Reliability of clinical signs in diagnosis of fungal keratitis

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ABSTRACT

Objective: To determine clinical signs predictive of fungal Keratitis (MK) in Uganda.
Methods: We prospectively recruited patients presenting with MK at two main eye units in Southern Uganda between December 2016 and March 2018. We collected information on clinical history and presentation and microbiology. Clinical signs predictive of a positive microbiological diagnosis of fungal keratitis were analyzed in a multi variable logistic regression model.
Results: Three hundred and thirteen individuals were enrolled. Median age was 47 years (range 18-96 years) and 174 (56%) were male. Trauma was reported by 29% and use of traditional eye medicine by 60%. Majority presented with severe infections (median infiltrate size 5.2 mm); 47% were blind in the affected eye (vision <3/60). Microbiology results were available in 265/313 (84.7%) participants. Overall, most infections were fungal (49%), 10% were bacterial and 4% were mixed (fungal and bacterial). Ninety-seven (37%) of the corneal scrapping samples were negative on both microscopy and culture. Presence of a slough (aOR 3.58, 95% CI [1.60-8.04], p=0.002), a serrated infiltrate margin (aOR 1.58, 95% CI [1.00-2.51], p=0.051), satellite lesions (aOR 2.90, 95% CI [1.65-5.11], p<0.0001) and a hypopyon (aOR 3.24, 95% CI [1.78-5.90], p<0.0001) were associated with a positive microbiology result for fungal keratitis.
Conclusion: This study conducted in a predominantly African population provided clues to support clinicians in making a diagnosis of fungal keratitis in settings where there is no microbiology support.

Key words: Microbial keratitis, Fungal keratitis, Clinical diagnosis, Microbiology

INTRODUCTION

Microbial Keratitis (MK) can be caused by a range of pathogens including; bacteria, viruses, protozoa, and fungi. It is characterized by acute or sub-acute onset of pain, conjunctival hyperemia and corneal ulceration with a stromal inflammatory cell infiltrate[1].

MK has been described as a "silent epidemic", which leads to substantial morbidity, related to blindness, pain and stigma[2]. It is the leading cause of unilateral blindness after cataract in tropical regions estimated at 2 million cases of monocular blindness per year[3]. MK frequently leads to sight-loss from dense corneal scarring, or even loss of the eye, especially when the infection is severe and/or appropriate treatment is delayed.

Our previous work in Uganda showed that the majority of MK is caused by filamentous fungi[4]. Compared to other infections, patients with Fungal Keratitis (FK) were more likely to have a worse outcome[4]. A good outcome depends on early identification of the causative organism so that appropriate treatment can be initiated[5,6]. However, in many Low and Middle-Income Countries (LMIC), good microbiology diagnostic support is not readily available[7]. Even in good "Centres of excellence", a microbiology diagnosis is negative in about 1/3 of the patients[4,8,9].

In many settings therefore, clinicians need to rely on clinical signs to confidently make a diagnosis. Reports from India and Ghana have described clinical signs that are predictive of fungal/bacterial keratitis with variable rates of reliability[9–11]. In this report, we describe the clinical signs that were predictive of a microbiology diagnosis of fungal keratitis in a cohort in Uganda.

MATERIALS AND 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 10647), Mbarara University Research Ethics Committee (Ref 10/04-16) and Uganda National Council for Science and Technology (Ref HS-2303). 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: In this cohort, we prospectively enrolled patients with MK that presented to Ruharo Eye Centre (REC) and Mbarara University and Referral Hospital Eye Centre (MURHEC) from December 2016 to March 2018. These are the two tertiary eye hospitals in Mbarara, Uganda.

Study participants: MK was defined as loss of corneal epithelium (of at least 1mm diameter) with underlying stromal infiltrate, associated with any or all signs of inflammation (conjunctival hyperemia, anterior chamber inflammatory cells, +/- hypopyon)[12]. We excluded those not willing to participate, those not willing to return for follow-up, pregnant women, lactating mothers, and those aged below 18 years.

