Clinical differentiation of *Pythium* keratitis from fungal keratitis and development of a scoring system

Samrat Chatterjee, Deepshika Agrawal, Sharad Nivrutti Gomase

**Purpose:** To differentiate *Pythium* keratitis from fungal keratitis using clinical signs, to explore usefulness of various signs as diagnostic prognosticators, and develop a clinical scoring system.  

**Methods:** A retrospective review of medical records and archived clinical photographs of patients with culture-positive *Pythium* keratitis and hyaline filamentous fungal keratitis was conducted at a tertiary eye institute to explore characteristics of ulcers that may aid diagnosis.  

**Results:** Full-thickness corneal stromal keratitis (*P* = 0.055), a dry ulcer surface (*P* = 0.010), tentacles (*P* < 0.0001), intrastromal dots (*P* < 0.0001), ring infiltrates (*P* = 0.024), reticular patterns (*P* < 0.0001), and peripheral furrows (*P* < 0.0001) were clinical signs associated with *Pythium* keratitis. Multiple regression analysis identified tentacles (odds ratio: 24.1, 95% confidence interval (CI): 3.8–158.1, *P* = 0.001) and peripheral furrows (odds ratio: 60.6, 95% CI: 5.1–712.3, *P* = 0.001) as independent diagnostic prognosticators for *Pythium* keratitis. The positive and negative likelihood ratios of a dry ulcer surface, tentacles, intrastromal dots, ring infiltrates, reticular patterns, and peripheral furrows predicting *Pythium* keratitis were 1.6, 13.6, 17.9, 4.3, 30.7, 15.3 and 0.4, 0.4, 0.7, 0.9, 0.6 and 0.8, respectively. The presence of two or more of these clinical signs (excluding a dry ulcer surface) had a sensitivity of 55.6% and a false positive rate of 1.4%.  

**Conclusion:** Tentacles, intrastromal dots, ring infiltrates, reticular patterns, and peripheral furrows are clinical signs to be considered for the diagnosis of *Pythium* keratitis and the presence of two or more signs has a very low false positive rate.

**Key words:** Clinical diagnosis, fungal keratitis, microbial keratitis, *pythium* keratitis, scoring system, signs

In recent times, one of the most challenging corneal infections to diagnose and treat is *Pythium* keratitis.\[1-15\] The concern about the under-diagnosis of this infection—that many patients with fungal keratitis diagnosed as either unidentified fungi or suspected fungi but culture negative may actually be *Pythium* keratitis\[9\]—was not misplaced when a study\[9\] reported *Pythium insidiosum* by DNA sequencing of archived unidentified fungal isolates. Clinically, *Pythium* keratitis resembles fungal keratitis,\[6-16\] and during direct microscopy evaluation of corneal scrapings, even experienced microbiologists frequently confuse the aseptate or sparsely septate filaments of *Pythium insidiosum* with hyaline filamentous fungi.\[6-9,13-16,17\] The definitive diagnosis is made through a microbiology culture, but zoospore formation requires a difficult technique, which is not routinely practiced in most laboratories.\[8,9,13-16\] and also the culture positivity is low.\[10,12,13\] When there is confusion about the diagnosis, anti-fungal drugs are usually prescribed given that fungal keratitis is more common. The outcome in such cases is usually poor because the organism lacks ergosterol in the cell wall,\[18-23\] which is the primary target of anti-fungal drugs. Instead, a better outcome is reported with a combination of antibiotics.\[19,19\]

