main one?	0 = None 1 = Pain 2 = Swelling 3 = Worsening 4 = Other (specify below)	
	Other:	
24. If the complications were there, what did you do about them?	1 = Stopped TEM 2 = Continued TEM 3 = Changed TEM 4 = Changed to Eye drops 5 = Other	
25. How much did it cost?	NPR	

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

Other ocular History		
26. Did you have any symptoms in last 3 months before onset of current illness? **1 = Yes** **0 = No**	None	
	Reduced vision	
	itching	
	Excessive tearing	
	Abnormal discharge	
	Feeling dry	
	Lid swelling	
	Foreign body sensation	
	Other	
	Specify other:	
27. Were you using any eye medication in the previous 3 months before onset of current illness? **1 = Yes** **0 = No**	None	
	antibiotic	
	steroid	
	antibiotic-steroid	
	artificial tears	
	anaesthetic	
	antiviral	
	antifungal	
	glaucoma drug	
	Other	
	Specify other:	
28. Did you have any eye operation in previous 3 months before onset of current illness? **1 = Yes** **0 = No**	None	
	lid surgery	
	Probing and syringing	
	cataract	
	glaucoma surgery	
	DCR	
	Other	
	Specify other:	
29. Has the eye ever been sick like this before ***(Ask specifically for MK)***	1 = Yes 0 = No	
If Q 29 is 'YES', ask Q 30, if 'NO', write '99' and move to question '31'		
30. When was the last time it was sick?	Number in years	
Other medical/surgical history		
31. Did you have any ENT conditions in previous 3 months before onset of current illness? **1 = Yes** **0 = No**	None	
	Flu like symptoms	
	Cough	
	Sinus congestion	
	Other (specify below)	
	Specify other:	
32. Did you have of any ENT/facial surgery in the previous 3 months?	1 = Yes 0 = No	
33. Do you have a history of Diabetes Mellitus?	1 = Yes 0 = No 2 = Don't Know	

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

If the answer to Q 33 is YES, proceed to ask Q 34-36, if NO or DON'T KNOW, skip to Q 37		
34. When were you diagnosed with DM? (write number of years)		
35. Which treatment are you using for DM?	0 = none 1 = diet/exercise 2 = Oral tablets 3 = insulin 4 = Tabs and insulin 5 = other 99=N/A	
36. What was your last **fasting** blood sugar check?	Write in mmol/l, if they have in mg/dl, convert to mmol/l by dividing by 18)	
37. Do you have a history of HIV infection?	1 = Yes 0 = No 2 = Don't Know	
If the answer to Q 38 is YES, proceed to ask Q 39-41, if NO or DON'T KNOW write 99		
38. When were you diagnosed with HIV? (write number of years)		
39. Which treatment are you using for HIV?	0 = No treatment 1 = Septrin/dapsone only 2 = Septrin + HAART 99 = N/A	
40. What was your last CD4 count?		

After this point, you thank the patient and inform them that you will speak to them at a later time for more history and to complete the QoL testing.

Investigator's Signature: _____ **Date**: _ _ / _ _ _ / _ _ _

Investigator's Name: _____

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

BASELINE CLINICAL EXAMINATION DATA ENTRY FORM
PATIENT DETAILS, DEMOGRAPHICS, REFRACTION AND VISUAL ACUITY

1. Triage Number	Write the triage number	
2. Date	Write in this format: DD/MMM/YYYY	
3. Presenting Visual Acuity (use spectacles if relevant) LogMAR (PEEK)	Right eye	
	Left eye	
	Spectacle corrected? 1 = Yes 2 = No	
4. Best pinhole corrected presenting visual acuity LogMAR (PEEK)	Right eye	
	Left eye	
5. Best Spectacle Corrected Visual Acuity LogMAR Score (after refraction, using ETDRS, after calculation)	Right eye	
	Left eye	
6. Refraction (affected eye only)	Please indicate + or – cyl Format: Sph / +/- cyl x axis	
7. Contrast sensitivity (PEEK)	Right eye	
	Left eye	

BASELINE CLINICAL EXAMINATION DATA ENTRY FORM
SLIT LAMP BIOMICROSCOPY EXAMINATION

1. Eye affected	1 = Right 2 = Left	
2. Presence of eye lid swelling	0 = No 1 = Yes	
3. Presence of entropion	0 = No 1 = Yes	
4. Amount of lagophthalmos	0 = None If present specify number of mm (1-10)	mm
5. Presence of Trichiasis	0 = No 1 = Yes	
6. Bell's phenomenon	1 = Normal 2 = Abnormal	
7. Lacrimal syringing	1 = Normal 2 = Abnormal	
8. Conjunctival hyperaemia	0 = None 1 = Mild 2 = Moderate 3 = Severe	
9. Corneal sensation	1 = Normal 2 = Reduced	
10. Slough elevated	0 = None 1 = Flat 2 = Raised	
11. Infiltrate edge	1 = Defined 2 = Serrated 99=Not able to see	
12. Satellite lesions?	0 = None 1 = Yes 99 = Not able to see	
13. Infiltrate colour?	99 = None 1 = White 2 = Cream 3 = Green 4 = Yellow 5 = Dark brown 6 = Black 7 = other (specify)	
	Specify Other:	

14. Immune ring?	0 = No 1 = Yes	
15. Superficial Corneal vascularisation	0 = No 1 = Yes	
16. How many clock hours?	Write "0" is absent	
17. Deep Corneal vascularisation	0 = No 1 = Yes	
18. How many clock hours?	Write "0" is absent	
19. Hypopyon height (mm)	0 = None If present specify number of mm (1-10) 99 = Unable to see	
20. Hypopyon shape	99 = NA 1 = Level / Flat 2 = Heaped	
21. Keratic precipitates	0 = No 1 = Yes 99 = NA	
22. Keratic precipitate age	1 = New 2 = Old 99 = NA	
23. Keratic precipitate size	1 = Small 2 = Large 99 = NA	
24. Perineural infiltrates	0 = No 1 = Yes 99 = Not able to see	
25. Fibrin	0 = No 1 = Yes 99 = Not able to see	
26. Flare	0 = No 1 = Yes 99 = Not able to see	
27. Flare grade	0 = None 1 = Mild 2 = Moderate (iris and pupil seen) 3 = Moderate-severe (iris and pupil hazy) 4 = Severe (fibrin in AC) 99 = Not able to see	
28. Cells in AC	0 = No 1 = Yes 99 = Not able to see	
29. Cells grade	0 = None 1 = 1-5 cells 2 = 6-15 cells 3 = 16-25 cells 4 = 26-50 cells 5 = more than 50 99 = Not able to see	
30. Posterior corneal abscess	0 = No 1 = Yes 99 = Not able to see	
31. Endothelial plaque	0 = No 1 = Yes 99 = Not able to see	
32. Solid inflammatory mass in AC	0 = No 1 = Yes 99 = Not able to see	
33. Size of infiltrate 1	Measure max. diameter	mm
34. Size of infiltrate 2	Measure perpendicular diameter to maximum diameter	mm
35. Size of epithelial defect 1	Measure max. diameter	mm
36. Size of epithelial defect 2	Measure max. diameter. *NB measure with FLN ONLY after photos and samples have been taken*	mm
37. Depth of ulceration	Ratio of remaining corneal tissue / normal corneal thickness as percentage 1 = 1 – 25% 2 = 26 – 50% 3 = 51 – 75% 4 = 75 – 100%	

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

38. Site of the ulcer	1 = Outside central 4mm 2 = Inside central 4mm not in centre 3 = Centre involved	
39. Perforation	0 = No 1 = Descemetocele 2 = Perforated	
40. IOP	Enter pneumotonometry reading here from initial examination in clinic	mmHg
41. Dilated Fundus examination - Lens	0 = Normal 1 = Abnormal (specify) 2 = No view 9 = Not examined	
	Specify abnormality:	
42. Dilated Fundus examination - Retina	0 = Normal 1 = Abnormal (specify) 2 = No view 9 = Not examined	
	Specify abnormality:	
43. Dilated Fundus examination – Macula	0 = Normal 1 = Abnormal (specify) 2 = No view 9 = Not examined	
	Specify abnormality:	
44. Dilated Fundus examination – Optic Nerve	0 = Normal 1 = Abnormal (specify) 2 = No view 9 = Not examined	
	Specify abnormality:	
45. B Scan Ultrasound – only required if no fundal view. NB if only abnormality is PVD then grade as normal.	0 = Normal (no vitreous opacities) 1 = evidence of vitreous opacities (e.g. vitritis) 2 = retinal detachment 3 = other – specify 9 = not performed	
	Specify other abnormality:	
46. *In vivo* confocal microscopy – presence of hyphae	0 = No 1 = Yes 2 = Unsure / inconclusive 3 = Poor quality scan 99 = Not examined	
47. In vivo confocal microscopy – presence of cysts	0 = No 1 = Yes 2 = Unsure / inconclusive 3 = Poor quality scan 99 = Not examined	
48. IVCM diagnosis	1 = Fungal keratitis 2 = Bacterial keratitis 3 = Amoebic keratitis 4 = Other (specify) 5 = Inconclusive / Poor Scan	
	Specify other:	
49. Clinical diagnosis	1 = Bacterial keratitis 2 = Fungal keratitis 3 = Viral keratitis 4 = Non-specific 5 = Mooren's Ulcer 6 = Penetrating injury 7 = Chemical injury 8 = Other (specify)	
	Specify other:	
50. Comment on the non-affected eye (if normal or not, comorbidity if present)	0 = Normal 1 = Adnexal abnormality 2 = Corneal disease 3 = Cataract 4 = Glaucoma 5 = Retinal disease 6 = Optic atrophy / oedema 7 = Refractive error 9 = Empty socket / phthisis 10 = Conjunctival growth 11 = Conjunctivitis	

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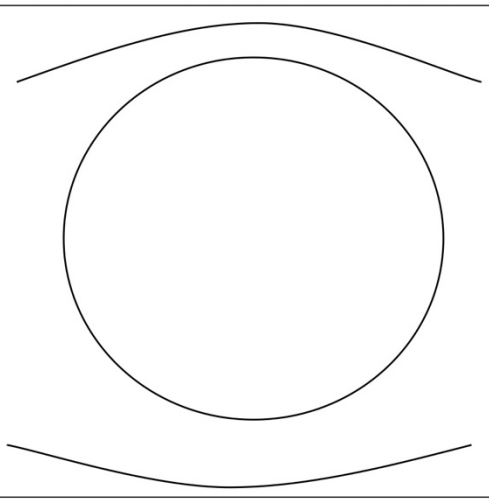
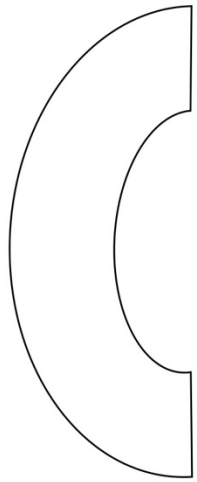
51. Picture DSC number no stain		
52. Picture DSC number with fluorescein		
		

CLINICAL NOTES

NOW PERFORM CORNEAL SAMPLE COLLECTION

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

BASELINE SAMPLE COLLECTION
CORNEAL AND BUCCAL SAMPLES

SAMPLE	1 = COLLECTED 2 = SAMPLE NOT ENOUGH 3 = EYE-PERFORATED / IMPENDING 4 = STROMAL ABSCESS 5 = PATIENT UNCO-OPERATIVE 6 = NOT INDICATED	REPORTED RESULTS *(INPUT DATA ONTO FORM 4 – LAB RESULTS ACCESS TABLE)*
GRAM SLIDE		
LP SLIDE 1		
CALCOFLUOR SLIDE		
FRESH BLOOD AGAR		
SABOURAUD AGAR		
CHOCOLATE AGAR (HBA)		
BRAIN HEART INFUSION		
PCR SWABS x 3		
BUCCAL SWABS X2		

SAMPLE ID FOR PCR AND BUCCAL SWABS: _____

BASELINE SAMPLE COLLECTION
BLOOD SAMPLES

SAMPLE	1 = COLLECTED 2 = NOT DONE, PT ALREADY POSITIVE 3 = NOT DONE, PT NOT CONSENTED 4 = NOT DONE, NOT INDICATED	REPORTED RESULTS HIV *(INPUT DATA ONTO FORM 4 – LAB RESULTS ACCESS TABLE)* 0 = NEGATIVE 1 = POSITIVE 2 = NOT DONE, PT ALREADY POSITIVE 3 = NOT DONE, PT NOT CONSENTED 4 = NOT DONE, NOT INDICATED	REPORTED RESULTS BLOOD SUGAR WRITE BLOOD GLUCOSE LEVEL *(INPUT DATA ONTO FORM 4 – LAB RESULTS ACCESS TABLE)*
HIV			
BM			

BASELINE SAMPLE COLLECTION
PREGNANCY TESTING

FEMALE PATIENTS ONLY AGED 18-50; MEN AND WOMEN OVER 50 SKIP

IS PATIENT KNOWN TO BE PREGNANT?	0 = NO (UNKNOWN STATUS) 1 = YES (EXCLUDE FROM RCT)	
IF UNKNOWN, IS THERE A CHANCE SHE IS PREGNANT?	0 = NO 1 = YES (PERFORM PREGNANCY TEST AFTER COUNSELLING)	
URINE HCG TESTING	0 = NEGATIVE 1 = POSITIVE (EXCLUDE FROM RCT) 2 = PATIENT REFUSED (EXCLUDE FROM RCT) 9 = NOT INDICATED	

Investigator's Signature: _____ **Date**: _ _ / _ _ _ / _ _ _ _
(DD / MMM / YYYY)

Investigator's Name: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

QUALIFICATION PROCEDURES
STAGE 2 – FUNGAL KERATITIS CASES FOR RCT
INCLUSION & EXCLUSION CHECKLIST

Go through all the questions below for triaged patient. Ask the questions, do examination and check for laboratory results as appropriate. Tick in the answer boxes based on the responses you get. All questions should be completed before enrolling or excluding the person and do not leave any empty answer boxes.

SN	Criteria	Present			Remark
		Yes	*No*	*NA*	
1	Acute microbial keratitis characterised by: • Corneal epithelial ulceration >1mm diameter • Corneal stromal infiltrate • Acute inflammation: e.g. conjunctival injection, anterior chamber inflammatory cells, hypopyon.				
2	Filamentous fungal hyphae visualised on smear microscopy and/or *in vivo* confocal microscopy.				
3	Adult (>18 years age)				
4	Agree to be randomised to either treatment arm and able to give informed consent				
5	Agree to be followed up at 2 days, 1 week, 2 weeks, 3 weeks, 2 months and 3 months				
If the answer is "Yes" to all of the above questions and if the answer is "No" to all of the questions below enrol the person into the trial. If the answer to any of the questions above is "No" or if the answer to any of the questions below is "Yes" exclude the person from the trial and refer to the releavant SOP					
6	Pregnant woman?				
7	Breast-feeding woman?				
8	Has the participant used any topical anti-fungal treatment recently other than traditional eye medicines?				
9	History of hypersensitivity/allergy to natamycin or chlorhexidine or preservatives?				
10	No light perception in the affected eye				
11	Fellow eye visual acuity <6/60				
12	Acanthamoebic infection visualised by smear microscopy or IVCM				
13	Clinical evidence of herpetic keratitis				
14	Previous corneal graft in the affected eye				
15	Bilateral corneal ulcers				
17	Very severe ulcers warranting immediate evisceration or conjunctival flap including perforation / descemetocele				
18	Fungal endophthalmitis				

Investigator's Signature: _____ **Date**: _ _ / _ _ _ / _ _ _ _
(DD / MMM / YYYY)

Investigator's Name: _____

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

QUALIFICATION PROCEDURES
STAGE 2 – FUNGAL KERATITIS CASES FOR RCT
ENROLMENT CHECKLIST

ENROLMENT CHECKLIST:

Prior to enrolment into the study, confirm that the following has taken place:

- ☐ Explanation and presentation to patient about the study (counselling)
- ☐ Eligibility for **STAGE 2** confirmed (above)
- ☐ Consent taken from patient for **STAGE 2**
- ☐ The patient has read (or been read) and expressed an understanding of the informed consent,
- ☐ All the patient's questions were answered,
- ☐ Patient has signed and dated the Informed Consent form prior to any study-related procedures,
- ☐ Patient was given a copy of the signed consent
- ☐ Study number issued to patient
- ☐ Triaging logbook updated
- ☐ Logbook of trial participants completed
- ☐ Triaging card update given to patient

Please tick each box above as it is completed

Consented? ☐ **Yes**	**Date participant signed written consent form:** _ _ / _ _ _ / _ _ _ _ (DD / MMM / YYYY)	**Version and version date of signed Informed Consent Form** Version ___.___ _ _ / _ _ _ / _ _ _ _ (DD / MMM / YYYY)

Name of person taking informed consent: _____

QUALIFICATION PROCEDURES
PARTICIPANT ELIGIBILITY REVIEW

Qualification Procedures Checklist:

		Yes	No
1.	**Does the participant satisfy the inclusion and exclusion criteria to date?**	☐	☐
2.	**Have all Qualification procedures been completed?**	☐	☐
3.	**Have the baseline demographic, history and examination forms been completed fully?**	☐	☐
4.	**Is the participant still willing to proceed in the trial?**	☐	☐

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

Participant's eligibility Investigator Sign-Off:	
Is the participant eligible to take part in the trial? **Investigator's Signature**: _____ **Date** : __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY) **Investigator's Name:** _____ **Principal Investigator's or Project Co-Ordinator's Signature**: _____ **Date** : __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY) **Name:** _____	☐ **Yes** ☐ **No** ***Please give reason for screen failure below***
Reason(s) for qualification failure:	
1.	
2.	