Assessment: We documented demographic information and ophthalmic history, presenting Log MAR (Logarithm of Minimum Angle of Resolution) visual acuity at 2 meters in a dark room was measured using Peek Acuity software[13]. Participants were examined with a slit lamp to assess the anterior segment using a structured protocol, including eyelid assessment, corneal ulcer features, anterior chamber (flare, cells, hypopyon shape and size) and perforation status. Infiltrate size was determined from the greatest diameter of the infiltrate (major axis) and the widest perpendicular diameter (minor axis)[12]. The final infiltrate size was then derived as the geometric mean of these two diameters[12]. The same was repeated after fluorescein staining of the ulcer to determine epithelial defect sizes. High-resolution digital photographs with and without fluorescein staining were taken with a Nikon SLR 7200 digital camera with Macro lens.

Corneal scrape specimens were collected from the ulcer at a slit lamp or an operating microscope, using 21G needles after application of a proxymetacaine (minims) anaesthetic eye drops. Samples underwent processing for the Gram stain, Potassium Hydroxide [KOH] stain, Calcofluor White [CFW] stain and direct inoculation on culture media (Sheep's Blood Agar [BA], Chocolate Agar [HBA], Potato Dextrose Agar [PDA] and Brain Heart Infusion broth [BHI]).The choice of microbiological investigations and culture media used was to maximise isolation of fungus and bacteria.

Two sterile corneal swab samples were taken for pan fungal gene sequencing at a reference laboratory at Kilimanjaro Christian Medical Centre, Moshi, Tanzania. The number of corneal samples were dependent on how much material could be safely scraped from the cornea. The order was samples for microscopy, agar, broth and finally corneal swabs.

Microscopy, culture and antimicrobial sensitivity work was done at the Mbarara University Department of Microbiology. The technician underwent initial training in ocular microbiology at the Aravind Eye Hospital System, Department of Ocular Microbiology in Madurai, India and had a site supervision visit by a mycologist from the London School of Hygiene & Tropical Medicine. Immediate CFW staining was also done in the side lab at MURHEC on a fluorescein microscope (Zeiss Primostar ILED) by the attending ophthalmologist. Agar plates and broths were incubated and read daily at 35-37°C for bacteria for up to 7 days and at 25°C for up to 21 days for fungi. Organism identification and sensitivity testing (MIC/zone of inhibition) were performed using standard microbiological techniques. We followed a previously described approach for reporting positive microbiology results[8]. Briefly, bacteria were identified using routine biochemical identification tests. Identification of fungi was according to the macroscopic appearance of cultures on potato dextrose and microscopic appearance of conidia and spore bearing structures. Positive culture was growth at the site of inoculation or growth on one solid medium consistent with microscopy; or semiconfluent growth at the site of inoculation on one solid medium (if bacteria); or growth of the same organism on repeated scraping. If, by microscopy, hyphae were observed in corneal tissue, but failed to grow in culture, the causative organism was reported as fungal.

Analysis: Data were analyzed in STATA v14.The outcome of interest in this analysis was a positive microbiology result of fungal keratitis. A logistic regression modelling was done for baseline clinical features associated with a positive microbiology result for fungal keratitis. Variables with a p-value less than 0.05 were initially included in the multivariable model. A backward stepwise approach was then used until only the variables with a p-value of less than 0.05 were retained. Adjusted ORs were reported for the final model.

RESULTS

Participants: The baseline characteristics of the patients have been previously presented[14]. Briefly, the median age was 47 years (IQR 35-60, total range 18-96 years), and the majority (56%) were male. The main occupation was farming (70%).

Clinical features: Table 1 shows the clinical features at presentation, including detailed characteristics of the ulcers and microbiology results. Specimen for microbiology was collected in 265 patients. Due to limited amounts of sample material, it was not possible to perform all tests on all those sampled. Almost half of the participants (47%) had a visual acuity of less than 3/60 (blind) in the affected eye at presentation.