When microbiological findings are ambiguous, negative, or delayed, the clinical history, risk factors and morphological features of the keratitis can help in diagnosis.\[20\] For example, ocular injury with vegetative matter, an indolent clinical course and a dry, raised surface with feathery margins generally indicate fungal keratitis\[21-24\] and differentiate it from bacterial keratitis.\[24-26\] Alternatively, the presence of a corneal ring infiltrate suggests the possible diagnosis of *Acanthamoeba* keratitis.\[27\] At times, it is difficult to differentiate the filaments of *Pythium insidiosum* from the filamentous fungi on direct microscopy.\[9,11,13,16,17\] In such cases, clinical signs may aid in the diagnosis. Some signs such as a reticular pattern of stromal infiltrates,\[5,6,8,10,13-15\] tentacles at the ulcer margin,\[10,13-15\] and intrastromal pinpoint-sized dot lesions\[10,13-15\] have been reported to be peculiar to *Pythium* keratitis. Hence, the aim of this study was to explore the possible clinical differentiation of *Pythium* keratitis from fungal keratitis, and identify clinical signs as prognosticators for diagnosis. If successful, the findings would aid ophthalmologists in making a presumptive diagnosis of *Pythium* keratitis, alert microbiologists, and guide treatment decisions when the microbiological results are ambiguous or delayed.

**Methods**  
This retrospective study was carried out at a tertiary eye care institute in central India. The institute’s ethics committee approved\[28\] This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

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Figure 1: Slit-lamp images of the cornea in diffuse illumination in patients with *Pythium* keratitis (left column) and fungal keratitis (right column) showing the different clinical signs. Tentacles in *Pythium* keratitis (a) and in *Fusarium solani* keratitis (b). Intrastromal dots present in *Pythium* keratitis (c) and *Fusarium solani* keratitis (d). Ring infiltrate in *Pythium* keratitis (e) and *Aspergillus nigra* keratitis (f). Reticular pattern at the ulcer margin in *Pythium* keratitis (g) and in *Fusarium solani* keratitis (h). Furrows at the ulcer periphery in *Pythium* keratitis (i) and in *Aspergillus flavus* keratitis. (j)
the study, and the research adhered to the tenets of the Declaration of Helsinki. Patients who were diagnosed with *Pythium* keratitis from January 2017 to December 2020 were included, and for comparison, another group of patients with fungal keratitis from January 2019 to December 2020 were selected. Patients who were culture-negative, or whose cultures were positive for dematiaceous fungi, yeasts and bacteria, or where species identification was not possible, and patients with incomplete medical records and missing clinical photographs were excluded.

The medical records were reviewed for demographic details, pre-disposing risk factors, symptom duration, laterality, visual acuity, morphological appearance of the corneal ulcer, and microbiological findings. The protocol of the institute [Appendix 1] for managing all patients with suspected microbial keratitis includes detailed documentation of the findings using labelled clinical diagrams and anterior segment slit-lamp photographs. Anterior segment photography was performed by trained optometrists with a digital slit-lamp imaging system (VISUPAC c⃝, Carl Zeiss Meditec AG, Jena, Germany) for all patients with microbial keratitis. Photographs were taken in diffuse and optic section illumination at 5 × and 8 × or 12 × magnification. For the study, the morphological characteristics of the corneal ulcers were retrieved from the clinical notes, and a careful examination of the archived digital clinical photographs was undertaken to corroborate findings and gather additional information. All photographs were reviewed for quality, ensuring that the complete cornea and anterior segment were included, the illumination was appropriate and did not lead to either over- or under-exposure. The morphologies of *Pythium* keratitis and fungal keratitis were described using specific signs shown in Fig. 1 and Appendix 2.

Statistical analysis
Quantitative and qualitative variables were expressed as mean ± standard deviation and percentages, respectively. Pearson’s Chi-squared test was used to compare categorical variables, while an independent sample *t*-test was used to analyze continuous variables between both groups. Binary logistic regression analysis was used to determine the association between clinical signs and *Pythium* keratitis. Sensitivity, specificity, likelihood ratios and predictive values with 95% confidence intervals (CI) were computed for the clinical signs significantly associated with *Pythium* keratitis. Post-test probability testing was estimated via Fagan’s nomogram. A receiver operating characteristic (ROC) curve was plotted for each clinical sign to calculate the area under the curve (AUC), following which, a clinical scoring system was computed by selecting specific signs and assigning a score of 1 to each. Two clinical scoring systems were tested, an ROC curve was drawn and coordinates plotted. All tests were computed using the Statistical Package for the Social Sciences (SPSS) version 23.0 for Macintosh (IBM Corporation, New York, USA). Fagan’s nomogram was computed using an online calculator available at: http://araw.medc.uic.edu/cgi-bin/testcalc.pl, (accessed on August 20, 2021). A two-tailed *P*-value of <0.05 was considered statistically significant.