FOR PATIENTS NOT ELIGIBLE OR WILLING FOR THE RCT, REFER TO SOP03 (EXCERPT BELOW)

Non-RCT patients handling procedure

- Register all non-MK and non-RCT patients on the non-MK / RCT logbook
- Patients with fungal keratitis and exclusion criteria - Offer standard treatment with Topical natamycin 5% and treat as per local protocol.
- Patients with non-fungal microbial keratitis - Offer standard treatment as appropriate hourly and treat as per local protocol
- Patients with non-MK eye disease (other pathologies) –
    - Non-emergency: refer patient to outpatient department
    - Emergency condition: commence treatment if available and immediately refer the patient to the outpatient department, informing the duty ophthalmologist

ELIGIBLE PATIENTS SHOULD NOW BE REFERRED TO THE RANDOMISATION ADMINISTRATOR AND TREATMENT DISPENSED

BASELINE QOL FORMS TO BE COMPLETED ONCE ADMITTED / PRIOR TO DISCHARGE

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

RANDOMISATION AND DISPENSING CHECKLIST

RANDOMISATION AND DISPENSING CHECKLIST

- ☐ Randomisation envelope and numbers checked to be the same
- ☐ Randomisation logbook completed x 2
- ☐ Correct drug box identified and given to patient
- ☐ Patient counselled about use
- ☐ Patient details entered onto drug box
- ☐ First dose of medication given

Please tick each box above as it is completed

RANDOMIZATION CODE: (e.g. N-999-L): _____

TRIAL DRUG (A OR B): _____

Name of randomization administrator: _____ **Signature**: _____

Date : _ _ / _ _ _ / _ _ _ _

(DD / MMM / YYYY)

NOW COMPLETE THE BASELINE QUALITY OF LIFE FORMS

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

BASELINE QUALITY OF LIFE ENTRY FORMS
BASELINE WHOQOL-BREF QUESTIONNAIRE

1.	**Quality of Life Questionnaire**	
2.	TRIAGE NUMBER	
3.	FIRST NAME	
4.	SURNAME	
5.	INTERVIEW DATE	

The following questions ask how you feel about your quality of life, health, or other areas of your life. I will read out each question to you, along with the response options. **Please choose the answer that appears most appropriate.** If you are unsure about which response to give to a question, the first response you think of is often the best one.
निम्न प्रश्नहरू तपाईको जीवन, स्वास्थ्य, वा तपाईंको जीवनका अन्य क्षेत्रहरूको बारेमा कस्तो महसुस गर्नुहुन्छ भन्ने प्रश्न सोध्छ. म तपाईलाई प्रत्येक प्रश्न पढ्नेछु, प्रतिक्रिया विकल्प सहित। कृपया जवाफ दिनुहोस् जुन सबैभन्दा उपयुक्त देखिन्छ। यदि तपाईं प्रश्नको जवाफ दिने बारे निश्चित हुनुहुन्न भने, तपाईले सोच्नु भएको पहिलो प्रतिक्रिया अक्सर उत्तम हो।

Please keep in mind your standards, hopes, pleasures and concerns. We ask that you think about your life **in the last four weeks.**
कृपया आफ्नो स्तर, आशा, आनन्द र चिन्तालाई ध्यान दिनुहोस। हामी सोध्छौं कि तपाईं आफ्नो जीवनको बारेमा अन्तिम चार हप्तामा के सोच्नुहुन्छ।

		Very poor धेरै गरिब	*Poor गरिब*	*Neither poor nor good नत गरीब नत राम्रो*	*Good राम्रो*	*Very good धेरै राम्रो*
1.1.	How would you rate your quality of life? तपाईं आफ्नो जीवन को गुणस्तर कसरी मूल्याङ्कन गर्नु हुन्छ?	1	2	3	4	5
		Very dissatisfied धेरै असन्तुष्ट	*Dissatisfied असन्तुष्ट*	*Neither satisfied nor dissatisfied नत सन्तुष्ट नत असन्तुष्ट*	*Satisfied सन्तुष्ट*	*Very satisfied धेरै सन्तुष्ट*
1.2.	How satisfied are you with your health? तपाईं आफ्नो स्वास्थ्यसंग कति सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5

The following questions ask about **how much** you have experienced certain things in the last four weeks.
निम्न प्रश्नहरूले तपाइँलाई पछिल्लो चार हप्तामा केहि चीजहरू अनुभव गरेकाछममेरोसामान्यगतिविधिहरूगर्नअसमर्थछुन्भन्ने बारे सोध्दछ।

		Not at all हुदै हैन	*A little थोरै*	*A moderate amount मध्यम मात्रामा*	*Very much धेरै*	*An extreme amount धेरै अत्यधिक मात्रा*
1.3.	To what extent do you feel that physical pain prevents you from doing what you need to do? कुन हदसम्म तपाई महसुस गर्नुहुन्छ कि शारीरिक पीडाले तपाईंलाई आवश्यक कामबाट रोक्छ?	5	4	3	2	1

1.4.	How much do you need any medical treatment to function in your daily life? के तपाईँ आफ्नो दैनिक जीवनमा कार्य गर्न कुनै पनि उपचारको आवश्यकता पर्दछ?	5	4	3	2	1
1.5.	How much do you enjoy life? तपाईं जीवनमा कतिको आनन्द लिनुहुन्छ?	1	2	3	4	5
1.6.	To what extent do you feel your life to be meaningful? तपाईं कुन हदसम्म आफ्नो जीवन सार्थक महसुस गर्नुहुन्छ?	1	2	3	4	5
		Not at all *हुदै हैन*	*A little* *थोरै*	*A moderate amount* *एक मध्यम मात्रा*	*Very much* *धेरै*	*Extremely* *अत्यधिक*
1.7.	How well are you able to concentrate? तपाईं कुन हदसम्म ध्यान केन्द्रित गर्न सक्नुहुन्छ?	1	2	3	4	5
1.8.	How safe do you feel in your daily life? तपाईं दैनिक जीवनमा कति सुरक्षित महसुस गर्नुहुन्छ?	1	2	3	4	5
1.9.	How healthy is your surrounding environment? तपाईंको आसपासको वातावरण कति स्वस्थ छ?	1	2	3	4	5

The following questions ask about how completely you experience or were able to do certain things in the last four weeks.
निम्न प्रश्नहरू तपाइँ कस्तो अनुभव गर्नुहुन्छ वा कसरी अन्तिम चार हप्तामा केहि चीज गर्न सक्षम सक्नु भएको थियो

		Not at all *हुदै हैन*	*A little* *थोरै*	*Moderately* *सामान्य*	*Mostly* *प्राय*	*Completely* *पूर्णतया*
1.10.	Do you have enough energy for everyday life? के तपाईंसँग पर्याप्त ऊर्जा छ दैनिक जीवनको लागि?	1	2	3	4	5
1.11.	Are you able to accept your bodily appearance? के तपाईं आफ्नो शारीरिक उपस्थिति स्वीकार गर्न सक्नुहुन्छ?	1	2	3	4	5
1.12.	Have you enough money to meet your needs? के तपाइँका आवश्यकताहरू पूरा गर्न पर्याप्त पैसा छ?	1	2	3	4	5
1.13.	How available to you is the information that you need in your day-to-day life? तपाइँलाई दिनहुँको जीवनमा आवश्यक जानकारी कसरी छ?	1	2	3	4	5

1.14.	To what extent do you have the opportunity for leisure activities? तपाईँसँग कुन हदसम्म खाली समय गतिविधिहरू को लागि अवसर छ?	1	2	3	4	5
		Very poor *धेरै गरिब*	*Poor* *गरिब*	*Neither poor nor good* *नत गरीब नत राम्रो*	*Good* *राम्रो*	*Very good* *धेरै राम्रो*
1.15.	How well are you able to get around? तपाईं कति राम्ररी वरिपरि हिंड्न सक्नुहुन्छ?	1	2	3	4	5
		Very dissatisfied *धेरै असन्तुष्ट*	*Dissatisfied* *असन्तुष्ट*	*Neither satisfied nor dissatisfied* *नत सन्तुष्ट नत असन्तुष्ट*	*Satisfied* *सन्तुष्ट*	*Very satisfied* *धेरै सन्तुष्ट*
1.16.	How satisfied are you with your sleep? तपाईं निद्रासंग कति सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
1.17.	How satisfied are you with your ability to perform your daily living activities? तपाइँ दैनिक जीवनका गतिविधिहरू प्रदर्शन गर्न आफ्नो क्षमताको साथ कत्ति सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
1.18.	How satisfied are you with your capacity for work? आफ्नो काम को लागि तपाई आफ्नो क्षमतासंग कतिको सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
1.19.	How satisfied are you with yourself? तपाईं आफै सँग कत्तिको सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
1.20.	How satisfied are you with your personal relationships? तपाईं आफ्नो व्यक्तिगत सम्बन्धमा कति सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
1.21.	How satisfied are you with your sex life? तपाईंआफ्नो यौन जीवनसँग कतिको सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
1.22.	How satisfied are you with the support you get from your friends? तपाई साथीहरूको सहयोगबाट कतिको सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
1.23.	How satisfied are you with the conditions of your living place? तपाईंआफ्नो बस्ने ठाउँ को अवस्थाको साथ तपाईं कति को सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
1.24.	How satisfied are you with your access to health services?	1	2	3	4	5

	तपाई स्वास्थ्य सेवाको पहुँच संग कतिको सन्तुष्ट हुनुहुन्छ?					
1.25.	How satisfied are you with your transport? तपाईं यातायात संग कतिको सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
The following question refers to how often you have felt or experienced certain things in the last four weeks. निम्न प्रश्नले तपाइँलाई पछिल्लो चार हप्तामा केहि कुराहरूको बारेमा कस्तो लाग्यो वा अनुभव गरेको छ भनेर बुझाउँछ।						
		Never कहिल्यै होइन	*Seldom* कहिले काहीँ	*Quite often* प्राय प्राय	*Very often* अक्सर	*Always* सँधै
1.26.	How often do you have negative feelings such as blue mood, despair, anxiety, depression? तपाइँसँग प्राय: उदास मन , निराशा, चिन्ता, अवसाद जस्ता नकारात्मक भावनाहरू छन्?	5	4	3	2	1

Investigator's Signature: _____ **Date**: _ _ / _ _ _ / _ _ _ _

Investigator's Name: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

BASELINE QUALITY OF LIFE ENTRY FORMS
BASELINE WHO/PBD-VISUAL FUNCTIONING QUESTIONNAIRE

2.	**Visual Functioning Questionnaire**					
The first two questions are about your overall eyesight. I will read out a choice of five answers and you will choose the one that describes you best. पहिलो दुई प्रश्नहरू तपाईंको समग्र दृष्टिको बारेमा हो।म पाँचवटा जवाफहरूको छनौट पढ्नेछु र तपाईं एक छान्नु भएको छ जसले तपाइँलाई राम्रो वर्णन गर्दछ।						
	Question	**Answer options** (Please circle the number which corresponds to the answer)				
2.1.	Overall, how would you rate your eyesight using both eyes – with glasses or contact lenses if you wear them? कुल मिलाएर, तपाईं कसरी आँखामा चश्मा वा सम्पर्कलें दुवै आँखा प्रयोग गरेर आफ्नोआँखाको मूल्याङ्कन गर्नुहुन्छ ?	*Very good धेरै राम्रो*	*Good राम्रो*	*Moderate मध्यम*	*Bad नराम्रो*	*Very bad धेरै नराम्रो*
		1	2	3	4	5
2.2.	How much pain or discomfort do you have in your eyes (e.g. burning, itching, aching)? तपाईंको आँखामा कति दुखाइ वा असुविधा छ (जलिरहेको, खुजली अनुभूति, पीडा दुखाइ)?	*None कुनै पनि छैन*	*Mild हल्का*	*Moderate मध्यम*	*Severe गंभीर*	*Extreme चरम*
		1	2	3	4	5
In the next section, I am going to ask you how much difficulty, if any, you have doing certain activities. I will read out choice of five answers and you will choose the one that describes you best.						
		None कुनै पनि छैन	*Mild हल्का*	*Moderate मध्यम*	*Severe गंभीर*	*Extreme / Cannot Do चरम / गर्न सक्दैन*
2.3.	Because of your eyesight, how much difficulty do you have in going down steps/stairs/ steep slopes? तपाईंको आँखाको कारणले, तल कदम / सीढी / खडा ढोकाहरूमा तपाईंलाई कति कठिनाइ हुन्छ?	1	2	3	4	5
2.4.	How much difficulty do you have in noticing obstacles while you are walking alone (e.g. animals or vehicles)? जब तपाईं एक्लै हिँडिरहनु भएको छ बाधाहरू हेर्दा तपाईंलाई कति कठिनाइ हुन्छ (जस्तै जनावर वा वाहनहरू)?	1	2	3	4	5
2.5.	How much difficulty do you have in seeing because of glare from bright lights उज्ज्वल रोशनीको चमकले गर्दा देख्न तपाईंलाई कति कठिनाइ हुन्छ ?	1	2	3	4	5

2.6.	Because of your eyesight, how much difficulty do you have in searching for something on a crowded shelf? तपाईंको आँखा को कारणले, भीडमा केहि खोजी गर्नको लागी तपाईंलाई कति कठिनाइलाईलाई हुन्छ ?	1	2	3	4	5
2.7.	How much difficulty do you have in seeing differences in colours? रङहरूमा भिन्नता देख्न तपाईंलाई कति कठिनाई हुन्छ ?	1	2	3	4	5
2.8.	Because of your eyesight, how much difficulty do you have in recognizing the face of a person standing near you? तपाईंको आँखाको कारणले, तपाईंलाई नजिकैको व्यक्तिको अनुहारलाई पहिचान गर्न कति कठिनाइ हुन्छ ?	1	2	3	4	5
2.9.	How much difficulty do you have in seeing the level in a container when pouring? तपाईंसँग कति कठिनाई छ एक कंटेनर मा स्तर जब डालना?	1	2	3	4	5
2.10.	Because of your eyesight, how much difficulty do you have in going to activities outside of the_house on your own (e.g. sporting events, shopping, religious events)? तपाईंको आँखाको कारणले, तपाईंलाईआफ्नै घरको बाहिर गतिविधिहरू (जस्तै खेलकुद घटनाहरू, किनमेल, धार्मिक कार्यक्रमहरू) गतिविधिहरूमा जान कति कठिनाइ हुन्छ?	1	2	3	4	5
2.11.	Because of your eyesight, how much difficulty do you have in recognizing people you know from a distance of 20 metres? (e.g. from that building/tree – give marker of 20 meters) तपाईंकोआँखाको कारणले, तपाईले 20 मीटरको दूरीबाट तपाईंले जान्नुभएका व्यक्तिहरूलाई पहिचान गर्न कत्तिको कठिनाई हुन्छ ? (जस्तै कि भवन / रूखबाट - 20 मिटरको मार्क दिनुहोस्)	1	2	3	4	5
2.12.	How much difficulty do you have in seeing close objects (e.g. making out differences in coins or notes, reading newsprint)? नजिकका वस्तुहरू हेर्नका लागि कति कठिनाई हुन्छ (जस्तै सिक्का वा टिप्पणीहरूमा मतभेदहरू बनाउने, समाचारपत्र पढ्ने)?	1	2	3	4	5

2.13.	How much difficulty do you have in seeing irregularities in the path when walking (e.g. potholes)? जब हिड्ने क्रममा (उदाहरणका लागि सडकमा छेदहरू)बाटोमा अनियमितताहरू देख्न तपाईंलाई कति कठिनाइ हुन्छ ?	1	2	3	4	5
2.14.	How much difficulty do you have in seeing after a few moments when coming inside after being in bright sunlight? केहि क्षणपछि देख्दा कत्तिको कठिनाई हुन्छ जब तपाईं उज्ज्वल सूर्यको प्रकाश बाट आउँदै हुनुहुन्छ?	1	2	3	4	5
2.15.	How much difficulty do you have in doing activities that require you to see well close up (e.g. sewing – not including threading the needle, using hand tools)? गतिविधिहरू गर्न तपाईलाई कत्तिको कठिनाइ हुन्छ जब तपाईं राम्ररी नजिक बाट हेर्न चाहानुहुन्छ (जस्तै सिलाई - सुई सूत्र सहित, हात उपकरण प्रयोग गर्दै)?	1	2	3	4	5
2.16.	Because of your eyesight, how much difficulty do you have in carrying out your usual work? तपाईंको आँखाको कारणले, सामान्य कार्य को लागी तपाईलाई कतिको कठिनाइ हुन्छ?	1	2	3	4	5
In the next section, I am going to ask you how you feel because of your vision problem. I will read out a choice of five answers and you will choose the one that describes you best.						
		Never कहिल्यैहो इन	*Rarely दुर्लभ*	*Sometimes कहिले काहीं*	*Often अक्सर*	*Very often प्राय*
2.17.	Because of your eyesight, how often have you been hesitant to participate in social functions? तपाईंको आँखाको कारणले, सामाजिक कार्यमा भाग लिन कतिको संकोच भएको छ?	1	2	3	4	5
2.18.	Because of your eyesight, how often have you found that you are ashamed or embarrassed? तपाईंको आँखाको कारण, तपाईंले कति पटक भेट्नुभएको छ कि तपाईं लाजमर्दो वा शर्मिला हुनुहुन्छ?	1	2	3	4	5
2.19.	Because of your eyesight, how often have you felt that you are a burden on others? तपाईंको आँखाको कारणले गर्दा तपाइँले कति पटक महसुस गर्नुभयो कि तपाइँ अरुमा बोझ हुनुहुन्छ?	1	2	3	4	5

2.20.	Because of your eyesight, how often do you worry that you may lose your remaining eyesight? तपाईंको आँखाको कारणले, तपाई कति चिन्ता गर्नुहुन्छ कि तपाइँ आफ्नो बाँकी आँखा गुमाउन सक्नुहुन्छ?	1	2	3	4	5
2.21.	Does your vision problem affect your life in ways we have not mentioned? If YES, describe how के तपाईको दृष्टिको समस्याले तपाईको जीवनलाई असर गर्ने तरिकाहरूमा असर पार्छ जुन हामीले उल्लेख गरेको छैन? यदि हो, कसरी वर्णन गर्नुहोस्					
	Record as fully as possible the answer given जवाफ दिईएको पूर्ण रूपमा रेकर्ड गर्नुहोस्					

Investigator's Signature: _____ **Date**: _ _ / _ _ _ / _ _ _

Investigator's Name: _____

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

BASELINE QUALITY OF LIFE ENTRY FORMS
BASELINE EQ-5D-5L FORM

Under each heading, please tick the **ONE** option that best describes your health TODAY
प्रत्येक शीर्षककोअधीनमा ,कृपया एक विकल्प छनौट गर्नुहोस्जुन तपाईंको स्वास्थ्यलाई उत्तम वर्णन गर्दछ