Table 1: Clinical features and diagnosis at presentation (n=313)

Variable	Median	(IQR [Total Range])
Infiltrate size (mm)*	5.2	(3.3-7.7 [0.5-13])
Epithelial defect size (mm)*	3.9	(2.4-6.5 [0-14])
Variable	n/313	(%)
Snellen Visual Acuity in affected eye (n=312)		
6/5-6/18	102	(33%)
6/24-6/60	42	(12%)
5/60-3/60	24	(8%)
2/60-1/60	33	(11%)
Counting fingers-light perception	103	(33%)
No Light Perception	9	(3%)
Snellen visual acuity in non-affected eye (n=313)		
6/5-6/18	278	(89%)
6/24-6/60	16	(5%)
5/60-3/60	2	(0.6%)
2/60-1/60	4	(1.3%)
Counting fingers-light perception	6	(1.9%)
No light perception	6	(1.9%)
Missing	1	(0.3%)
Slough (n=313) ┼		
No slough	62	(19.8%)
Flat	124	(39.6%)
Raised	126	(40.2%)
Missing Infiltrate edge (n=313)	1	(0.4%)
Defined	35	(12%)
Serrated	258	(82%)
Not visible	20	(6%)
Satellite lesions present (n=313)		
Yes	178	(57%)
No	126	(40%)
Missing	9	(3%)
Infiltrate colour (n=288)		
White	148	(47%)
Cream	106	(34%)
Other colour	34	(11%)
Missing	25	(8%)
Hypopyon (median height 1.3mm IQR 0.9-2.9, n=313)		
Yes	94	(30%)
No	217	(69%)
Missing	2	(1%)
Site of ulcer (n=313)≠		
Peripheral	27	(9%)
Paracentral	64	(20%)
Central	219	(70%)
Missing	3	(1%)

Variable	Median	(IQR [Total Range])
Perforation status (n=313)		
Not perforated	237	(76%)
Impending	31	(10%)
Perforated	38	(12%)
Perforated and sealed	7	(2%)

*These were calculated as thegeometrical means using the MUTT protocol. The upper limits exceeded normal corneal diameter for some lesions, which extended up to the sclera.
┼ Raised slough was when the corneal infiltrate profile was raised, flat slough was when the profile was flat while no slough is when there was no debris noted.
┼ Site of ulcer was peripheral when the ulcer was marginal, paracentral was when the ulcer was not marginal but not within 4mm of the center of the cornea, central was when the ulcer was within the central 4mm of the cornea.
Impending perforation is when the clinicians felt the ulcer would perforate in the next 48hours.

Microbiology: Microbiology results were available in 265/313 (86.3%) participants (Table 2). Corneal scrapping was not performed on participants who either did not consent, had deep seated infiltrates or small infiltrates (less than 0.5mm). Overall, most infections were fungal (49%),10% were bacterial and 4% were mixed (fungal and bacterial). Ninety-seven (37%) of the corneal scrapping samples were negative on both microscopy and culture.

Table 2: Microbiology of MK (n=265)

Variable	Category	Count	(%)
Gram microscopy	Unknown	139	56 %
	Bacteria	31	13 %
	Fungal	79	31 %
KOH	Unknown	154	65 %
	Fungal	83	35 %
Calcofluor White-KOH ┼	Unknown	32	30 %
	Fungal	75	70 %
BHI culture	Unknown	115	53 %
	Bacteria	23	11 %
	Fungal	78	36 %
Blood agar culture	Unknown	111	51 %
	Bacteria	21	10 %
	Fungal	81	39 %
Chocolate agar culture	Unknown	97	50 %
	Bacteria	20	10 %
	Fungal	79	40 %
Potato dextrose agar culture	Unknown	128	57 %
	Fungal	95	43 %
Fungal PCR	Fungal positive	159	74 %
	Fungal negative	56	26 %
Overall laboratory diagnosis ┼	Unknown	97	37 %
	Bacterial	27	10 %
	Fungal	131	49 %
	Mixed (Bacteria/Fungal)	10	4 %
Cultured organisms	*Staph Aureus* (2 mixed)	8	3 %
	Strep Pneumonae	8	3 %
	Pseudomonas	6	2.5 %
	Klebsiella	4	2 %
	Norcadia	1	0.5 %
	Fusarium (2 mixed)	48	19 %