Results
All patients
Between January 2017 and December 2020, 1628 patients were diagnosed with microbial keratitis, of whom 27 (1.6%)...
Table 1: Contd...

| Variable                        | Pythium keratitis n=27 | Fungal keratitis n=69 | \chi^2 | P     |
|---------------------------------|-------------------------|-----------------------|--------|-------|
| Absent                          | 20 (74.1)               | 68 (98.6)             | 15.220 | <0.0001|
| Satellite lesions               |                         |                       |        |       |
| Present                         | 3 (11.1)                | 15 (21.7)             |        |       |
| Absent                          | 24 (88.9)               | 54 (78.3)             | 1.439  | 0.230 |
| Ring-shaped infiltrate          |                         |                       |        |       |
| Present                         | 5 (18.5)                | 3 (4.3)               |        |       |
| Absent                          | 22 (81.5)               | 66 (95.7)             | 5.101  | 0.024 |
| Immune (Wessely) ring           |                         |                       |        |       |
| Present                         | 0 (0)                   | 8 (11.6)              |        |       |
| Absent                          | 27 (100)                | 61 (88.4)             | 3.415  | 0.065 |
| Reticular pattern               |                         |                       |        |       |
| Present                         | 12 (44.4)               | 1 (1.4)               |        |       |
| Absent                          | 15 (55.6)               | 68 (98.6)             | 30.641 | <0.0001|
| Peripheral furrow               |                         |                       |        |       |
| Present                         | 6 (22.2)                | 1 (1.4)               |        |       |
| Absent                          | 21 (77.8)               | 68 (98.6)             | 12.388 | <0.0001|
| Anterior chamber exudates       |                         |                       |        |       |
| Hypopyon only                   | 10 (37.0)               | 22 (31.9)             |        |       |
| Hypopyon and endothelial exudates| 0 (0)                   | 11 (15.9)             |        |       |
| No hypopyon or endothelial exudates| 17 (63.0)             | 36 (52.2)             | 4.868  | 0.088 |
| Perforation                     |                         |                       |        |       |
| Perforation                     | 1 (3.7)                 | 2 (2.9)               |        |       |
| Thinning/ Descemetocoele        | 2 (7.4)                 | 4 (5.8)               |        |       |
| Absence of the above            | 24 (88.9)               | 63 (91.3)             | 0.133  | 0.936 |
| Continuous variables*           |                         |                       |        |       |
| Age in years                    | 38.8±17.9               | 44.9±14.8             | 0.094  |       |
| Symptom duration in days        | 15.3±10.5               | 13.9±13.5             | 0.430  |       |
| Visual acuity in LogMAR units   | 1.9±0.6                 | 1.41±0.9              | 0.005  |       |
| Ulcer diameter 1 in mm          | 5.7±2.1                 | 4.4±2.6               | 0.025  |       |
| Ulcer diameter 2 in mm          | 5.8±2.3                 | 4.4±2.6               | 0.015  |       |

*Continuous variables are expressed as mean and standard deviation and both the groups were compared by Student’s T test

Demography and risk factors
A comparison between the demographic features and risk factors of keratitis between the two groups of patients is given in Table 1. Patients with Pythium keratitis were younger than those with fungal keratitis, although the difference was statistically not significant (P = 0.094), nor was there any difference in the sex distribution ratio (P = 0.731). A history of minor corneal trauma was more frequently present in patients with fungal keratitis (P = 0.050), while the right eye was commonly involved in patients with Pythium keratitis (P = 0.046).

Comparison of clinical signs between Pythium keratitis and Fungal keratitis:

Signs such as poor presenting visual acuity (P = 0.005), a large ulcer diameter (P = 0.015), full-thickness stromal infiltrate (P = 0.055), a dry ulcer surface (P = 0.010), tentacles in the ulcer margins (P < 0.0001), intrastromal dots (P < 0.0001), ring-shaped stromal infiltrates (P = 0.024), and peripheral furrows (P < 0.0001) were significantly associated with Pythium keratitis [Table 1]. The only sign significantly associated with fungal keratitis was a raised ulcer surface or plaque (P = 0.044). Although endothelial exudates in the anterior chamber were more frequently observed in eyes with fungal keratitis, the difference was not statistically significant (P = 0.088).