MOBILITY (गतिशीलता)	
1. I have no problems in walking about मलाई हिँड्न कुनै समस्या छैन	
2. I have slight problems in walking about मलाई अलि कति हिड्न समस्या छ	
3. I have moderate problems in walking about मलाई पैदल हिड्दा मध्यम समस्या छ	
4. I have severe problems in walking about मलाई हिड्न गम्भीर समस्या छ	
5. I am unable to walk about म हिड्न असमर्थ छु	

SELF-CARE (आफ्नै हेर विचार)	
1. I have no problems washing or dressing myself मलाई कुनै समस्या छैन लुगा धुन वा लुगा लगाउन	
2. I have slight problems washing or dressing myself मलाई केहि समस्या छ लुगा धुन वा लुगा लगाउन	
3. I have moderate problems washing or dressing myself मलाई मध्यम समस्या छ लुगा धुन वा लुगा लगाउन	
4. I have severe problems washing or dressing myself मलाई गम्भीर समस्या छ लुगा धुन वा लुगा लगाउन	
म असक्षम छु लुगा धुन वा लुगा लगाउन	

USUAL ACTIVITIES (सामान्यतया गतिविधिहरू) (e.g. work, study, housework, family or leisure activities) (जस्तै काम, अध्ययन, गृहकार्य, परिवार वा अवकाश गतिविधिहरू)	
1. I have no problems doing my usual activities मेरो सामान्य गतिविधिहरू गर्नमा कुनै समस्या छैन	
2. I have slight problems doing my usual activities मेरो सामान्य गतिविधिहरू गरिरहँदा केहि समस्याहरूछन्	
3. I have moderate problems doing my usual activities मेरो सामान्य गतिविधिहरू गरिरहँदा मध्यम समस्याहरू छन्	
4. I have severe problems doing my usual activities मेरो सामान्य गतिविधिहरू गरिरहँदा गम्भीर समस्याहरू छन्	
5. I am unable to do my usual activities म मेरो सामान्य गतिविधिहरू गर्न असमर्थछु	

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

PAIN / DISCOMFORT (दुखाइ / असुविधा)	
1. I have no pain or discomfort मलाई कुनै पीडा वा असुविधा छैन	
2. I have slight pain or discomfort मेरो बिस्तारै दुखाइ वा असुविधा छ.	
3. I have moderate pain or discomfort मलाई मध्यम दुखाइ वा असुविधा छ	
4. I have severe pain or discomfort मलाई कडा दुखाइ वा असुविधा छ	
5. I have extreme pain or discomfort मलाई चरम दुखाइ वा असुविधा छ	

ANXIETY / DEPRESSION (चिन्ता / अवसाद)	
1. I am not anxious or depressed मलाई कुनै चिंता वा निराश छैन	
2. I am slightly anxious or depressed म थोडा चिन्तित वा निराश हुँ	
3. I am moderately anxious or depressed मलाई मध्यम चिन्तित। वा निराश छ	
4. I am severely anxious or depressed म गम्भीर चिन्तित वा निराश हुँ	
5. I am extremely anxious or depressed म अत्यन्त चिन्तित वा निराश हुँ	

Investigator's Signature: _____ **Date**: _ _ / _ _ _ / _ _ _ _

Investigator's Name: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

BASELINE QUALITY OF LIFE ENTRY FORMS
PAIN IMPACT QUESTIONNAIRE

Pain Impact Questionnaire (दुखाइ प्रभाव प्रश्न)	
On a scale of 0-10, 0 being no pain at all and 10 being the most severe pain, how much pain have you felt in the last 2 weeks? 0–10 को मापन, 0 कुनै पनि दुखाइ नहुन सक्छ र 10 सबै भन्दा कडा दुखाइ हुन्छ, तपाइँले पछिल्लो 2 हप्तामा कतिको दुखाइ महसुस गर्नुभएको छ?	
1=Never, 2=Occasionally, 3=Often, 4=Constantly **1 = कहिल्यै होइन, 2 = कहिलेकाहीं, 3 = अक्सर, 4 = निरन्तर**	
In the course of your current illness, how often have you experienced eye pain? तपाईको वर्तमान रोगको समयमा, तपाईले कति पटक आँखाको दुखाइ अनुभव गर्नुभयो?	
In the course of your current illness, how often has eye pain interfered with your personal care such as bathing, eating, and dressing? तपाईको वर्तमान रोगको समयमा, कतिपय आँखाले तपाईको व्यक्तिगत हेरचाह जस्तै स्नान, खाने, र ड्रेसिङको साथमा कतिपय दुखाइको हस्तक्षेप गरेको छ?	
In the course of your current illness, how often has eye pain disturbed your sleep? तपाईको वर्तमान रोगको समयमा, कतिपय आँखाले तपाईको निद्रालाई कष्ट बनाएको छ?	
In the course of your current illness, how often has eye pain interfered with your household work such as cooking, house cleaning, washing cloth, fetching water, fetching firewood, caring to other family members? तपाईको हालको बिरामीको समयमा, कहिलेकाहीं आँखाले दुखाइको साथ तपाईको परिवारको साथ हस्तक्षेप गर्दछ जस्तै खाना पकाउने, घरको सफाई, कपडा धोएर कपडा, पानी ल्याउने, फन्धुवाट ल्याउने, अन्य परिवारका सदस्यहरूलाई हेरचाह गर्ने?	
In the course of your current illness, how often has eye pain affected your agricultural or paid work? तपाईको वर्तमान रोगको समयमा, कतिपय आँखाले तपाईंको कृषि वा सशुल्क कामलाई असर गरेको छ?	
In the course of your current illness, how often has eye pain affected your participation in social activities such as attending weddings, social meetings, and funerals? तपाईको हालको बीमारीको समयमा, कहिलेकाँही आँखाले दुखाइले सामाजिक गतिविधिहरूमा तपाईको सहभागितालाई प्रभावित गरेको छ जस्तै विवाह, सामाजिक सभा र रौतहटमा सहभागी हुन सक्छ?	

Investigator's Signature: _____ **Date**: _ _ / _ _ _ / _ _ _

Investigator's Name: _____

NOW COMPLETE THE ADMISSION AND FOLLOW-UP CHECKLIST PRIOR TO DISCHARGING THE PATIENT

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

ADMISSION AND FOLLOW-UP

Admission of patients follows local hospital protocol. All patients with an infiltrate of greater than 1mm maximum diameter and / or presence of a hypopyon will be admitted. Additionally, all study patients will be admitted for the first 48 hours if they are willing and there are enough beds available on the cornea ward.

	Admission and follow-up checklist	Yes	No
1.	**Patient admitted?** **If not, specify reason** _____ **Estimated date of discharge:** _____	☐	☐
2.	**Have the baseline QoL forms been completed?**	☐	☐
2.	**Counselled on correct drug use and importance of follow-up?**	☐	☐
3.	**Has the follow-up card been completed correctly including the date?**	☐	☐
4.	**Correct contact details checked with patient and relative**	☐	☐
5.	**Date of follow-up agreed with patient**	_ _ / _ _ _ / _ _ _	

Investigator's Signature: _____ **Date** : __ __ / __ __ __ / __ __ __ __
(DD / MMM / YYYY)

Investigator's Name: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

RANDOMISED CONTROLLED NON-INFERIORITY TRIAL OF TOPICAL CHLORHEXIDINE 0.2% VERSUS TOPICAL NATAMYCIN 5% FOR FUNGAL KERATITIS IN NEPAL

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

NEPAL NETRA JYOTI SANGH
EASTERN REGIONAL EYE CARE PROGRAMME
SAGARMATHA CHOUDHARY EYE HOSPITAL, LAHAN
BIRATNAGAR EYE HOSPITAL, BIRATNAGAR
EREC-P

CASE REPORT FORM
FOLLOW-UP

Chief Investigator: Prof Matthew Burton

Primary Investigators: Dr Reena Yadav; Dr Sanjay Singh; Dr Jeremy Hoffman

Name of site: Sagarmatha Choudhary Eye Hospital, Lahan

CRF Version Number: V1

Patient Initials	Triage No.
Enrolment date _ _ / _ _ _ / _ _	SRN

CRF Completion Instructions

General

Complete the CRF using a **black ballpoint pen** and that all entries are complete and legible.
Avoid the use of abbreviations and acronyms.
The CRF should be completed during, or if not possible, as soon as possible after the scheduled visit.
Ensure that the header information (i.e. subject's initials and ID number) is completed consistently throughout the CRF. Missing initials should be recorded with a dash (i.e. D-L).
If a subject prematurely withdraws from the trial a single line must be drawn across each uncompleted page.

Ensure that all fields are completed on each page

- If a test was Not Done record **ND** in the relevant box(es)
- Where information is Not Known write **NK** in relevant box(es)
- Where information is not applicable write **NA** in the relevant box(es)

The Principal Investigator is responsible for the accuracy of the data reported on the CRF. The PI must sign and date the Principal Investigator's Sign Off page to certify accuracy, completeness and legibility of the data reported in the CRF.
Medications should be recorded using the generic name whenever possible excepting combination products which will be recorded using the established trade name.

Verbatim Adverse Event terms should be recorded as the final diagnosis whenever possible.

Complete all **dates** as day, month, year i.e. 13/NOV/2008. Partial dates should be recorded as NK/NOV/2008
All **times** are to be recorded in 24-hour format without punctuation always using 4-digits; i.e. 0200 or 2130. Midnight is recorded as 0000.
Weights should be recorded to the nearest 0.1 kg.

CRF Footer section

The 'completed by' Name must be legible and **CRFs should only be completed by individuals on the Site Delegation log, signed by the PI.**
Each CRF should be signed and dated by the person completing the form.

Serious Adverse Events (SAEs)

A SAE form should be completed and faxed to the study coordination centre for all SAEs within 24 hours. However, relapse and death due to a known pre-existing condition and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs. All Adverse Event reporting should be carried out as per SOP 19: Adverse Event Reporting. The Reporting Form is attached at the end of this CRF collection.

Corrections to entries

If an error is made draw a single line through the item, then write the correct entry on an appropriate blank space near the original data point on the CRF and initial and date the change.

Do NOT

- Obscure the original entry by scribbling it out
- Try to correct/ modify the original entry
- Use Tippex or correction fluid

Storage

The CRF documents should be stored in a locked, secure area when not in use where confidentiality can be maintained. Ensure that they are separate to any other documents that might reveal the identity of the subject.

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

FOLLOW-UP DAY 2 CLINICAL EXAMINATION DATA ENTRY FORM
PATIENT DETAILS AND VISUAL ACUITY

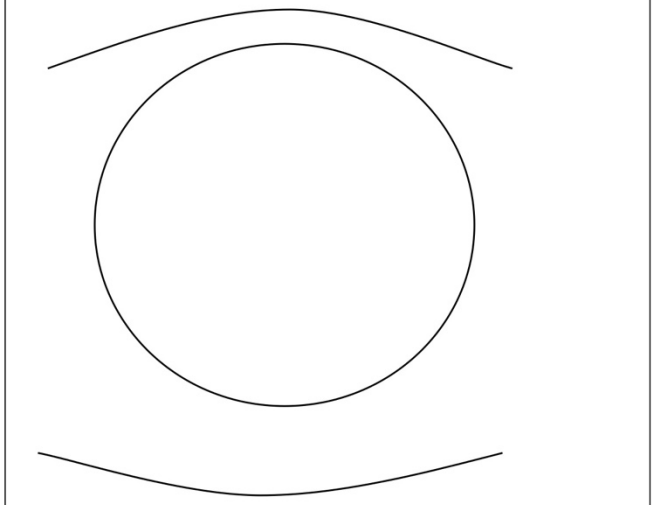
1. Study Number	Write the study number	
2. Date	Write in this format: DD/MMM/YYYY	
3. Presenting Visual Acuity (use spectacles if relevant) LogMAR	AFFECTED EYE ONLY	
	Spectacle corrected? 1 = Yes 2 = No	
4. Best pinhole corrected visual acuity LogMAR	AFFECTED EYE ONLY	
5. Scheduled review	1 = Yes 0 = No	
6. Reason if unscheduled	1 = Eye Worse 2 = Perforation/impending 3 = Missed previous visit 3 = On request 5 = NA	

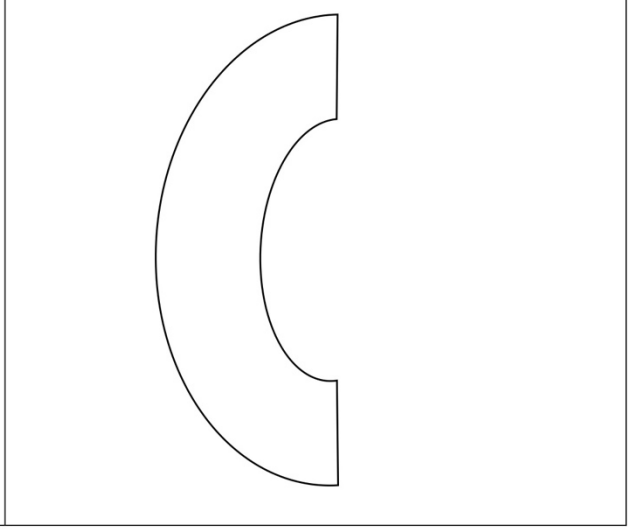
F/U DAY 2 CLINICAL EXAMINATION DATA ENTRY FORM
SLIT LAMP BIOMICROSCOPY EXAMINATION

1. Conjunctival hyperaemia	0 = None 1 = Mild 2 = Moderate 3 = Severe	
1. Immune ring?	0 = No 1 = Yes	
2. Hypopyon height (mm)	0 = None If present specify number of mm (1-10) 99 = Unable to see	
3. Hypopyon shape	99 = NA 1 = Level / Flat 2 = Heaped	
4. Fibrin	0 = No 1 = Yes 99 = Not able to see	
5. Flare	0 = No 1 = Yes 99 = Not able to see	
6. Flare grade	0 = None 1 = Mild 2 = Moderate (iris and pupil seen) 3 = Moderate-severe (iris and pupil hazy) 4 = Severe (fibrin in AC) 99 = Not able to see	
7. Cells in AC	0 = No 1 = Yes 99 = Not able to see	
8. Cells grade	0 = None 1 = 1-5 cells 2 = 6-15 cells 3 = 16-25 cells 4 = 26-50 cells 5 = more than 50 99 = Not able to see	
9. Size of infiltrate 1	Measure max. diameter	mm
10. Size of infiltrate 2	Measure perpendicular diameter to maximum diameter	mm
11. Size of epithelial defect 1	Measure max. diameter. *NB measure with FLN ONLY after photos and samples have been taken*	mm

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

12. Size of epithelial defect 2	Measure perpendicular diameter to maximum diameter	mm
13. Depth of ulceration	Ratio of remaining corneal tissue / normal corneal thickness as percentage 1 = 1 – 25% 2 = 26 – 50% 3 = 51 – 75% 4 = 75 – 100%	
14. Perforation	0 = No 1 = Descemetocele 2 = Perforated	
15. IOP	Enter pneumotonometry reading here	mmHg
16. **SUSPECTED ADVERSE EVENTS?**	0 = No 1 = Yes IF SUSPECTED COMPLETE ADVERSE EVENT REPORTING FORM	
17. Picture DSC number no stain		
18. Picture DSC number with fluorescein		





CLINICAL NOTES:

FOLLOW-UP CHECKLIST		**Yes**	**No**
1.	**Has the follow-up card been completed correctly including the date?**	☐	☐
2.	**Correct contact details checked with patient and relative**	☐	☐
3.	**Date of follow-up agreed with patient**	_ _ / _ _ _ / _ _ _	

Investigator's Signature: _____ **Date** : _ _ / _ _ _ / _ _ _ _
(DD / MMM / YYYY)

Investigator's Name: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

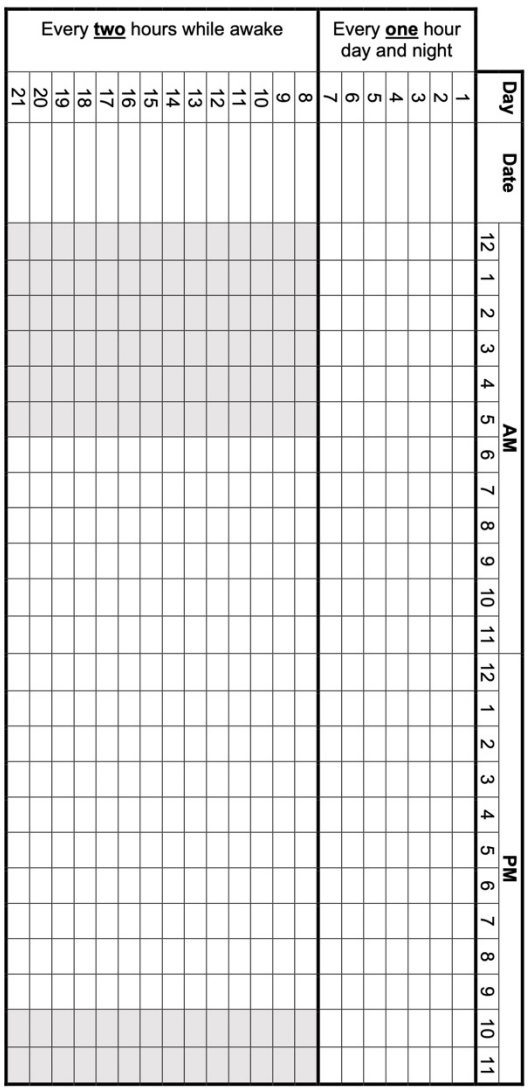
FOLLOW-UP CLINICAL EXAMINATION DATA ENTRY FORM

INPATIENT ADHERENCE FORM

Complete for all admitted patients. If patient is treated as an outpatient, use the outpatient adherence form

Instructions:

- One drop should be given every hour for one week (hourly day and night)
- From the second week, drops should be given every two hours (two-hourly)
- Please mark the form when the medication is given
- Medication is replaced when it is finished – if it is due to be finished ahead of the patient's review in clinic, please contact the study coordinator