Variable	Category	Count	(%)
	Aspegillus	19	8 %
	Acremonium	13	6 %
	Bipolaris	6	2.5 %
	Scedospovium	1	0.5 %
	Candida	3	1.5 %
	Lasiodiplodia	2	1 %
	Unidentified fungi	9	3.5 %
	No growth	112	47 %
Yield rates	Gram	112/247	43 %
	KOH	83/249	33 %
	Calcofluor-KOH	72/103	70 %
	BHI culture	101/223	45 %
	Blood agar	102/213	49 %
	Chocolate agar	99/203	48 %
	Potato dextrose agar	97/233	41 %

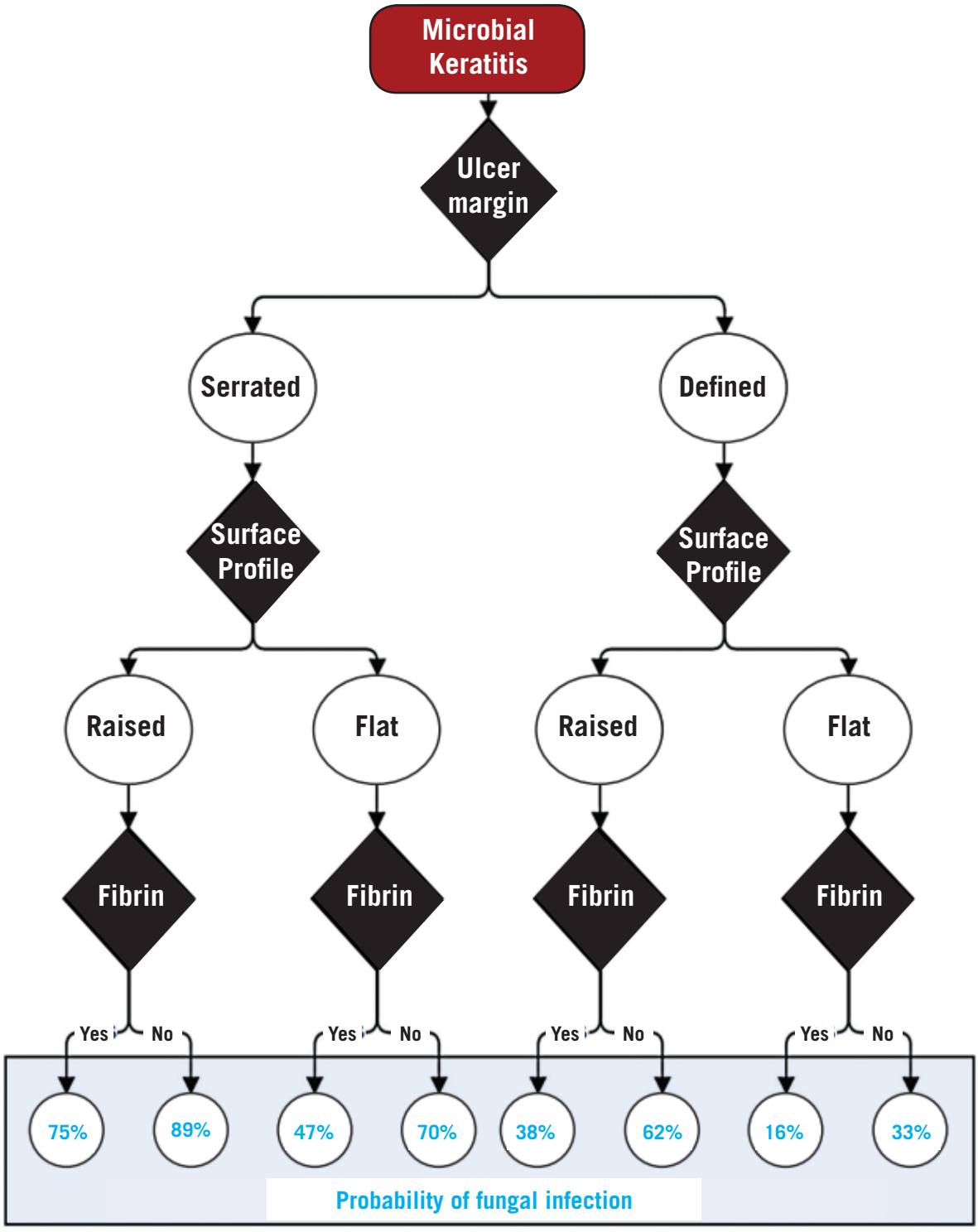
Specimen for microbiology was collected in 265 patients. Due to limited amounts of sample material, it was not possible to perform all tests on all those sampled. The order of material collection was 3 slide smears (gram, KOH, CFW), 3 agar inoculations (blood, chocolate, PDA) and 1 broth (BHI) depending on available material. ǂ Calcofluor stain has less numbers because it was introduced mid-way into the study. ᶜ Fungal PCR results were not included in the overall laboratory diagnosis.