Predictability of clinical signs in diagnosing Pythium keratitis
We selected seven signs based on their significant association with Pythium keratitis to assess their predictability in arriving at a correct clinical diagnosis. These were full-thickness stromal infiltrates, a dry ulcer surface, intrastromal dots, tentacles, reticular patterns, ring-shaped infiltrates, and peripheral furrows. The sensitivity, specificity, and likelihood ratios are given in Table 2. A dry ulcer surface had the highest sensitivity (81.5%; 95% CI: 61.9%–93.7%) followed by a full-thickness stromal infiltrate (66.7%; 95% CI: 46.0%–83.5%). However, both of these clinical signs had very low specificities (<60%). On the contrary, intrastromal dots [Fig. 1c], reticular patterns [Fig. 1i], and peripheral furrows [Fig. 1k] had high specificity values (98.5%; 95% CI: 92.2%–99.9%). However, their sensitivity values were low (<50%). The only sign with moderate sensitivity but good specificity was tentacles with 59.3% (95% CI: 38.8%–77.6%) and 95.6% (95% CI: 87.8%–99.1%), respectively.

The signs with the highest positive predictive values and positive likelihood ratios were reticular patterns, intrastromal dots, peripheral furrows, and tentacles [Table 2]. They also had a low negative predictive value and negative likelihood ratios. While a dry ulcer surface had a high positive predictive value, its negative predictive value was lower than the other signs. We used the Fagan’s nomogram method to assess the probabilities of a positive diagnosis of Pythium keratitis in the presence of these clinical signs [Appendix 5]. The presence of a reticular pattern, followed by intrastromal dots, and tentacles had ≥85% probability of a positive diagnosis.
Sensitivity

Specificity

Positive

Negative

Likelihood

Ratio

Ratio

Table 2: Predictive values of ulcer characteristics in the diagnosis of Pythium keratitis

| Variable                          | Sensitivity | Specificity | Positive predictive value | Negative predictive value | Positive likelihood ratio | Negative likelihood ratio |
|-----------------------------------|-------------|-------------|---------------------------|---------------------------|--------------------------|--------------------------|
| Full-thickness stromal infiltrate | 66.7 (46.0-83.5) | 55.1 (42.3-67.1) | 2.4 (1.6-3.4) | 99.0 (6.3-99.4) | 1.5 (1.0-2.2) | 0.6 (0.3-1.1) |
| Dry ulcer surface                 | 81.5 (61.9-93.7) | 50.7 (38.4-63.0) | 39.3 (32.4-46.6) | 87.5 (75.4-94.1) | 1.6 (1.2-2.2) | 0.4 (0.1-0.8) |
| Tentacles                         | 59.3 (38.8-77.6) | 95.6 (87.8-99.1) | 18.1 (6.6-41.2) | 99.3 (98.9-99.6) | 13.6 (4.3-43.0) | 0.4 (0.3-0.7) |
| Intrastromal dots                 | 25.9 (11.1-46.3) | 98.5 (92.2-99.9) | 22.5 (3.6-69.3) | 98.8 (95.5-99.0) | 17.9 (2.3-138.6) | 0.7 (0.6-0.9) |
| Ring-shaped infiltrate            | 18.5 (6.3-38.1) | 95.6 (87.8-99.1) | 6.5 (1.7-21.3) | 98.6 (98.4-98.9) | 4.3 (1.1-16.6) | 0.9 (0.7-1.0) |
| Reticular pattern                 | 44.4 (25.5-64.7) | 98.5 (92.2-99.9) | 33.3 (6.4-78.5) | 99.1 (98.7-99.3) | 30.7 (4.2-224.5) | 0.6 (0.4-0.8) |
| Peripheral furrow                 | 22.2 (8.6-42.3) | 98.5 (92.2-99.9) | 20.0 (3.0-66.4) | 98.7 (98.4-99.0) | 15.3 (1.9-121.5) | 0.8 (0.6-1.0) |