DATE OF ADMISSION: _ _ / _ _ _ / _ _

TIME OF ADMISSION: _ _ : _ _

PLACE A LINE THROUGH ON THE FIRST DAY UNTIL THE TIME PATIENT ADMITTED

DATE OF DISCHARGE: _ _ / _ _ _ / _ _

TIME OF DISCHARGE: _ _ : _ _

PLACE A LINE THROUGH THE REST OF THE FORM FOLLOWING THE TIME OF DISCHARGE

AT DISCHARGE, COUNT UP THE NUMBER OF BOXES BETWEEN ADMISSION AND DISCHARGE (INTENDED DOSES). THEN COUNT UP THE NUMBER OF MISSED DOSES (I.E. THOSE WHERE THERE IS NO MARK). ENTER THE NUMBERS HERE:

NUMBER OF INTENDED DOSES: _____

NUMBER OF MISSED DOSES: _____

Study co-ordinator's signature: _____

Date: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

FOLLOW-UP DAY 2 CLINICAL EXAMINATION DATA ENTRY FORM

OUTPATIENT ADHERENCE FORM

Complete for all outpatients. If patient is admitted, use the inpatient adherence form

Instructions:

- One drop should be given every hour for one week (hourly)
- From the second week, drops should be given every two hours (two-hourly)
- Medication is replaced when it is finished – if it is due to be finished ahead of the patient's review in clinic, please contact the study coordinator
- Refer to the drug bottle registry for the dispensing weight
- **The Randomisation Administrator must weigh the bottle at each follow-up and enter into the Log Book**

Number of missed doses		*Self-reported adherence*
Why missed? 0 = NO MISSED DOSES REPORTED 1 = FORGOT 2 = TOO BUSY 3 = DID NOT KNOW THE TIME 4 = COULD NOT FIND THE BOTTLE 5 = UNCOMFORTABLE / STINGING 6 = UNSURE / CAN'T REMEMBER 9 = OTHER (SPECIFY IN NOTES)		
Given to others?		
Note		

Study co-ordinator's signature: _____

Date: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

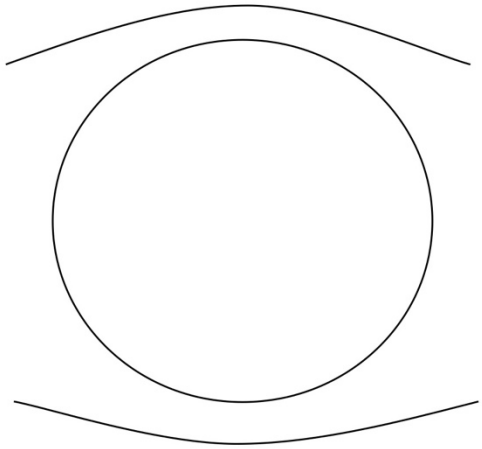
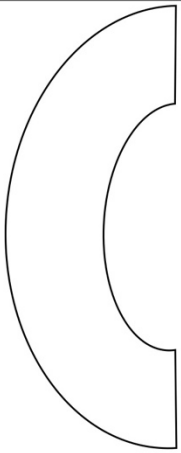
FOLLOW-UP DAY 7 CLINICAL EXAMINATION DATA ENTRY FORM
PATIENT DETAILS AND VISUAL ACUITY

1. Study Number	Write the study number	
2. Date	Write in this format: DD/MMM/YYYY	
3. Presenting Visual Acuity (use spectacles if relevant) LogMAR	AFFECTED EYE ONLY	
	Spectacle corrected? 1 = Yes 2 = No	
4. Best pinhole corrected visual acuity LogMAR	AFFECTED EYE ONLY	
5. Scheduled review	1 = Yes 0 = No	
6. Reason if unscheduled	1 = Eye Worse 2 = Perforation/impending 3 = Missed previous visit 3 = On request 5 = NA	

F/U DAY 7 CLINICAL EXAMINATION DATA ENTRY FORM
SLIT LAMP BIOMICROSCOPY EXAMINATION

1. Conjunctival hyperaemia	0 = None 1 = Mild 2 = Moderate 3 = Severe	
2. Immune ring?	0 = No 1 = Yes	
3. Hypopyon height (mm)	0 = None If present specify number of mm (1-10) 99 = Unable to see	
4. Hypopyon shape	99 = NA 1 = Level / Flat 2 = Heaped	
5. Fibrin	0 = No 1 = Yes 99 = Not able to see	
6. Flare	0 = No 1 = Yes 99 = Not able to see	
7. Flare grade	0 = None 1 = Mild 2 = Moderate (iris and pupil seen) 3 = Moderate-severe (iris and pupil hazy) 4 = Severe (fibrin in AC) 99 = Not able to see	
8. Cells in AC	0 = No 1 = Yes 99 = Not able to see	
9. Cells grade	0 = None 1 = 1-5 cells 2 = 6-15 cells 3 = 16-25 cells 4 = 26-50 cells 5 = more than 50 99 = Not able to see	
10. Size of infiltrate 1	Measure max. diameter	mm
11. Size of infiltrate 2	Measure perpendicular diameter to maximum diameter	mm
12. Size of epithelial defect 1	Measure max. diameter. *NB measure with FLN ONLY after photos and samples have been taken*	mm

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

13. Size of epithelial defect 2	Measure perpendicular diameter to maximum diameter	mm
14. Depth of ulceration	Ratio of remaining corneal tissue / normal corneal thickness as percentage 1 = 1 – 25% 2 = 26 – 50% 3 = 51 – 75% 4 = 75 – 100%	
15. Perforation	0 = No 1 = Descemetocele 2 = Perforated	
16. IOP	Enter pneumotonometry reading here	mmHg
17. **SUSPECTED ADVERSE EVENTS?**	0 = No 1 = Yes IF SUSPECTED COMPLETE ADVERSE EVENT REPORTING FORM	
18. Picture DSC number no stain		
19. Picture DSC number with fluorescein		
		
20. *In vivo* confocal microscopy – presence of hyphae	0 = No 1 = Yes 3 = Unsure / inconclusive 4 = Poor quality scan 99 = Not examined	
21. *In vivo* confocal microscopy – presence of cysts	0 = No 1 = Yes 3 = Unsure / inconclusive 4 = Poor quality scan 99 = Not examined	
22. IVCM diagnosis	1 = Fungal keratitis 2 = Bacterial keratitis 3 = Amoebic keratitis 4 = Other (specify)	
	Specify other:	

CLINICAL NOTES:

Continued on following page…

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

IF EPITHELIAL DEFECT NOT HEALED, THEN FURTHER SAMPLE REQUIRED:

SAMPLE	1 = COLLECTED 2 = SAMPLE NOT ENOUGH 3 = EYE-PERFORATED / IMPENDING 4 = STROMAL ABSCESS 5 = PATIENT UNCO-OPERATIVE 6 = NOT INDICATED	REPORTED RESULTS *(INPUT DATA ONTO FORM 4 – LAB RESULTS ACCESS TABLE)*
GRAM SLIDE		
KOH SLIDE 1		
CALCOFLUOR SLIDE		
FRESH BLOOD AGAR		
SABOURAUD AGAR		
CHOCOLATE AGAR		
BRAIN HEART INFUSION		

FOLLOW-UP CHECKLIST		Yes	No
1.	Has the follow-up card been completed correctly including the date?	☐	☐
2.	Correct contact details checked with patient and relative	☐	☐
3.	Date of follow-up agreed with patient	_ _ / _ _ _ / _ _ _	

Investigator's Signature: _____ **Date** : _ _ / _ _ _ / _ _ _ _
(DD / MMM / YYYY)

Investigator's Name: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

FOLLOW-UP DAY 7 CLINICAL EXAMINATION DATA ENTRY FORM
PATIENT ADHERENCE FORM

Complete for the inpatient adherence form on <u>Page 35</u> of this CRF booklet for all <u>admitted</u> patients. If patient is treated as an outpatient, use the outpatient adherence form below

Instructions:

- One drop should be given every hour for one week (hourly)
- From the second week, drops should be given every two hours (two-hourly)
- Medication is replaced when it is finished – if it is due to be finished ahead of the patient's review in clinic, please contact the study coordinator
- Refer to the drug bottle registry for the dispensing weight
- **The Randomisation Administrator must weigh the bottle at each follow-up and enter into the Log Book**

Number of missed doses		*Self-reported adherence*
Why missed? 0 = NO MISSED DOSES REPORTED 1 = FORGOT 2 = TOO BUSY 3 = DID NOT KNOW THE TIME 4 = COULD NOT FIND THE BOTTLE 5 = UNCOMFORTABLE / STINGING 6 = UNSURE / CAN'T REMEMBER 9 = OTHER (SPECIFY IN NOTES)		
Given to others?		
Note		

Study co-ordinator's signature: _____

Date: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

FOLLOW-UP DAY 14 CLINICAL EXAMINATION DATA ENTRY FORM
PATIENT DETAILS AND VISUAL ACUITY

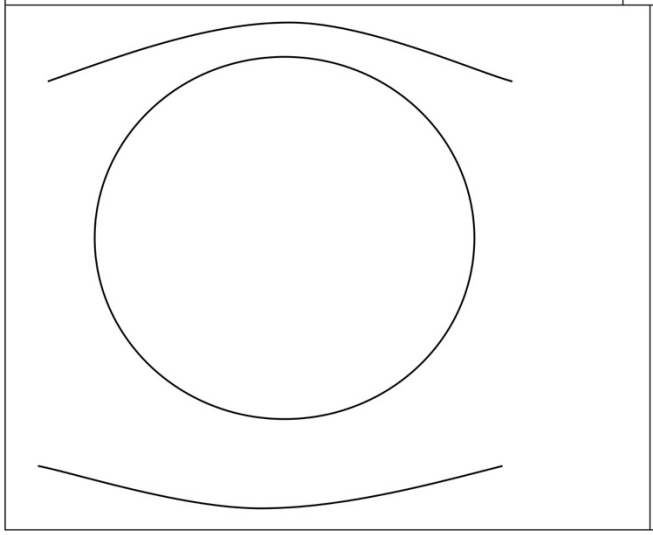
1. Study Number	Write the study number	
2. Date	Write in this format: DD/MMM/YYYY	
3. Presenting Visual Acuity (use spectacles if relevant) LogMAR	AFFECTED EYE ONLY	
	Spectacle corrected? 1 = Yes 2 = No	
4. Best pinhole corrected visual acuity LogMAR	AFFECTED EYE ONLY	
5. Scheduled review	1 = Yes 0 = No	
6. Reason if unscheduled	1 = Eye Worse 2 = Perforation/impending 3 = Missed previous visit 3 = On request 5 = NA	

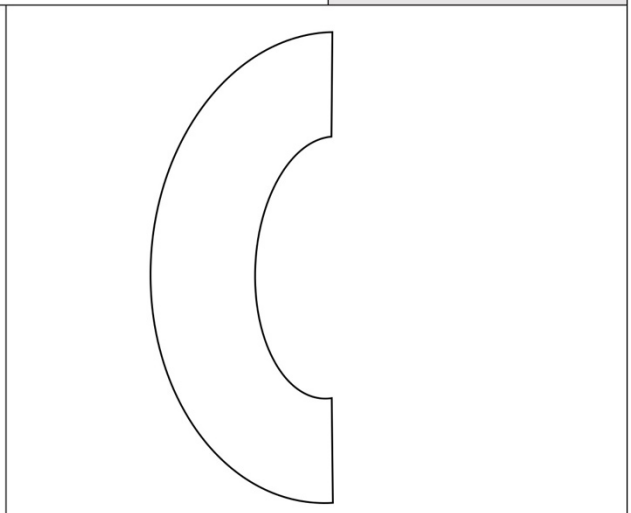
F/U DAY 14 CLINICAL EXAMINATION DATA ENTRY FORM
SLIT LAMP BIOMICROSCOPY EXAMINATION

1. Conjunctival hyperaemia	0 = None 1 = Mild 2 = Moderate 3 = Severe	
2. Immune ring?	0 = No 1 = Yes	
3. Hypopyon height (mm)	0 = None If present specify number of mm (1-10) 99 = Unable to see	
4. Hypopyon shape	99 = NA 1 = Level / Flat 2 = Heaped	
5. Fibrin	0 = No 1 = Yes 99 = Not able to see	
6. Flare	0 = No 1 = Yes 99 = Not able to see	
7. Flare grade	0 = None 1 = Mild 2 = Moderate (iris and pupil seen) 3 = Moderate-severe (iris and pupil hazy) 4 = Severe (fibrin in AC) 99 = Not able to see	
8. Cells in AC	0 = No 1 = Yes 99 = Not able to see	
9. Cells grade	0 = None 1 = 1-5 cells 2 = 6-15 cells 3 = 16-25 cells 4 = 26-50 cells 5 = more than 50 99 = Not able to see	
10. Size of infiltrate 1	Measure max. diameter	mm
11. Size of infiltrate 2	Measure perpendicular diameter to maximum diameter	mm
12. Size of epithelial defect 1	Measure max. diameter. *NB measure with FLN ONLY after photos and samples have been taken*	mm

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

13. Size of epithelial defect 2	Measure perpendicular diameter to maximum diameter	mm
14. Depth of ulceration	Ratio of remaining corneal tissue / normal corneal thickness as percentage 1 = 1 – 25% 2 = 26 – 50% 3 = 51 – 75% 4 = 75 – 100%	
15. Perforation	0 = No 1 = Descemetocele 2 = Perforated	
16. IOP	Enter pneumotonometry reading here	mmHg
17. **SUSPECTED ADVERSE EVENTS?**	0 = No 1 = Yes IF SUSPECTED COMPLETE ADVERSE EVENT REPORTING FORM	
18. Picture DSC number no stain		
19. Picture DSC number with fluorescein		





CLINICAL NOTES:

FOLLOW-UP CHECKLIST		**Yes**	**No**
1.	**Has the follow-up card been completed correctly including the date?**	☐	☐
2.	**Correct contact details checked with patient and relative**	☐	☐
3.	**Date of follow-up agreed with patient**	_ _ / _ _ _ / _ _ _	

Investigator's Signature: _____ **Date** : _ _ / _ _ _ / _ _ _ _
(DD / MMM / YYYY)

Investigator's Name: _____

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

FOLLOW-UP DAY 14 CLINICAL EXAMINATION DATA ENTRY FORM
PATIENT ADHERENCE FORM

Complete for the inpatient adherence form on Page 35 of this CRF booklet for all admitted patients. If patient is treated as an outpatient, use the outpatient adherence form below

Instructions:

- One drop should be given every hour for one week (hourly)
- From the second week, drops should be given every two hours (two-hourly)
- Medication is replaced when it is finished – if it is due to be finished ahead of the patient's review in clinic, please contact the study coordinator
- Refer to the drug bottle registry for the dispensing weight
- **The Randomisation Administrator must weigh the bottle at each follow-up and enter into the Log Book**

Number of missed doses		*Self-reported adherence*
Why missed? 0 = NO MISSED DOSES REPORTED 1 = FORGOT 2 = TOO BUSY 3 = DID NOT KNOW THE TIME 4 = COULD NOT FIND THE BOTTLE 5 = UNCOMFORTABLE / STINGING 6 = UNSURE / CAN'T REMEMBER 9 = OTHER (SPECIFY IN NOTES)		
Given to others?		
Note		

Study co-ordinator's signature: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

FOLLOW-UP DAY 21 CLINICAL EXAMINATION DATA ENTRY FORM
PATIENT DETAILS AND VISUAL ACUITY

1. Study Number	Write the study number	
2. Date	Write in this format: DD/MMM/YYYY	
3. Presenting Visual Acuity (use spectacles if relevant) LogMAR	AFFECTED EYE ONLY	
	Spectacle corrected? 1 = Yes 2 = No	
4. Best pinhole corrected visual acuity LogMAR	AFFECTED EYE ONLY	
5. Scheduled review	1 = Yes 0 = No	
6. Reason if unscheduled	1 = Eye Worse 2 = Perforation/impending 3 = Missed previous visit 3 = On request 5 = NA	

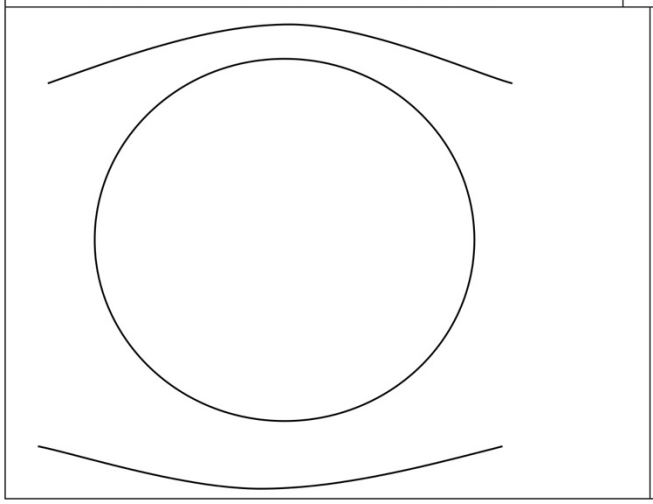
F/U DAY 21 CLINICAL EXAMINATION DATA ENTRY FORM
SLIT LAMP BIOMICROSCOPY EXAMINATION

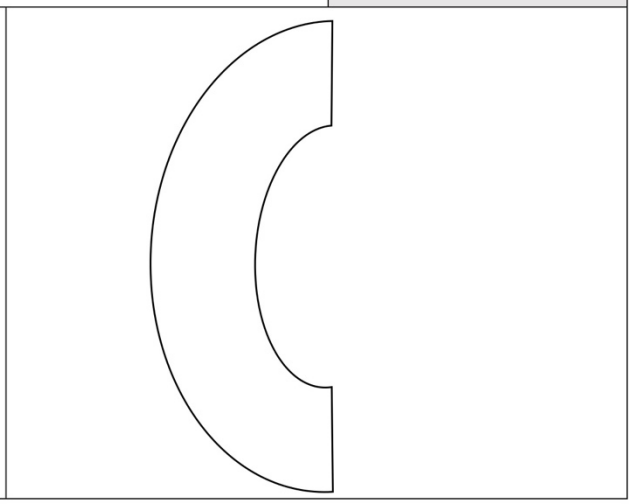
1. Conjunctival hyperaemia	0 = None 1 = Mild 2 = Moderate 3 = Severe	
2. Corneal sensation	1 = Normal 2 = Reduced	
3. Slough elevated	0 = None 1 = Flat 2 = Raised	
4. Infiltrate edge	1 = Defined 2 = Serrated 99=Not able to see	
5. Satellite lesions?	0 = None 1 = Yes 99 = Not able to see	
6. Infiltrate colour?	99 = None 1 = White 2 = Cream 3 = Green 4 = Yellow 5 = Dark brown / black 7 = other (specify)	
	Specify Other:	
7. Immune ring?	0 = No 1 = Yes	
8. Superficial Corneal vascularisation	0 = No 1 = Yes	
9. How many clock hours?	Write “0” is absent	
10. Deep Corneal vascularisation	0 = No 1 = Yes	
11. How many clock hours?	Write “0” is absent	
12. Hypopyon height (mm)	0 = None If present specify number of mm (1-10) 99 = Unable to see	
13. Hypopyon shape	99 = NA 1 = Level / Flat 2 = Heaped	