Clinical signs predictive of fungal keratitis: In the final multivariable model, presence of a slough (aOR 3.58, 95% CI [1.60-8.04], p=0.002), a serrated infiltrate margin (aOR 1.58, 95% CI [1.00-2.51], p=0.051), satellite lesions (aOR 2.90, 95% CI [1.65-5.11], p<0.0001) and a hypopyon (aOR 3.24, 95% CI [1.78-5.90], p<0.0001) were associated with a positive microbiology result for fungal keratitis (Table 3).

Table 3: Clinical features associated with fungal keratitis (n=265)

Variable	Univariate analysis			Multivariable analysis		
	Crude OR*	(95% CI)	P-value	Adjusted OR+	(95% CI)	P-value
Slough	3.30	(1.60-6.79)	0.001	3.58	(1.60-8.04)	0.002
Serrated infiltrate edge	1.79	(1.16-2.75)	0.008	1.58	(1.00-2.51)	0.051
Satellite lesions	3.12	(1.86-5.25)	<0.0001	2.90	(1.65-5.11)	<0.0001
Infiltrate color						
White	1		0.102			
Cream	1.74	(1.01-2.99)				
Colored	1.67	(0.76-3.67)				
Immune ring	1.30	(0.48-3.54)	0.601			
Hypopyon	3.55	(2.05-6.15)	<0.0001	3.24	(1.78-5.90)	<0.0001
Perineural infiltrate	2.04	(0.99-4.22)	0.054			
Fibrin	2.24	(0.87-5.79)	0.096			
Flare	2.41	(1.39-4.20)	0.002			
Endothelial plaque	2.50	(1.50-4.13)	0.001			
Solid inflammatory mass in AC	2.50	(1.33-4.59)	0.004			

Figure 1: Algorithm for determining the probability of fungal keratitis[10]



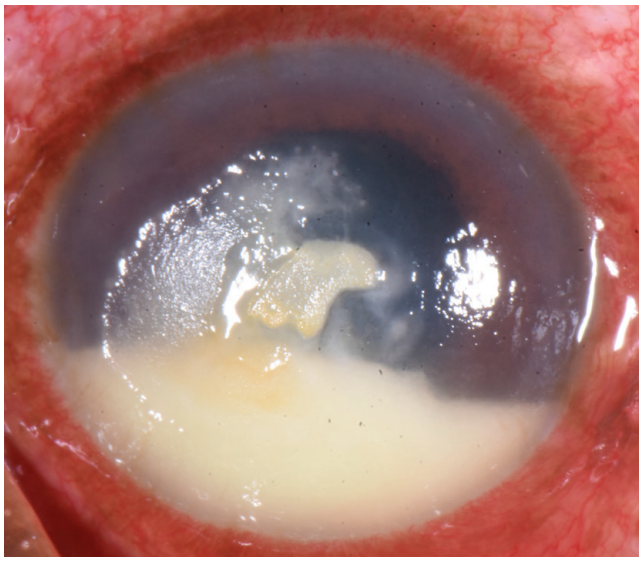
DISCUSSION

This study describes the clinical signs that were associated with a microbiological diagnosis of Fungal Keratitis (FK) in a cohort in Uganda. Some of these features have been highlighted in Figure 2.