14. Keratic precipitates	0 = No 1 = Yes 99 = NA	
15. Keratic precipitate age	1 = New 2 = Old 99 = NA	
16. Keratic precipitate size	1 = Small 2 = Large 99 = NA	
17. Perineural infiltrates	0 = No 1 = Yes 99 = Not able to see	
18. Fibrin	0 = No 1 = Yes 99 = Not able to see	
19. Flare	0 = No 1 = Yes 99 = Not able to see	
20. Flare grade	0 = None 1 = Mild 2 = Moderate (iris and pupil seen) 3 = Moderate-severe (iris and pupil hazy) 4 = Severe (fibrin in AC) 99 = Not able to see	
21. Cells in AC	0 = No 1 = Yes 99 = Not able to see	
22. Cells grade	0 = None 1 = 1-5 cells 2 = 6-15 cells 3 = 16-25 cells 4 = 26-50 cells 5 = more than 50 99 = Not able to see	
23. Posterior corneal abscess	0 = No 1 = Yes 99 = Not able to see	
24. Endothelial plaque	0 = No 1 = Yes 99 = Not able to see	
25. Solid inflammatory mass in AC	0 = No 1 = Yes 99 = Not able to see	
26. Size of infiltrate 1	Measure max. diameter	mm
27. Size of infiltrate 2	Measure perpendicular diameter to maximum diameter	mm
28. Size of epithelial defect 1	Measure max. diameter. *NB measure with FLN ONLY after photos and samples have been taken*	mm
29. Size of epithelial defect 2	Measure perpendicular diameter to maximum diameter	mm
30. Depth of ulceration	Ratio of remaining corneal tissue / normal corneal thickness as percentage 1 = 1 – 25% 2 = 26 – 50% 3 = 51 – 75% 4 = 75 – 100%	
31. Site of the ulcer	1=outside central 4mm 2=Inside central 4mm not in centre, 3=Centre involved	
32. Perforation	0 = No 1 = Descemetocele 2 = Perforated	
33. IOP	Enter pneumotonometry reading here from initial examination in clinic	mmHg
34. **SUSPECTED ADVERSE EVENTS?**	0 = No 1 = Yes IF SUSPECTED COMPLETE ADVERSE EVENT FORM	

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

35. *In vivo* confocal microscopy – presence of hyphae	0 = No 1 = Yes 3 = Unsure / inconclusive 4 = Poor quality scan 99 = Not examined	
36. *In vivo* confocal microscopy – presence of cysts	0 = No 1 = Yes 3 = Unsure / inconclusive 4 = Poor quality scan 99 = Not examined	
37. IVCM diagnosis	1 = Fungal keratitis 2 = Bacterial keratitis 3 = Amoebic keratitis 4 = Other (specify)	
	Specify other:	
38. Picture DSC number no stain		
39. Picture DSC number with fluorescein		





CLINICAL NOTES:

FOLLOW-UP CHECKLIST		Yes	No
1.	**Has the follow-up card been completed correctly including the date?**	☐	☐
2.	**Correct contact details checked with patient and relative**	☐	☐
3.	**Date of follow-up agreed with patient**	_ _ / _ _ _ / _ _ _	

Investigator's Signature: _____ **Date** : _ _ / _ _ _ / _ _ _ _
(DD / MMM / YYYY)

Investigator's Name: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

FOLLOW-UP DAY 21 CLINICAL EXAMINATION DATA ENTRY FORM
PATIENT ADHERENCE FORM

Complete for the inpatient adherence form on <u>Page 35</u> of this CRF booklet for all <u>admitted</u> patients. If patient is treated as an outpatient, use the outpatient adherence form below

Instructions:

- One drop should be given every hour for one week (hourly)
- From the second week, drops should be given every two hours (two-hourly)
- Medication is replaced when it is finished – if it is due to be finished ahead of the patient's review in clinic, please contact the study coordinator
- Refer to the drug bottle registry for the dispensing weight
- **The Randomisation Administrator must weigh the bottle at each follow-up and enter into the Log Book**

Number of missed doses		*Self-reported adherence*
Why missed? 0 = NO MISSED DOSES REPORTED 1 = FORGOT 2 = TOO BUSY 3 = DID NOT KNOW THE TIME 4 = COULD NOT FIND THE BOTTLE 5 = UNCOMFORTABLE / STINGING 6 = UNSURE / CAN'T REMEMBER 9 = OTHER (SPECIFY IN NOTES)		
Given to others?		
Note		

Study co-ordinator's signature: _____

Date: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

FOLLOW-UP MONTH 2 CLINICAL EXAMINATION DATA ENTRY FORM
PATIENT DETAILS AND VISUAL ACUITY

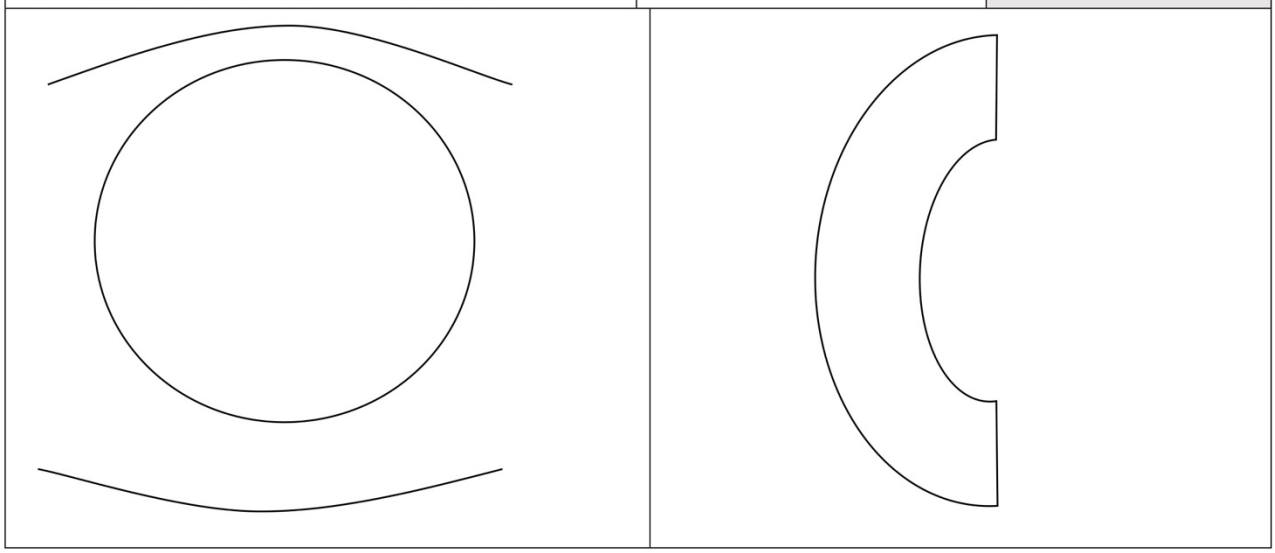
1. Study Number	Write the study number	
2. Date	Write in this format: DD/MMM/YYYY	
3. Presenting Visual Acuity (use spectacles if relevant) LogMAR	AFFECTED EYE ONLY	
	Spectacle corrected? 1 = Yes 2 = No	
4. Best pinhole corrected visual acuity LogMAR	AFFECTED EYE ONLY	
5. Scheduled review	1 = Yes 0 = No	
6. Reason if unscheduled	1 = Eye Worse 2 = Perforation/impending 3 = Missed previous visit 3 = On request 5 = NA	

FOLLOW-UP MONTH 2 CLINICAL EXAMINATION DATA ENTRY FORM
SLIT LAMP BIOMICROSCOPY EXAMINATION

1. Conjunctival hyperaemia	0 = None 1 = Mild 2 = Moderate 3 = Severe	
2. Immune ring?	0 = No 1 = Yes	
3. Hypopyon height (mm)	0 = None If present specify number of mm (1-10) 99 = Unable to see	
4. Hypopyon shape	99 = NA 1 = Level / Flat 2 = Heaped	
5. Fibrin	0 = No 1 = Yes 99 = Not able to see	
6. Flare	0 = No 1 = Yes 99 = Not able to see	
7. Flare grade	0 = None 1 = Mild 2 = Moderate (iris and pupil seen) 3 = Moderate-severe (iris and pupil hazy) 4 = Severe (fibrin in AC) 99 = Not able to see	
8. Cells in AC	0 = No 1 = Yes 99 = Not able to see	
9. Cells grade	0 = None 1 = 1-5 cells 2 = 6-15 cells 3 = 16-25 cells 4 = 26-50 cells 5 = more than 50 99 = Not able to see	
10. Size of infiltrate 1	Measure max. diameter	mm
11. Size of infiltrate 2	Measure perpendicular diameter to maximum diameter	mm
12. Size of epithelial defect 1	Measure max. diameter. *NB measure with FLN ONLY after photos and samples have been taken*	mm

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

13. Size of epithelial defect 2	Measure perpendicular diameter to maximum diameter	mm
14. Depth of ulceration	Ratio of remaining corneal tissue / normal corneal thickness as percentage 1 = 1 – 25% 2 = 26 – 50% 3 = 51 – 75% 4 = 75 – 100%	
15. Perforation	0 = No 1 = Descemetocele 2 = Perforated	
16. IOP	Enter pneumotonometry reading here	mmHg
17. **SUSPECTED ADVERSE EVENTS?**	0 = No 1 = Yes IF SUSPECTED COMPLETE ADVERSE EVENT REPORTING FORM	
18. Picture DSC number no stain		
19. Picture DSC number with fluorescein		



CLINICAL NOTES:

FOLLOW-UP CHECKLIST		**Yes**	**No**
1.	**Has the follow-up card been completed correctly including the date?**	☐	☐
2.	**Correct contact details checked with patient and relative**	☐	☐
3.	**Date of follow-up agreed with patient**	_ _ / _ _ _ / _ _ _	

Investigator's Signature: _____ **Date** : _ _ / _ _ _ / _ _ _ _
(DD / MMM / YYYY)

Investigator's Name: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

FOLLOW-UP 2 MONTH CLINICAL EXAMINATION DATA ENTRY FORM

OUTPATIENT ADHERENCE FORM

Instructions:

- One drop should be given every hour for one week (hourly)
- From the second week, drops should be given every two hours (two-hourly)
- Medication is replaced when it is finished – if it is due to be finished ahead of the patient's review in clinic, please contact the study coordinator
- Refer to the drug bottle registry for the dispensing weight
- **The Randomisation Administrator must weigh the bottle at each follow-up and enter into the Log Book**

Number of missed doses		*Self-reported adherence*
Why missed? 0 = NO MISSED DOSES REPORTED 1 = FORGOT 2 = TOO BUSY 3 = DID NOT KNOW THE TIME 4 = COULD NOT FIND THE BOTTLE 5 = UNCOMFORTABLE / STINGING 6 = UNSURE / CAN'T REMEMBER 9 = OTHER (SPECIFY IN NOTES)		
Given to others?		
Note		

Study co-ordinator's signature: _____

Date: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

FOLLOW-UP MONTH 3 CLINICAL EXAMINATION DATA ENTRY FORM
PATIENT DETAILS, REFRACTION AND VISUAL ACUITY

1. Study Number	Write the study number	
2. Date	Write in this format: DD/MMM/YYYY	
3. Presenting Visual Acuity (use spectacles if relevant) LogMAR (PEEK)	AFFECTED EYE	
	Spectacle corrected? 1 = Yes 2 = No	
4. Best pinhole corrected presenting visual acuity LogMAR (PEEK)	AFFECTED EYE	
5. Best Spectacle Corrected Visual Acuity LogMAR Score (post-refraction, using ETDRS, after calculation)	AFFECTED EYE	
6. Refraction (affected eye only)	Please indicate + or – cyl Format: Sph / +/- cyl x axis	
7. Contrast sensitivity (PEEK)	AFFECTED EYE	
8. Scheduled review	1 = Yes 0 = No	
9. Reason if unscheduled	1 = Eye Worse 2 = Perforation/impending 3 = Missed previous visit 3 = On request 5 = NA	

FOLLOW-UP MONTH 3 CLINICAL EXAMINATION DATA ENTRY FORM
SLIT LAMP BIOMICROSCOPY EXAMINATION

20. Eye affected	1 = Right 2 = Left	
21. Presence of eye lid swelling	0 = No 1 = Yes	
22. Presence of entropion	0 = No 1 = Yes	
23. Amount of lagophthalmos	0 = None If present specify number of mm (1-10)	mm
24. Presence of Trichiasis	0 = No 1 = Yes	
25. Bell's phenomenon	1 = Normal 2 = Abnormal	
26. Conjunctival hyperaemia	0 = None 1 = Mild 2 = Moderate 3 = Severe	
27. Corneal sensation	1 = Normal 2 = Reduced	
28. Slough elevated	0 = None 1 = Flat 2 = Raised	
29. Infiltrate edge	1 = Defined 2 = Serrated 99=Not able to see	
30. Satellite lesions?	0 = None 1 = Yes 99 = Not able to see	

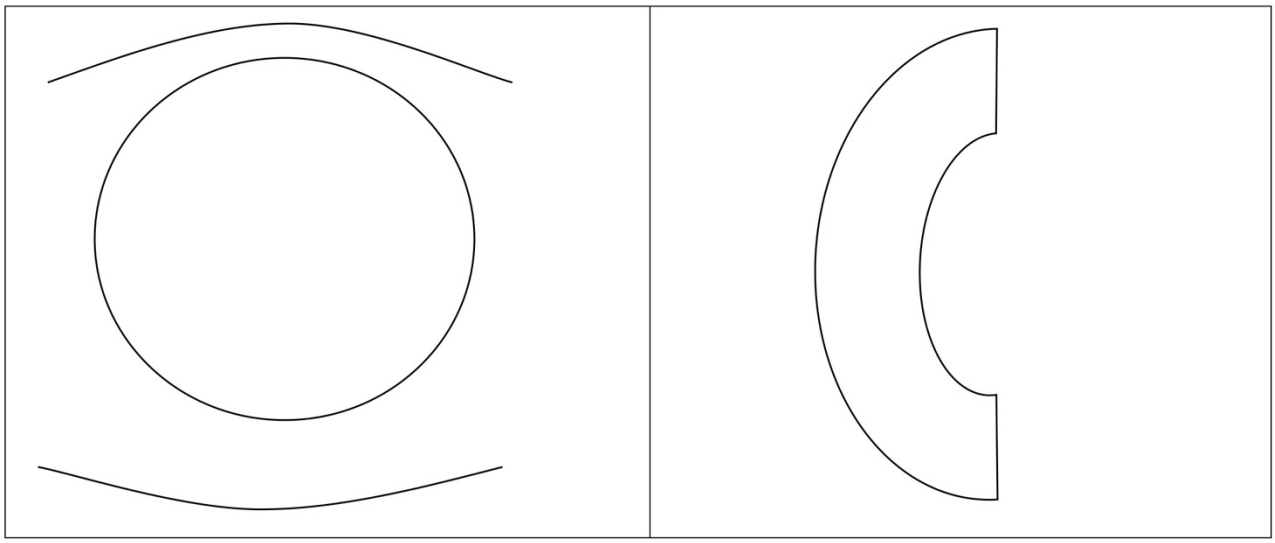
TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

31. Infiltrate colour?	99 = None 1 = White 2 = Cream 3 = Green 4 = Yellow 5 = Dark brown / black 7 = other (specify)	
	Specify Other:	
32. Immune ring?	0 = No 1 = Yes	
33. Superficial Corneal vascularisation	0 = No 1 = Yes	
34. How many clock hours?	Write "0" is absent	
35. Deep Corneal vascularisation	0 = No 1 = Yes	
36. How many clock hours?	Write "0" is absent	
37. Hypopyon height (mm)	0 = None If present specify number of mm (1-10) 99 = Unable to see	
38. Hypopyon shape	99 = NA 1 = Level / Flat 2 = Heaped	
39. Keratic precipitates	0 = No 1 = Yes 99 = NA	
40. Keratic precipitate age	1 = New 2 = Old 99 = NA	
41. Keratic precipitate size	1 = Small 2 = Large 99 = NA	
42. Perineural infiltrates	0 = No 1 = Yes 99 = Not able to see	
43. Fibrin	0 = No 1 = Yes 99 = Not able to see	
44. Flare	0 = No 1 = Yes 99 = Not able to see	
45. Flare grade	0 = None 1 = Mild 2 = Moderate (iris and pupil seen) 3 = Moderate-severe (iris and pupil hazy) 4 = Severe (fibrin in AC) 99 = Not able to see	
46. Cells in AC	0 = No 1 = Yes 99 = Not able to see	
47. Cells grade	0 = None 1 = 1-5 cells 2 = 6-15 cells 3 = 16-25 cells 4 = 26-50 cells 5 = more than 50 99 = Not able to see	
48. Posterior corneal abscess	0 = No 1 = Yes 99 = Not able to see	
49. Endothelial plaque	0 = No 1 = Yes 99 = Not able to see	
50. Solid inflammatory mass in AC	0 = No 1 = Yes 99 = Not able to see	
51. Size of infiltrate 1	Measure max. diameter	mm
52. Size of infiltrate 2	Measure perpendicular diameter to maximum diameter	mm

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

53. Size of epithelial defect 1	Measure max. diameter. *NB measure with FLN ONLY after photos and samples have been taken*	mm
54. Size of epithelial defect 2	Measure perpendicular diameter to maximum diameter	mm
55. Depth of ulceration	Ratio of remaining corneal tissue / normal corneal thickness as percentage 1 = 1 – 25% 2 = 26 – 50% 3 = 51 – 75% 4 = 75 – 100%	%
56. Site of the ulcer	1=outside central 4mm 2=Inside central 4mm not in centre, 3=Centre involved	
57. Perforation	0 = No 1 = Descemetocele 2 = Perforated	
58. IOP	Enter pneumotonometry reading here from initial examination in clinic	mmHg
59. Dilated Fundus examination - Lens	0 = Normal 1 = Abnormal (specify) 2 = No view 9 = Not examined	
	Specify abnormality:	
60. Dilated Fundus examination - Retina	0 = Normal 1 = Abnormal (specify) 2 = No view 9 = Not examined	
	Specify abnormality:	
61. Dilated Fundus examination – Macula	0 = Normal 1 = Abnormal (specify) 2 = No view 9 = Not examined	
	Specify abnormality:	
62. Dilated Fundus examination – Optic Nerve	0 = Normal 1 = Abnormal (specify) 2 = No view 9 = Not examined	
	Specify abnormality:	
63. Comment on the non-affected eye (if normal or not, comorbidity if present)	0 = Normal 1 = Adnexal abnormality 2 = Corneal disease 3 = Cataract 4 = Glaucoma 5 = Retinal disease 6 = Optic atrophy / oedema 7 = Refractive error 9 = Empty socket / phthisis 10 = Conjunctival growth 11 = conjunctivitis	
64. Picture DSC number no stain		
65. Picture DSC number with fluorescein		

TRIAGE NO. T | | | | | STUDY NO. S | | | | | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _



CLINICAL NOTES:

Investigator's Signature: _____ **Date**: _ _ / _ _ _ / _ _ _ _
(DD / MMM / YYYY)

Investigator's Name: _____

NOW PROCEED TO COMPLETE QUALITY OF LIFE FORMS AND STUDY COMPLETION CHECKLIST

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

MONTH 3 QUALITY OF LIFE ENTRY FORMS
MONTH 3 WHOQOL-BREF QUESTIONNAIRE

1.	**Quality of Life Questionnaire**	
2.	TRIAGE NUMBER	
3.	FIRST NAME	
4.	SURNAME	
5.	INTERVIEW DATE	

The following questions ask how you feel about your quality of life, health, or other areas of your life. I will read out each question to you, along with the response options. **Please choose the answer that appears most appropriate.** If you are unsure about which response to give to a question, the first response you think of is often the best one.
निम्न प्रश्नहरू तपाईको जीवन, स्वास्थ्य, वा तपाईंको जीवनका अन्य क्षेत्रहरूको बारेमा कस्तो महसुस गर्नुहुन्छ भन्ने प्रश्न सोध्छ. म तपाईलाई प्रत्येक प्रश्न पढ्नेछु, प्रतिक्रिया विकल्प सहित। कृपया जवाफ दिनुहोस् जुन सबैभन्दा उपयुक्त देखिन्छ। यदि तपाईं प्रश्नको जवाफ दिने बारे निश्चित हुनुहुन्न भने, तपाईले सोच्नु भएको पहिलो प्रतिक्रिया अक्सर उत्तम हो।

Please keep in mind your standards, hopes, pleasures and concerns. We ask that you think about your life **in the last four weeks.**
कृपया आफ्नो स्तर, आशा, आनन्द र चिन्तालाई ध्यान दिनुहोस। हामी सोध्छौं कि तपाईं आफ्नो जीवनको बारेमा अन्तिम चार हप्तामा के सोच्नुहुन्छ।

		Very poor धेरै गरिब	*Poor गरिब*	*Neither poor nor good नत गरीब नत राम्रो*	*Good राम्रो*	*Very good धेरै राम्रो*
2.22.	How would you rate your quality of life? तपाईं आफ्नो जीवन को गुणस्तर कसरी मूल्याङ्कन गर्नु हुन्छ?	1	2	3	4	5
		Very dissatisfied धेरै असन्तुष्ट	*Dissatisfied असन्तुष्ट*	*Neither satisfied nor dissatisfied नत सन्तुष्ट नत असन्तुष्ट*	*Satisfied सन्तुष्ट*	*Very satisfied धेरै सन्तुष्ट*
2.23.	How satisfied are you with your health? तपाईं आफ्नो स्वास्थ्यसंग कति सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5

The following questions ask about **how much** you have experienced certain things in the last four weeks.
निम्न प्रश्नहरूले तपाइँलाई पछिल्लो चार हप्तामा केहि चीजहरू अनुभव गरेकाछममेरोसामान्यगतिविधिहरूगर्नअसमर्थछुन्भन्ने बारे सोध्दछ।

		Not at all हुदै हैन	*A little थोरै*	*A moderate amount मध्यम मात्रामा*	*Very much धेरै*	*An extreme amount धेरै अत्यधिक मात्रा*
2.24.	To what extent do you feel that physical pain prevents you from doing what you need to do? कुन हदसम्म तपाई महसुस गर्नुहुन्छ कि शारीरिक पीडाले तपाईंलाई आवश्यक कामबाट रोक्छ?	5	4	3	2	1

2.25.	How much do you need any medical treatment to function in your daily life? के तपाईँ आफ्नो दैनिक जीवनमा कार्य गर्न कुनै पनि उपचारको आवश्यकता पर्दछ?	5	4	3	2	1
2.26.	How much do you enjoy life? तपाईं जीवनमा कतिको आनन्द लिनुहुन्छ?	1	2	3	4	5
2.27.	To what extent do you feel your life to be meaningful? तपाईं कुन हदसम्म आफ्नो जीवन सार्थक महसुस गर्नुहुन्छ?	1	2	3	4	5
		Not at all *हुदै हैन*	*A little* *थोरै*	*A moderate amount* *एक मध्यम मात्रा*	*Very much* *धेरै*	*Extremely* *अत्यधिक*
2.28.	How well are you able to concentrate? तपाईं कुन हदसम्म ध्यान केन्द्रित गर्न सक्नुहुन्छ?	1	2	3	4	5
2.29.	How safe do you feel in your daily life? तपाईं दैनिक जीवनमा कति सुरक्षित महसुस गर्नुहुन्छ?	1	2	3	4	5
2.30.	How healthy is your surrounding environment? तपाईंको आसपासको वातावरण कति स्वस्थ छ?	1	2	3	4	5

The following questions ask about how completely you experience or were able to do certain things in the last four weeks.
निम्न प्रश्नहरू तपाइँ कस्तो अनुभव गर्नुहुन्छ वा कसरी अन्तिम चार हप्तामा केहि चीज गर्न सक्षम सक्नु भएको थियो

		Not at all *हुदै हैन*	*A little* *थोरै*	*Moderately* *सामान्य*	*Mostly* *प्राय*	*Completely* *पूर्णतया*
2.31.	Do you have enough energy for everyday life? के तपाईंसँग पर्याप्त ऊर्जा छ दैनिक जीवनको लागि?	1	2	3	4	5
2.32.	Are you able to accept your bodily appearance? के तपाईं आफ्नो शारीरिक उपस्थिति स्वीकार गर्न सक्नुहुन्छ?	1	2	3	4	5
2.33.	Have you enough money to meet your needs? के तपाइँका आवश्यकताहरू पूरा गर्न पर्याप्त पैसा छ?	1	2	3	4	5
2.34.	How available to you is the information that you need in your day-to-day life? तपाइँलाई दिनहुँको जीवनमा आवश्यक जानकारी कसरी छ?	1	2	3	4	5

2.35.	To what extent do you have the opportunity for leisure activities? तपाईँसँग कुन हदसम्म खाली समय गतिविधिहरू को लागि अवसर छ?	1	2	3	4	5
		Very poor धेरै गरिब	*Poor गरिब*	*Neither poor nor good नत गरीब नत राम्रो*	*Good राम्रो*	*Very good धेरै राम्रो*
2.36.	How well are you able to get around? तपाईं कति राम्ररी वरिपरि हिंड्न सक्नुहुन्छ?	1	2	3	4	5
		Very dissatisfied धेरै असन्तुष्ट	*Dissatisfied असन्तुष्ट*	*Neither satisfied nor dissatisfied नत सन्तुष्ट नत असन्तुष्ट*	*Satisfied सन्तुष्ट*	*Very satisfied धेरै सन्तुष्ट*
2.37.	How satisfied are you with your sleep? तपाईं निद्रासंग कति सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
2.38.	How satisfied are you with your ability to perform your daily living activities? तपाइँ दैनिक जीवनका गतिविधिहरू प्रदर्शन गर्न आफ्नो क्षमताको साथ कत्ति सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
2.39.	How satisfied are you with your capacity for work? आफ्नो काम को लागि तपाई आफ्नो क्षमतासंग कतिको सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
2.40.	How satisfied are you with yourself? तपाईं आफै सँग कत्तिको सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
2.41.	How satisfied are you with your personal relationships? तपाईं आफ्नो व्यक्तिगत सम्बन्धमा कति सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
2.42.	How satisfied are you with your sex life? तपाईंआफ्नो यौन जीवनसँग कतिको सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
2.43.	How satisfied are you with the support you get from your friends? तपाई साथीहरूको सहयोगबाट कतिको सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
2.44.	How satisfied are you with the conditions of your living place? तपाईंआफ्नो बस्ने ठाउँ को अवस्थाको साथ तपाईं कति को सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
2.45.	How satisfied are you with your access to health services?	1	2	3	4	5

	तपाई स्वास्थ्य सेवाको पहुँच संग कतिको सन्तुष्ट हुनुहुन्छ?					
2.46.	How satisfied are you with your transport? तपाईं यातायात संग कतिको सन्तुष्ट हुनुहुन्छ?	1	2	3	4	5
The following question refers to how often you have felt or experienced certain things in the last four weeks. निम्न प्रश्नले तपाइँलाई पछिल्लो चार हप्तामा केहि कुराहरूको बारेमा कस्तो लाग्यो वा अनुभव गरेको छ भनेर बुझाउँछ।						
		Never कहिल्यै होइन	*Seldom* कहिले काहीँ	*Quite often* प्राय प्राय	*Very often* अक्सर	*Always* सँधै
2.47.	How often do you have negative feelings such as blue mood, despair, anxiety, depression? तपाइँसँग प्राय: उदास मन , निराशा, चिन्ता, अवसाद जस्ता नकारात्मक भावनाहरू छन्?	5	4	3	2	1

Investigator's Signature: _____ **Date**: _ _ / _ _ _ / _ _ _ _

Investigator's Name: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

MONTH 3 QUALITY OF LIFE ENTRY FORMS
MONTH 3 WHO/PBD-VISUAL FUNCTIONING QUESTIONNAIRE

1.	**Visual Functioning Questionnaire**					
The first two questions are about your overall eyesight. I will read out a choice of five answers and you will choose the one that describes you best. पहिलो दुई प्रश्नहरू तपाईंको समग्र दृष्टिको बारेमा हो।म पाँचवटा जवाफहरूको छनौट पढ्नेछु र तपाईं एक छान्नु भएको छ जसले तपाइँलाई राम्रो वर्णन गर्दछ।						
	Question	**Answer options** (Please circle the number which corresponds to the answer)				
1.1.	Overall, how would you rate your eyesight using both eyes – with glasses or contact lenses if you wear them? कुल मिलाएर, तपाईं कसरी आँखामा चश्मा वा सम्पर्कलें दुवै आँखा प्रयोग गरेर आफ्नोआँखाको मूल्याङ्कन गर्नुहुन्छ ?	*Very good धेरै राम्रो*	*Good राम्रो*	*Moderate मध्यम*	*Bad नराम्रो*	*Very bad धेरै नराम्रो*
		1	2	3	4	5
1.2.	How much pain or discomfort do you have in your eyes (e.g. burning, itching, aching)? तपाईंको आँखामा कति दुखाइ वा असुविधा छ (जलिरहेको, खुजली अनुभूति, पीडा दुखाइ)?	*None कुनै पनि छैन*	*Mild हल्का*	*Moderate मध्यम*	*Severe गंभीर*	*Extreme चरम*
		1	2	3	4	5
In the next section, I am going to ask you how much difficulty, if any, you have doing certain activities. I will read out choice of five answers and you will choose the one that describes you best.						
		None कुनै पनि छैन	*Mild हल्का*	*Moderate मध्यम*	*Severe गंभीर*	*Extreme / Cannot Do चरम / गर्न सक्दैन*
1.3.	Because of your eyesight, how much difficulty do you have in going down steps/stairs/ steep slopes? तपाईंको आँखाको कारणले, तल कदम / सीढी / खडा ढोकाहरूमा तपाईंलाई कति कठिनाइ हुन्छ?	1	2	3	4	5
1.4.	How much difficulty do you have in noticing obstacles while you are walking alone (e.g. animals or vehicles)? जब तपाईं एक्लै हिँडिरहनु भएको छ बाधाहरू हेर्दा तपाईंलाई कति कठिनाइ हुन्छ (जस्तै जनावर वा वाहनहरू)?	1	2	3	4	5
1.5.	How much difficulty do you have in seeing because of glare from bright lights उज्ज्वल रोशनीको चमकले गर्दा देख्न तपाईंलाई कति कठिनाइ हुन्छ ?	1	2	3	4	5

1.6.	Because of your eyesight, how much difficulty do you have in searching for something on a crowded shelf? तपाईंको आँखा को कारणले, भीडमा केहि खोजी गर्नको लागी तपाईंलाई कति कठिनाइलाईलाई हुन्छ ?	1	2	3	4	5
1.7.	How much difficulty do you have in seeing differences in colours? रङहरूमा भिन्नता देख्न तपाईंलाई कति कठिनाई हुन्छ ?	1	2	3	4	5
1.8.	Because of your eyesight, how much difficulty do you have in recognizing the face of a person standing near you? तपाईंको आँखाको कारणले, तपाईंलाई नजिकैको व्यक्तिको अनुहारलाई पहिचान गर्न कति कठिनाइ हुन्छ ?	1	2	3	4	5
1.9.	How much difficulty do you have in seeing the level in a container when pouring? तपाईंसँग कति कठिनाई छ एक कंटेनर मा स्तर जब डालना?	1	2	3	4	5
1.10.	Because of your eyesight, how much difficulty do you have in going to activities outside of the_house on your own (e.g. sporting events, shopping, religious events)? तपाईंको आँखाको कारणले, तपाईंलाईआफ्नै घरको बाहिर गतिविधिहरू (जस्तै खेलकुद घटनाहरू, किनमेल, धार्मिक कार्यक्रमहरू) गतिविधिहरूमा जान कति कठिनाइ हुन्छ?	1	2	3	4	5
1.11.	Because of your eyesight, how much difficulty do you have in recognizing people you know from a distance of 20 metres? (e.g. from that building/tree – give marker of 20 meters) तपाईंकोआँखाको कारणले, तपाईले 20 मीटरको दूरीबाट तपाईंले जान्नुभएका व्यक्तिहरूलाई पहिचान गर्न कत्तिको कठिनाई हुन्छ ? (जस्तै कि भवन / रूखबाट - 20 मिटरको मार्क दिनुहोस्)	1	2	3	4	5
1.12.	How much difficulty do you have in seeing close objects (e.g. making out differences in coins or notes, reading newsprint)? नजिकका वस्तुहरू हेर्नका लागि कति कठिनाई हुन्छ (जस्तै सिक्का वा टिप्पणीहरूमा मतभेदहरू बनाउने, समाचारपत्र पढ्ने)?	1	2	3	4	5

1.13.	How much difficulty do you have in <u>seeing irregularities in the path</u> when walking (e.g. potholes)? जब हिड्ने क्रममा (उदाहरणका लागि सडकमा छेदहरू)बाटोमा अनियमितताहरू देख्न तपाईंलाई कति कठिनाइ हुन्छ ?	1	2	3	4	5
1.14.	How much difficulty do you have in <u>seeing after a few moments when coming inside after being in bright sunlight</u>? केहि क्षणपछि देख्दा कत्तिको कठिनाई हुन्छ जब तपाईं उज्ज्वल सूर्यको प्रकाश बाट आउँदै हुनुहुन्छ?	1	2	3	4	5
1.15.	How much difficulty do you have in <u>doing activities that require you to see well close up</u> (e.g. sewing – not including threading the needle, using hand tools)? गतिविधिहरू गर्न तपाईलाई कत्तिको कठिनाइ हुन्छ जब तपाईं राम्ररी नजिक बाट हेर्न चाहानुहुन्छ (जस्तै सिलाई - सुई सूत्र सहित, हात उपकरण प्रयोग गर्दै)?	1	2	3	4	5
1.16.	Because of your eyesight, how much difficulty do you have in <u>carrying out your usual work</u>? तपाईंको आँखाको कारणले, सामान्य कार्य को लागी तपाईलाई कतिको कठिनाइ हुन्छ?	1	2	3	4	5
In the next section, I am going to ask you how you feel because of your vision problem. I will read out a choice of five answers and you will choose the one that describes you best.						
		Never *कहिल्यैहो इन*	*Rarely* *दुर्लभ*	*Sometimes* *कहिले काहीं*	*Often* *अक्सर*	*Very often* *प्राय*
1.17.	Because of your eyesight, how often have you been <u>hesitant to participate in social functions</u>? तपाईंको आँखाको कारणले, सामाजिक कार्यमा भाग लिन कतिको संकोच भएको छ?	1	2	3	4	5
1.18.	Because of your eyesight, how often have you found that you are <u>ashamed or embarrassed</u>? तपाईंको आँखाको कारण, तपाईंले कति पटक भेट्नुभएको छ कि तपाईं लाजमर्दो वा शर्मिला हुनुहुन्छ?	1	2	3	4	5
1.19.	Because of your eyesight, how often have you felt that you are a <u>burden on others</u>? तपाईंको आँखाको कारणले गर्दा तपाईंले कति पटक महसुस गर्नुभयो कि तपाईं अरुमा बोझ हुनुहुन्छ?	1	2	3	4	5