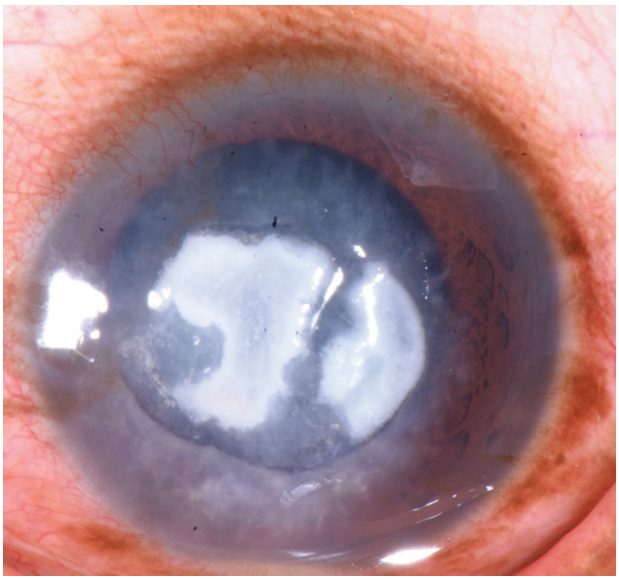
The overall microbiology yield was 80%. This was a composite of all the microscopy and culture results. Overall culture positive results were 55% like the expected yield reported in literature[8,15]. Maximum microscopic yield for fungal keratitis was achieved when we introduced the calcofluor white combined with Potassium Hydroxide staining (CFW + KOH). CFW is a non-specific fluorochrome dye that fluoresces when illuminated by light of a particular wave length making visualisation of fungal elements under a fluorescence easy[16,17]. In this study, we were also able to do PCR pan fungal testing for fungal DNA from corneal swabs. Although this showed the highest yield, it is a technology that is not readily available in most settings and was purely used for research purposes in our cohort.

We found that the clinical signs predictive for FK were presence of a slough, a serrated infiltrate margin, satellite lesions and hypopyon. These features seem to have been consistently reported in other settings of variable designs and size. Some of these include a study done by our colleague in India that analyzed data of 191 patients with confirmed FK; fungal ulcers were more likely to have feathery margins/serrated margins ($p< 0.001$) with ulcers caused by *Fusarium sp* four times more likely to have feathery margins as compared to those caused by *Aspergilus sp* (OR: 4.55, 95% CI: 1.92 – 10.75, $p = 0.001$). The study also reported that a raised profile/slough ($p = 0.039$) and dry texture of a surface slough ($p = 0.007$) was significantly associated with FK. However, this particular study noted that bacterial ulcers are more likely to have a hypopyon ($p = 0.02$) as compared to FK[18]. Another study by our group in Nepal found that serrated

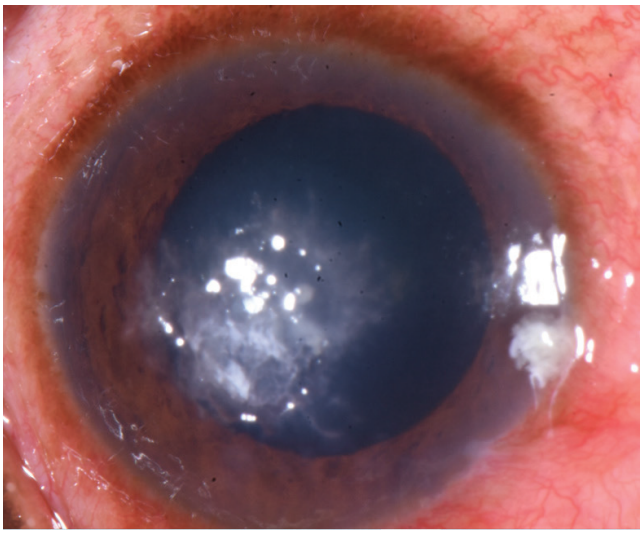
Figure 2: Pictures showing some of the clinical signs associated with microbial keratitis in our cohort