1.20.	Because of your eyesight, how often do you worry that you may lose your remaining eyesight? तपाईंको आँखाको कारणले, तपाई कति चिन्ता गर्नुहुन्छ कि तपाइँ आफ्नो बाँकी आँखा गुमाउन सक्नुहुन्छ?	1	2	3	4	5
1.21.	Does your vision problem affect your life in ways we have not mentioned? If YES, describe how के तपाईको दृष्टिको समस्याले तपाईको जीवनलाई असर गर्ने तरिकाहरूमा असर पार्छ जुन हामीले उल्लेख गरेको छैन? यदि हो, कसरी वर्णन गर्नुहोस्					
	Record as fully as possible the answer given जवाफ दिईएको पूर्ण रूपमा रेकर्ड गर्नुहोस्					

Investigator's Signature: _____ **Date**: _ _ / _ _ _ / _ _ _

Investigator's Name: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

MONTH 3 QUALITY OF LIFE ENTRY FORMS
MONTH 3 EQ-5D-5L FORM

Under each heading, please tick the **ONE** option that best describes your health TODAY
प्रत्येक शीर्षककोअधीनमा ,कृपया एक विकल्प छनौट गर्नुहोस्जुन तपाईंको स्वास्थ्यलाई उत्तम वर्णन गर्दछ

MOBILITY (गतिशीलता)	
6. I have no problems in walking about मलाई हिँड्न कुनै समस्या छैन	
7. I have slight problems in walking about मलाई अलि कति हिड्न समस्या छ	
8. I have moderate problems in walking about मलाई पैदल हिड्दा मध्यम समस्या छ	
9. I have severe problems in walking about मलाई हिड्न गम्भीर समस्या छ	
10. I am unable to walk about म हिड्न असमर्थ छु	

SELF-CARE (आफ्नै हेर विचार)	
5. I have no problems washing or dressing myself मलाई कुनै समस्या छैन लुगा धुन वा लुगा लगाउन	
6. I have slight problems washing or dressing myself मलाई केहि समस्या छ लुगा धुन वा लुगा लगाउन	
7. I have moderate problems washing or dressing myself मलाई मध्यम समस्या छ लुगा धुन वा लुगा लगाउन	
8. I have severe problems washing or dressing myself मलाई गम्भीर समस्या छ लुगा धुन वा लुगा लगाउन	
म असक्षम छु लुगा धुन वा लुगा लगाउन	

USUAL ACTIVITIES (सामान्यतया गतिविधिहरू) (e.g. work, study, housework, family or leisure activities) (जस्तै काम, अध्ययन, गृहकार्य, परिवार वा अवकाश गतिविधिहरू)	
6. I have no problems doing my usual activities मेरो सामान्य गतिविधिहरू गर्नमा कुनै समस्या छैन	
7. I have slight problems doing my usual activities मेरो सामान्य गतिविधिहरू गरिरहँदा केहि समस्याहरूछन्	
8. I have moderate problems doing my usual activities मेरो सामान्य गतिविधिहरू गरिरहँदा मध्यम समस्याहरू छन्	
9. I have severe problems doing my usual activities मेरो सामान्य गतिविधिहरू गरिरहँदा गम्भीर समस्याहरू छन्	
10. I am unable to do my usual activities म मेरो सामान्य गतिविधिहरू गर्न असमर्थछु	

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

PAIN / DISCOMFORT (दुखाइ / असुविधा)	
6. I have no pain or discomfort मलाई कुनै पीडा वा असुविधा छैन	
7. I have slight pain or discomfort मेरो बिस्तारै दुखाइ वा असुविधा छ.	
8. I have moderate pain or discomfort मलाई मध्यम दुखाइ वा असुविधा छ	
9. I have severe pain or discomfort मलाई कडा दुखाइ वा असुविधा छ	
10. I have extreme pain or discomfort मलाई चरम दुखाइ वा असुविधा छ	

ANXIETY / DEPRESSION (चिन्ता / अवसाद)	
6. I am not anxious or depressed मलाई कुनै चिंता वा निराश छैन	
7. I am slightly anxious or depressed म थोडा चिन्तित वा निराश हुँ	
8. I am moderately anxious or depressed मलाई मध्यम चिन्तित। वा निराश छ	
9. I am severely anxious or depressed म गम्भीर चिन्तित वा निराश हुँ	
10. I am extremely anxious or depressed म अत्यन्त चिन्तित वा निराश हुँ	

Investigator's Signature: _____ **Date**: _ _ / _ _ _ / _ _ _ _

Investigator's Name: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

MONTH 3 QUALITY OF LIFE ENTRY FORMS
PAIN IMPACT QUESTIONNAIRE

Pain Impact Questionnaire (दुखाइ प्रभाव प्रश्न)	
On a scale of 0-10, 0 being no pain at all and 10 being the most severe pain, how much pain have you felt in the last 2 weeks? 0–10 को मापन, 0 कुनै पनि दुखाइ नहुन सक्छ र 10 सबै भन्दा कडा दुखाइ हुन्छ, तपाइँले पछिल्लो 2 हप्तामा कतिको दुखाइ महसुस गर्नुभएको छ?	
1=Never, 2=Occasionally, 3=Often, 4=Constantly **1 = कहिल्यै होइन, 2 = कहिलेकाहीं, 3 = अक्सर, 4 = निरन्तर**	
In the course of your current illness, how often have you experienced eye pain? तपाईको वर्तमान रोगको समयमा, तपाईले कति पटक आँखाको दुखाइ अनुभव गर्नुभयो?	
In the course of your current illness, how often has eye pain interfered with your personal care such as bathing, eating, and dressing? तपाईको वर्तमान रोगको समयमा, कतिपय आँखाले तपाईको व्यक्तिगत हेरचाह जस्तै स्नान, खाने, र ड्रेसिङको साथमा कतिपय दुखाइको हस्तक्षेप गरेको छ?	
In the course of your current illness, how often has eye pain disturbed your sleep? तपाईको वर्तमान रोगको समयमा, कतिपय आँखाले तपाईको निद्रालाई कष्ट बनाएको छ?	
In the course of your current illness, how often has eye pain interfered with your household work such as cooking, house cleaning, washing cloth, fetching water, fetching firewood, caring to other family members? तपाईको हालको बिरामीको समयमा, कहिलेकाहीं आँखाले दुखाइको साथ तपाईको परिवारको साथ हस्तक्षेप गर्दछ जस्तै खाना पकाउने, घरको सफाई, कपडा धोएर कपडा, पानी ल्याउने, फन्धुवाट ल्याउने, अन्य परिवारका सदस्यहरूलाई हेरचाह गर्ने?	
In the course of your current illness, how often has eye pain affected your agricultural or paid work? तपाईको वर्तमान रोगको समयमा, कतिपय आँखाले तपाईंको कृषि वा सशुल्क कामलाई असर गरेको छ?	
In the course of your current illness, how often has eye pain affected your participation in social activities such as attending weddings, social meetings, and funerals? तपाईको हालको बीमारीको समयमा, कहिलेकाँही आँखाले दुखाइले सामाजिक गतिविधिहरूमा तपाईको सहभागितालाई प्रभावित गरेको छ जस्तै विवाह, सामाजिक सभा र रौतहटमा सहभागी हुन सक्छ?	

Investigator's Signature: _____ **Date**: _ _ / _ _ _ / _ _ _

Investigator's Name: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

3 MONTH QUALITY OF LIFE ENTRY FORMS
COST-EFFECTIVENESS DATA ENTRY FORM

Number of non-study visits since enrolment: _____

Number of internist visits related to study: _____

Total number of days in-hospital related to study: _____

How many hours of work did the patient miss because of the eye infection? (Record only working hours missed) _____

How much money did you spend on a return trip to the hospital for visits related to your eye infection? _____

Did someone have to miss work in order to help take care of your eye? (i.e., accompany you on hospital visits, in-home eye care, etc.) **YES / NO**

What is their occupation? _____

How many hours of work did they have to miss? _____

Investigator's Signature: _____ **Date:** __ __ / __ __ __ / __ __ __ __

Investigator's Name: _____

TRIAGE NO. T | | | | | STUDY NO. S | | | | | ENROLMENT DATE _ _ / _ _ _ / _ _ _ _

STUDY COMPLETION

Final quality of life questionnaires completed: ☐ Yes ☐ No

Did the subject complete the study? ☐ Yes ☐ No

Date of completion: _ _ / _ _ _ / _ _ _ _
(DD / MMM / YYYY)

If no, specify reason:

_____

_____

Investigator's Signature: _____ **Date:** _ _ / _ _ _ / _ _ _ _
(DD / MMM / YYYY)

Investigator's Name: _____

TRIAGE NO. T | STUDY NO. S | ENROLMENT DATE __ / ___ / ____

BEST SPECTACLE CORRECTED (BSCVA) VISUAL ACUITY TESTING FORM

PID:		Today's Date:	
TIME POINT:	□ Baseline □ 3 months	ACUITY WITH:	□ Refraction □ Pin-hole
Eye tested:	□ Right eye □ Left eye	□ Study eye □ Fellow (non-study) eye (baseline only)	

NB Use a new, separate form for each eye being tested. Only test the fellow (non-study) eye at baseline

CIRCLE each letter the subject identifies correctly and write the total correct for each row in the column at the right. If 3 letters or fewer are read correctly on any row from Row 3 or below, STOP TESTING on that row. If the total number of letters read correctly at 3 metres is less than 10, add a +1.00 sphere to the distance correction, then have the subject read the first 6 rows of the letters again at 1 metres.

SECTION 1 = 3 METRE TEST (MUST TRY FIRST TWO LINES)

Row	Snellen equiv	Tumbling Es	Number correct at 3m	Log MAR equiv
1	6/60		_____	1.0
2	6/48		_____	0.9
3	6/38		_____	0.8
4	6/30		_____	0.7
5	6/24		_____	0.6
6	6/19		_____	0.5
7	6/15		_____	0.4
8	6/12		_____	0.3
9	6/9.5		_____	0.2
10	6/7.5		_____	0.1
11	6/6		_____	0.0
12	6/4.8		_____	-0.1
13	6/3.8		_____	-0.2
14	6/3		_____	-0.3

Total number correct at 3m _____

If <10, proceed to 1 m testing

Refraction: _____ +/- _____ x____

Or plano □

Please circle + or - cylinder

SECTION 2 = 1 METRE TEST ADD +1.00 SPHERE (MUST TRY FIRST TWO LINES)

□ Done □ Not done

Row	Snellen equiv	Tumbling Es	Number correct at 1m	Log MAR equiv
1	6/242		_____	1.6
2	6/194		_____	1.5
3	6/152		_____	1.4
4	6/121		_____	1.3
5	6/97		_____	1.2
6	6/76		_____	1.1

Total number correct at 1 m _____

If <10, proceed to low vision testing

SECTION 3: LOW VISION TESTING

□ Done □ Not done

If visual acuity is not measurable, please test:

Count finger (CF)	□ Yes	□ No	*If no test for HM*
Hand motion (HM)	□ Yes	□ No	*If no test for LP*
Light perception (LP)	□ Yes	□ No	

SNELLEN EQUIVALENT:
(Most difficult line with 4 or 5 correct) _ / _ _

□ CF □ HM □ LP □ NPL

Signature: _____

Initials: _____

SCORING TO BE PERFORMED BY PROJECT CO-ORDINATOR USING FORM ON FOLLOWING PAGE

TRIAGE NO.	T					STUDY NO.	S					ENROLMENT DATE	_ _ / _ _ _ / _ _ _ _

BEST SPECTACLE CORRECTED (BSCVA) VISUAL ACUITY SCORING FORM

After each measurement of visual acuity, the score for the visit can be calculated. This will be completed by the project co-ordinator. The visual acuity score is defined as follows:

- If 10 or more letters are read correctly at 3m, the 3m visual acuity score is equal to the number of letters read correctly at 3m plus 30.
- If 3 or fewer letters of the largest line are read correctly at 3m, the visual acuity score is equal to the number of letters read correctly at 1m.
- If 4 – 9 letters are seen at 3m, the score from 3m is added to the score from 1m.

If 3 or fewer letters are read at 1m, then low vision testing must be performed
The highest attainable 3-metre visual acuity score is 100.

Letters seen at **3 m**	10 or more	4-9 letters	3 or less
Write letter score at 3m (Score A)			
Write letter score at 1m (Score B)	30		
TOTAL (Score A + Score B)			

If score is 3 or less, low vision testing should have been performed. Record the result of that here:

□ CF □ HM □ LP □ NPL

Calculate logMAR for analysis:

1.7 – (0.02 x letter score above) ***or*** *as per the low vision table*

Notation	LogMAR
NPL	2.0
PL	1.9
HM	1.8
CF	1.7

logMAR score:

Eye tested:	□ Right eye □ Left eye	□ Study eye □ Fellow (non-study) eye (baseline only)

Appendix 4: Journey History Completion Form

Triage Number:						
Journey Number	**Date**	**From**	**To**	**Distance in Km**	**Transport Cost (NRS)** **(If patient comes from India convert IC to NRS)**	**Mode of Transport** **List all that apply, separated by commas**
		1 = Home, 2 = Pharmacy, 3 = Health Post, 4 = Private Clinic 5 = Government Hospital 6 = Private Hospital 7 = Traditional healer's clinic, 8 = ECC 9 = SCEH 99 = don't know				1 = Walking, 2 = Auto Rickshaw 3 = Cycle 4 = Motorcycle 5 = Shared car / Scorpio 6 = Shared minibus 7 = Bus 8 = Private car 9 = Ambulance 99 = don't know / can't remember
1						
2						
3						
4						
5						
6						
8						
9						
10						
11						
12						

Appendix 5: Microbiology request form

CHX NATA MICROBIOLOGY FORM – CORNEAL TISSUE SPECIMENS

Patient ID (PID): Age (y): _____

Specimen time point: ☐ Enrolment ☐ Repeat examination

Triage number (T-XXXX): **Study number (S-XXXX):**

MICROSCOPY

Gram stain:

Gram-positive cocci ☐ Gram-positive bacilli ☐

Gram-negative cocci ☐ Gram-negative bacilli ☐

Fungal hyphae ☐ Yeast cells ☐

Calcofluor white:

Fungal hyphae ☐ Yeast cells ☐

Lactophenol cotton blue:

Fungal hyphae ☐ Yeast cells ☐

CULTURE

FBA + ++ +++ ++++ NSG AOG

Date: __/__/____

Organism type: _____

HBA + ++ +++ ++++ NSG AOG

Date: __/__/____ ☐ ☐

Organism type: _____

BHIB Growth ☐ No growth ☐

Date __/__/____

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial agent	Susceptibility	
Moxifloxacin	S	R
Ampicillin	S	R
Chloramphenicol	S	R
Gentamicin	S	R
Ceftazidime	S	R

Bacteriology report:

Date: __/__/____

Mycology report:

Date: __/__/____

Report completed by:

(signature)

KEY: NSS – Nothing significant seen NSG – No significant growth AOG – Absence of growth

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Observational / Interventions Research Ethics Committee

Prof Matthew Burton
Professor of International Eye Health
Department of Clinical Research (CRD)
Infectious and Tropical Diseases (ITD)
LSHTM

4 April 2018

Dear Matthew

Study Title: Randomised controlled trial of topical chlorhexidine 0.2% versus topical natamycin 5% for fungal keratitis in Nepal

LSHTM Ethics Ref: 14841

Thank you for responding to the Interventions Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Other	eGCP (Secondary Care)_Introduction to Good Clinical Practice eLearning Certificate	13/01/2017	1
Information Sheet	3. Participant Information and Consent Form 2 - Nepal	26/01/2018	1
Protocol / Proposal	4. Data Collection Appendix	27/01/2018	1
Protocol / Proposal	1. Protocol - v1 - Nepal	28/01/2018	1
Investigator CV	Matthew Burton - Short CV - 2017	31/01/2018	1
Safety Information	Investigator Brochure	31/01/2018	1
Safety Information	Investigators Brochure Annexes	31/01/2018	1
Investigator CV	Jeremy Hoffman Ethics CV 2018- CHX NATA	31/01/2018	1
Sponsor Letter	2018-KEP-009_Sponsor confirmation_Nepal	31/01/2018	1
Covering Letter	14841 Response Letter	22/03/2018	1
Information Sheet	2. Participant Information and Consent Form 1 - v1.1 - Nepal	22/03/2018	1.1.

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

[redacted]

Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Improving health worldwide

Appendix 7: Nepal Health Research Council (NHRC) Ethical Approval

Government of Nepal

Nepal Health Research Council (NHRC)

Estd. 1991

Ref. No : 1937

14 January 2019

Dr. Reena Yadav
Principal Investigator, Sagarmatha Chaudhary Eye Hospital, Lahan, Nepal

Prof. Matthew John Burton
Principal Investigator, London School of Hygiene and Tropical Medicine

Ref: **Approval of research proposal entitled Randomised controlled trial of topical chlorhexidine 0.2% versus topical natamycin 5% for fungal keratitis in Nepal**

Dear Dr. Yadav and Prof. Burton,

It is my pleasure to inform you that the above-mentioned proposal submitted on **14 September 2018 (Reg. no. 613/2018)** please use this Reg. No. during further correspondence) has been approved by Nepal Health Research Council (NHRC) Ethical Review Board on **12 December 2018.**

As per NHRC rules and regulations, the investigator has to strictly follow the protocol stipulated in the proposal. Any change in objective(s), problem statement, research question or hypothesis, methodology, implementation procedure, data management and budget that may be necessary in course of the implementation of the research proposal can only be made so and implemented after prior approval from this council. Thus, it is compulsory to submit the detail of such changes intended or desired with justification prior to actual change in the protocol. Expiration date of this proposal is **December 2019.**

If the researcher requires transfer of the bio samples to other countries, the investigator should apply to the NHRC for the permission. The researchers will not be allowed to ship any raw/crude human biomaterial outside the country; only extracted and amplified samples can be taken to labs outside of Nepal for further study, as per the protocol submitted and approved by the NHRC. The remaining samples of the lab should be destroyed as per standard operating procedure, the process documented, and the NHRC informed.

Further, the researchers are directed to strictly abide by the National Ethical Guidelines published by NHRC during the implementation of their project proposal and **submit progress report in between and full or summary report upon completion.**

As per your research proposal, the total research amount is **NRs 83,90,200** and accordingly the processing fee amounts to **NRs 2,51,706.** It is acknowledged that the above-mentioned processing fee has been received at NHRC.

If you have any questions, please contact the Ethical Review M & E Section at NHRC.

Thanking you,

[redacted]

Prof. Dr. Anjani Kumar Jha
Executive Chairperson

Tel: +977 1 4254220, Fax: +977 1 4262469, Ramshah Path, PO Box: 7626, Kathmandu, Nepal
Website: http://www.nhrc.gov.np, E-mail: nhrc@nhrc.gov.np

Government of Nepal

Nepal Health Research Council (NHRC)

Estd. 1991

Ref. No.: 127

22 July 2022

Dr. Reena Yadav

Principal Investigator, Sagarmatha Chaudhary Eye Hospital, Lahan

Prof. Matthew John Burton

Principal Investigator, London School of Hygiene and Tropical Medicine

Subject: **Approval of the requested amendment** for the study entitled **Randomised controlled trial of topical chlorhexidine 0.2% versus topical natamycin 5% for fungal keratitis in Nepal (Reg. no. 613/2018, Approved on 12 December 2018)**

Dear Dr. Yadav and Prof. Burton,

The meeting of the Expedited Review Sub-Committee of Nepal Health Research Council held on 19 July 2022 discussed the amendment requested on 12 July 2022. The meeting has approved the amendment for the extension of the study till September 2022 to send samples for analysis to the London School of Hygiene and Tropical Medicine and to complete remaining activities of the study.

Note: Please adhere with the timeline mentioned in the approval letter. Any communication regarding the study will not be entertained after the completion of the timeline.

If you have any queries, please feel free to contact the Ethical Review M & E Section of NHRC.

Thanking you!

[redacted]

Dr. Pradip Gyanwali

Member Secretary

Tel: +977 1 4254220, Fax: +977 1 4262469, Ramshah Path, PO Box: 7626, Kathmandu, Nepal
Website: http://www.nhrc.gov.np, E-mail: nhrc@nhrc.gov.np

Appendix 8: Nepal Department of Drug Administration (DDA) Approval

Schedule-13
(Relating to Sub-Rule (2) of Rule 8)
Government of Nepal
Ministry of Health
Department of Drugs Administration

License for clinical trials

This license is hereby issued, setting out the following matters, allowing the following person to conduct clinical trial of the following new drug, subject to the Drugs Act, 2035 (1978) and the Drugs Registration Rules, 2038 (1981).

1. Of the new drug licensed for clinical trial:

Name	System	Group or Subgroup	Composition	Type or Kind	Active ingredient's		Remarks
					Name	Quantity	
CHLORHEXIDINE 0.2 % EYE DROPS	ALLOPATHY	KHA	CHLORHEXIDINE DIGLUCONATE	ANTI-MICROBIAL AGENT	CHLORHEXIDINE DIGLUCONATE	0.2 % (2 MG)	
NATAMYCIN 5 % OPTHALMIC SUSPENSION	ALLOPATHY	KHA	NATAMYCIN	ANTI-FUNGAL AGENT	NATAMYCIN	5 % (50 MG)	

2. Of the disease licensed for clinical trial:
(a) Name: FUNGAL KERATITIS
(b) Method of diagnosis: ACUTE MICROBIAL KERATITIS (CLINICAL DIAGNOSIS) WITH EVIDENCE OF FUNGAL INFECTION (EITHER POSITIVE MICROSCOPY OR FUNGAL HYPHAE VISIBLE ON *IN VIVO* CONFOCAL MICROSCOPY)
3. of the consumption of the new drug to be administered in the course of clinical trial:
(a) Method: SINGLE MASKED PROSPECTIVE RCT
(b) Mode: TOPICAL APPLICATION TO THE EYES
(c) Dosage (daily): INITIALLY HOURLY DAY AND NIGHT FOR THE FIRST WEEK, THEN TWO HOURLY FOR TWO WEEKS
(d) Period: ONE YEAR
4. Mode of clinical trial: CHLORHEXIDINE 0.2% IS NON-INFERIOR TO NATAMYCIN 5% IN PARALLEL TWO-ARM, SINGLE MASKED RCTs.
5. Place where clinical trial is to be conducted: SAGARMATHA CHOUDHARY EYE HOSPITAL, LAHAN, SIRAHA, NEPAL.
6. Of the person allowed conducting clinical trial:
(a) Name, surname and address: DR. REENA YADAV (NMC NO.9182)
(b) Occupation: OPTHALMOLOGIST
(c) Qualifications: MD OPTHALMOLOGY
7. Validity period of license: DECEMBER 2019
License receiver's: DR. REENA YADAV
Signature:
Date:

License issuing officer's:
Signature: [redacted]
Name: PAN BAHADUR KSHETRY
Date: 25 MARCH 2019
Designation: SENIOR DRUG ADMINISTRATOR

Senior Drug Administrator
10th Level

Appendix 9: Deterioration whilst on treatment and treatment failure standard operating procedure

Title:	Deterioration whilst on treatment and treatment failure		
SOP Ref:	RCT CHX-NATA/SOP/28		
Version:	Final v 1.1		
Author:	Dr Jeremy Hoffman	Title:	Clinical Research Fellow
Effective Date:	28/01/19	Review by:	Prof. Matthew Burton
Approved by:	Prof. Matthew Burton	Date:	30/07/19
Signature of Authoriser			

SOP Chronology			
Version	**Date**	**Reason for Change**	**Author**
1.1	30/7/19	Increased detail and added flow chart	JH
1.2	27/11/19	Removed intra-stromal injections as an option and clarified when to use oral treatment	JH

Purpose

This SOP (Standard Operating Procedure) defines clinical deterioration whilst on treatment as well as treatment failure at the end of the study, and what happens in either of these instances.

Introduction

Clinical deterioration is known to occur despite prompt treatment with topical anti-fungals in up to 30% of cases. We define clinical deterioration as an increase in the ulcer size, ulcer depth and / or perforation despite compliant treatment for at least one week with the study medication (either CHX or NATA). Treatment failure is defined as perforation at any time during the study or a persistent epithelial defect of > 0.5mm at the three-month follow-up.

Procedure in the event of clinical deterioration

If there are objective signs of clinical deterioration as defined above and confirmed by the treating ophthalmologist or study co-ordinator, the following protocol should be followed:

1. The patient's compliance with the medication must be confirmed. Poor compliance must be addressed with counselling. If the patient is not currently admitted, admission is strongly encouraged.
2. Ocular hygiene of the patient is assessed. If it is poor, the patient must be counselled on how to clean the eye appropriately. If the patient is admitted, the ward staff can assist in cleaning the eyes.
3. Any previous microbiological cultures should be reviewed at this point as this may provide evidence to an initial mixed infection at baseline. Sensitivities to anti-microbials should be reviewed where available.
4. A repeat battery of corneal scrapes should be taken including microscopy and culture. Follow the procedure below for "mixed infection deterioration" should bacteria be identified on gram stain. In the absence of a mixed infection, follow the procedure below for "non-mixed infection treatment failure".
5. All deteriorating cases should be discussed with the management team before changing any medications.
6. Change in medication can only be made by the supervising ophthalmologist.
7. All changes in medication need to be documented using the Medication Change Form (below, SOP28A).

Mixed infection deterioration

N.B. The below should only be followed in the event of definitive evidence of a mixed bacterial-fungal infection e.g. bacteria reported on microscopy or culture.

- First-line anti-microbial treatment is g. moxifloxacin applied hourly for a minimum of 48 hours. The intensity can be reduced depending on clinical response at the discretion of the treating ophthalmologist.
- In cases that are **deteriorating rapidly, resistant to the first-line agent on sensitivity testing** or failing to respond to g. moxifloxacin after a **minimum of 48 hours**, dual therapy ("fortified") can be given. This should be an aminoglycoside **and** a cephalosporin (ideally 2nd or 3rd generation). At present, the available options at SCEH are g. gentamycin and g. cefazolin (note g. cefazolin is a first-generation cephalosporin). It should be noted that these are not manufactured as per GMP: both are made in the clinic from diluting IV preparation *powder for injection* and are not licensed for ocular use. Quality assurance including sterility of these medications has not been performed or checked. It is therefore advised that these are second-line preparations that should only be given if treatment with first-line, licensed, anti-microbials has failed or is deemed inadequate.
- Patients who have been started on an anti-microbial should be reviewed at 48 hours
- Culture results (and sensitivities if available) should be reviewed at this point

- If there is progressive clinical deterioration, the first-line antimicrobial can be switched to the second-line (fortified) regimen. If there is deterioration whilst on the fortified regime, g. moxifloxacin can be added
- There is no role for oral anti-biotics, unless the eye has perforated

Non-mixed infection deterioration

The choice of the "rescue" anti-fungal in this study is difficult due to the lack of evidence. Indeed, natamycin 5% has been shown to be more effective than topical voriconazole. Amphotericin B is a possible alternative, although evidence for its use is lacking and it is currently not licensed or available in Nepal. The clinician should bear this in mind as patients who are deteriorating are unlikely to improve with medical treatment alone. However, despite these limitations the following should be followed:

- An additional anti-fungal should not be started before the day 7 review.
- First-line should be g. voriconazole 1%. It should be noted that this is not prepared as per GMP. The frequency should be hourly for 48 hours minimum, and then reduced at the discretion of the clinician, usually then 2-hourly until the epithelium has healed.
- If the ulcer is more than 75% deep and deteriorating at or after 7 days from enrolment, then oral ketoconazole 200mg twice daily with meals or oral voriconazole 200mg twice daily two hours after meals should be started. Liver function tests should be started before treatment and monitored over the course of the treatment. If the liver function tests are showing a deterioration, then the oral treatment should be stopped immediately.
- If the patient continues to deteriorate after one week of additional treatment, then TPK should be performed.
- If at any time the patient perforates, TPK should be performed when possible.
- If there is a descemetocele or small perforation, corneal gluing can be performed and the patient monitored closely. If the perforation worsens, then TPK should be performed when possible.

In the event that this happens, the Medication Change Log form (Appendix SOP28A) must be completed.

The flow chart below summarises the steps to follow.

Procedure in the event of treatment failure

If the patient perforates, then they can undergo TPK surgery at the discretion of the treating ophthalmologist and providing the facilities and corneal tissue is available.

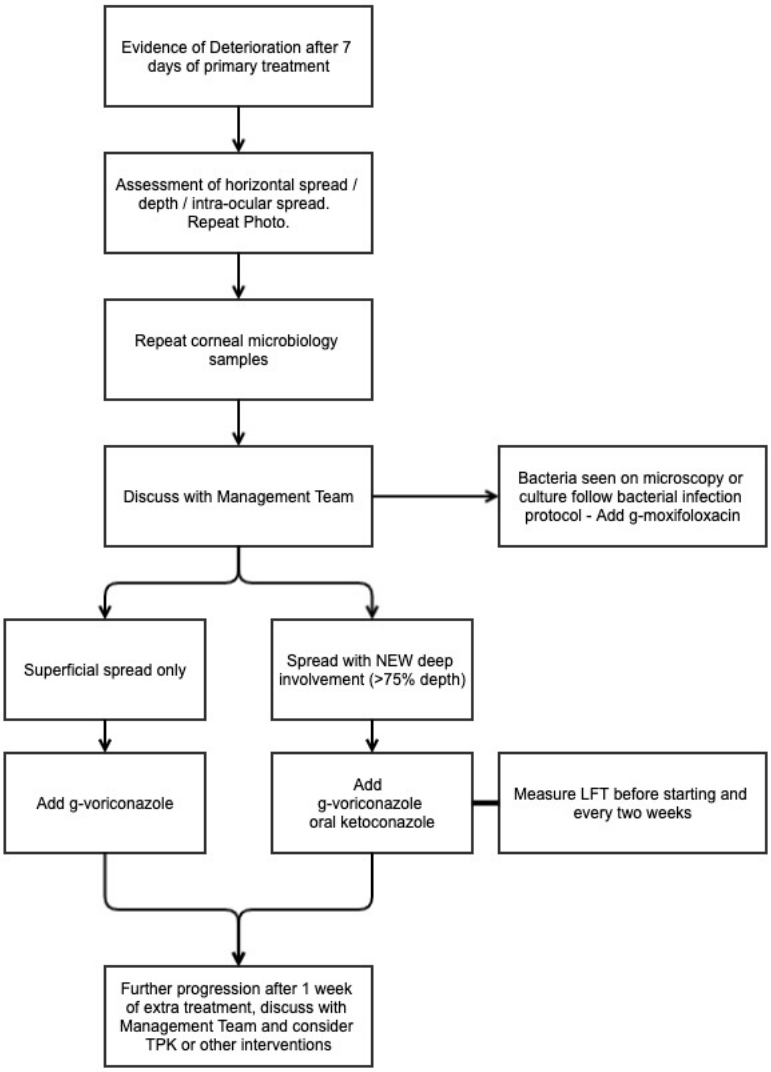

Figure 1: Flow chart of what to do if there is clinical deterioration

Appendix 10: Sample size calculation for the randomised controlled trial of topical chlorhexidine 0.2% vs. topical natamycin 5% for the treatment of fungal keratitis in Nepal

The study is powered to test the hypothesis that CHX is non-inferior to NATA in terms of the primary outcome (BSCVA at 3 months) at a pre-specified non-inferiority margin (Delta) of 0.15 logMAR units. It is possible that CHX is non-inferior to NATA, given pilot RCT data that showed no statistically significant difference between CHX and NATA, with a Cochrane review finding a non-significant trend favouring CHX over NATA.[1-3] Despite this trend favouring CHX, we have chosen to carry out a non-inferiority trial rather than a superiority trial as this is a more clinically pragmatic approach. It would be counter-productive to conduct a superiority trial and find no statistically significant difference between the two treatments leading clinicians to potentially disregard CHX as a treatment, when in fact it may be 'non-inferior" to NATA, and be the only available or most cost-effective treatment.

The choice of Delta: This clinically meaningful difference of 0.15 logMAR was chosen as a difference of 0.15 logMAR corresponds to approximately 1.5 lines on a Snellen chart; any difference greater than this is clinically significant, as a difference of less than 0.15 log MAR could potentially be accounted for by testing / re-testing error.[4] Furthermore, it was used in the MUTT1 trial, providing methodological consistency between studies.[5] In addition, previous studies have suggested treated ulcers improve at a mean of four Snellen lines from baseline.

Sample size was calculated for 90% power and adjusted final alpha of 0.0492, taking account of a single interim analysis using O'Brien-Fleming approach to maintain type-1 error rate of 5%. A sample size of 452 is required to detect a non-inferiority margin of 0.15-logMAR in BSCVA 3-months after enrolment between arms, assuming 0.5 SD for 3-month BSCVA and 15% dropout.

However, given approximately 10% of infections are mixed and these will be excluded from the primary analysis, 500 patients will be recruited. This sample size provides 90% power to detect superiority in BSCVA at 3-months if there is ≥0.17 LogMAR units difference as a secondary analysis.

This was given by the following formula and calculation:[6]

$n = f(\alpha, \beta) \times 2 \times \sigma 2 / d2$

where σ is the standard deviation, and

$f(\alpha, \beta) = [\Phi\text{-}1(\alpha) + \Phi\text{-}1(\beta)]2$

Φ-1 is the cumulative distribution function of a standardised normal deviate.

Alpha = 1-0.0492 = 0.9508

Beta = 0.9

Therefore f(alpha, beta) = 8.61

N = 8.61*2*(0.5^2)/(0.15^2) = 192 per group = 384 overall

Given 15% loss to follow up, N = 384/(1-0.15) = **452**

References:

1. Rahman MR, Minassian DC, Srinivasan M, Martin MJ, Johnson GJ. Trial of chlorhexidine gluconate for fungal corneal ulcers. *Ophthalmic Epidemiology* 1997; **4**(3): 141-9.
2. Rahman MR, Johnson GJ, Husain R, Howlader SA, Minassian DC. Randomised trial of 0.2% chlorhexidine gluconate and 2.5% natamycin for fungal keratitis in Bangladesh. *British Journal of Ophthalmology* 1998; **82**(8): 919-25.
3. FlorCruz NV, Evans JR. Medical interventions for fungal keratitis. *The Cochrane database of systematic reviews* 2015; **4**: CD004241.
4. Bastawrous A, Rono HK, Livingstone IAT, et al. Development and Validation of a Smartphone-Based Visual Acuity Test (Peek Acuity) for Clinical Practice and Community-Based Fieldwork. *JAMA Ophthalmology* 2015; **133**(8): 930-8.
5. Prajna NV, Krishnan T, Mascarenhas J, et al. The mycotic ulcer treatment trial: a randomized trial comparing natamycin vs voriconazole. *JAMA Ophthalmology* 2013; **131**(4): 422-9.
6. Sealed Envelope Ltd. Power calculator for continuous outcome non-inferiority trial. 2012. https://www.sealedenvelope.com/power/continuous-noninferior/

Appendix 11: Method for preparing chlorhexidine 0.2% eye drops

One of the advantages of chlorhexidine 0.2% eye drops is the ease and low cost of how it can be prepared. Below is an outline of how chlorhexidine 0.2% eye drops can be produced at a hospital pharmacy. Further details, including results of stability and sterility testing of test batches produced at one of our facilities in Uganda, will be published as open access in a peer-reviewed journal in due course, and falls beyond the scope of this PhD.

Eye Drop Production Department at Ruharo Mission Hospital, Uganda

Distilled water was produced onsite by a fixed distillation unit. For eye-drop production, distilled water was collected within 2 hours of production into a clean plastic container. All containers used for the preparation of the eye-drop solutions were rinsed three times with distilled water before use by filling with distilled water and discarding.

Acetate buffer was prepared by the addition of sodium acetate (141.4 mM) and acetic acid (7.8 mM) in a mixing bucket. The pH was measured using a portable pH meter (Hanna Halo 12302) and adjusted by dropwise addition of sodium hydroxide (10 M) to reach pH 6.75. CHX 0.2% (100-mL batches) was prepared by dilution of chlorhexidine digluconate (1 mL) in a volumetric flask to a volume of 100 mL. The solution was shaken to mix and filtered through a grade-3 sinter aided by a hand vacuum pump. The solution was dispensed using a Pressmatic bottle top dispenser in 10-mL portions into amber glass bottles. Lids were screwed on firmly by hand, and the bottles sterilized by a water steam bath at 100°C and atmospheric pressure for 30 minutes. Three batches were made in this way, and the first, middle, and last bottles of each batch were sent for sterility testing.