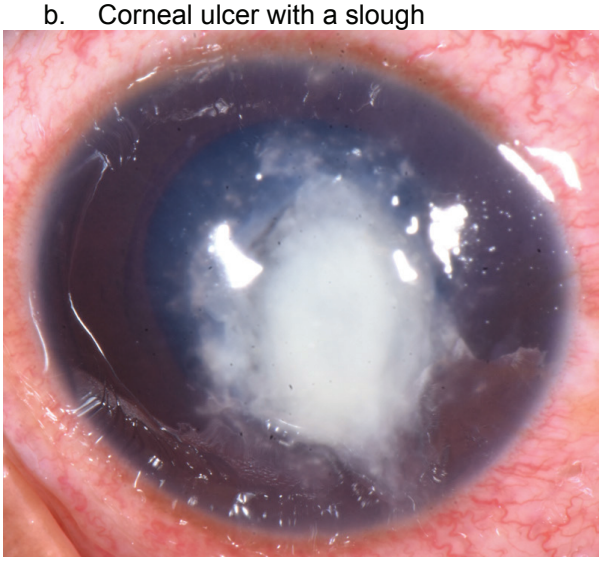
a. Corneal ulcer with a hypopyon



b. Corneal ulcer with a slough



c. Corneal ulcer with serrated margins/Feathery margins



d. Corneal ulcer with satellite lesions

margins (OR: 7.5, 95% CI: 4.09 – 13.78, p < 0.01) and a raised slough (OR: 4.27, 95% CI: 2.51 – 7.24, p < 0.001) are predictive of FK. However unlike in our study that found that satellite lesions are associated with FK, the study in Nepal noted that despite satellite lesions being more common in patients with FK, it was not a significant predictor for FK[19].

In summary most of the findings from these studies show that the key predictive clinical features for FK are a serrated margin, satellite lesions and the presence of a slough. It should be noted that these clinical features (serrated margins, slough and satellite lesions are also found in some patients with BK[8,19].

Basing on the above clinical signs, scientists have tried to devise a scoring system to aid in the diagnosis of FK. One such study was a large multicentre prospective study that created a clinical scoring system to aid in the diagnosis of microbial keratitis, with ability to calculate the probability of the causative infection[10,20]. In the study, the clinical data of 360 patients with FK and 132 patients with BK was analysed. The clinical signs that were significantly indicative of fungal keratitis as opposed to bacterial were serrated margins, raised slough, dry textured slough, satellite lesions and colouration other than yellow ($p<0.05$)[20]. Conversely, bacterial infections were associated with a hypopyon and fibrinous exudate ($p<0.05$). Hence the three clinical signs were used to create a score for diagnosing MK. Based on this model, Leck *et al*[10] developed an algorithm for determining the probability of FK. This algorithm uses the clinical signs: nature of ulcer margins, nature of the surface profile and presence of fibrin to calculate the probability of fungal infection[21]. The algorithm has been added as Figure 1 with permission and can be useful in settings where there is no microbiology support.

The recent study in Nepal has also created a scoring system for predicting patients with FK. The scoring system however uses four clinical features (serrated margins, raised slough, a nasolacrimal obstruction and trauma with vegetative objects) to predict whether the patient has FK[19].

Knowledge of the causative organism in patients with MK can help aid healing by reducing the drug toxicity associated with polypharmacy, facilitate informed disease monitoring for appropriate intervention, allow rationale use of antifungals, reduce the risk of developing drug resistance and reduce the cost of treatment. It should be noted that the use of clinical diagnosis is not completely reliable and can have variable conclusions even among corneal specialists. In one study that presented corneal photographs with a confirmed microbiology diagnosis to corneal specialists, only 66% could correctly differentiate between bacterial and fungal keratitis[21]. However, in the absence of a reliable microbiology support, these clinical signs could be useful to help clinicians make a rational clinical judgment.

Strengths and limitations

This is the first large prospective cohort study in SSA to describe the predictive signs of FK. The large number of patients gave sufficient power to analyze the clinical signs associated with MK. However, there was a sizable proportion of patients with whom a microbiology diagnosis could not be made and were subsequently dropped from the analysis. Ocular microbiology is not performed in many settings in SSA. As part of this study, we undertook to build the capacity of the hospital to provide this service as the first ocular microbiology laboratory in Uganda.

CONCLUSION

This study conducted in a predominantly African population provided clues to support clinicians in making a diagnosis of fungal keratitis in settings where there is no microbiology support. The main clinical signs that are predictive for FK are presence of a slough, serrated infiltrate margins, satellite lesions and hypopyon.

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