Development of a clinical score:

An ROC curve was plotted for each sign suggestive of Pythium keratitis and the AUC was calculated [Fig. 2a and Appendix 6]. The largest AUC was observed in tentacles in the ulcer margin (AUC: 0.78; 95% CI: 0.65-0.90), followed by reticular patterns (AUC: 0.71; 95% CI: 0.58-0.84), and a dry ulcer surface (0.66; 95% CI: 0.54-0.78).

Each clinical sign was assigned a score of 1. Subsequently, all positive signs were summated to arrive at a total score. Two clinical scores were computed using different combinations of signs. Clinical score 1 included all the signs which had AUC values greater than 0.5, while clinical score 2 included the same signs with the exception of a “dry ulcer surface”, which is classically associated with fungal keratitis.

An ROC curve was plotted and the coordinates of the curve were analyzed to determine a diagnostic cutoff value [Fig. 2b]. The AUC for clinical score 1 was 0.889 (95%; CI: 0.813-0.966, P < 0.0001), and for clinical score 2 was 0.896 (95%; CI: 0.810-0.981, P < 0.0001), respectively. The sensitivity and specificity of a score of 2 in clinical score 1 was 74% and 89.9%, respectively, while the same for clinical score 2 was 55.6% and 98.6%, respectively.

Discussion

Pythium keratitis is often misdiagnosed prior to culture results because of (1) morphological likeness to fungal keratitis, and (2) resemblance of the Pythium insidiosum filaments to hyaline fungal filaments on direct microscopy. Different studies have reported misdiagnosis, which can range from 22.5%[13] to 100%,[12] Even experienced microbiologists can be confused on direct microscopy.[12,13] A recent study from south India evaluated the concordance amongst microbiologists in identifying the filaments of Pythium insidiosum in corneal scrapings stained with 10% potassium hydroxide and calcofluor white.[17] The authors reported a good agreement amongst three experienced microbiologists, but the sensitivity of correct identification ranged from 79.3% to 96.5%, indicating that the initial diagnosis may be missed in several cases.

Culture techniques of Pythium insidiosum are difficult, take three to seven days, and zoospore formation can be as low as 32%.[18] These factors make the diagnosis of Pythium keratitis very challenging. Ophthalmologists have traditionally relied on clinical signs to differentiate between infectious and non-infectious keratitis, or bacterial and fungal keratitis. The present study was carried out to check if clinical signs play a role in diagnosis of Pythium keratitis, and, if it is feasible, to
differentiate it from hyaline fungal keratitis, with which it is most often misdiagnosed.

A dry ulcer surface, full-thickness stromal infiltrates, tentacles, intrastromal dot lesions, ring infiltrates, reticular patterns, and peripheral furrows were identified as clinical signs significantly associated with *Pythium* keratitis. However, the first two signs, although statistically significant, may not be clinically helpful. A dry ulcer surface is frequently present in fungal keratitis,[21–24] while a full-thickness stromal infiltrate is non-specific, indicating only the severity of the infection. Both of these signs had very low specificity values and odds of being associated with *Pythium* keratitis. We have, however, observed, as have others, that the clinical course of *Pythium* keratitis is more fulminant than fungal keratitis, with larger stromal infiltrates and poorer vision within the same duration. In the latter, the infection tends to be mostly indolent.[21–24]

Coming to the other signs, tentacles and peripheral furrows were identified as important prognosticators via multiple regression analysis. These two signs had high specificities and negative predictive values but low sensitivities. Tentacles are sinuous linear lesions extending from the ulcer margin into the stroma,[10,13–15] but can, at times, be confused with the feathery margins of fungal keratitis [Fig. 1 and Appendix 2]. The other signs such as intrastromal dots and a reticular pattern also had high specificity values but low sensitivity and positive predictive values. The prevalence of a disease affects predictive values.[20] As the prevalence rate of *Pythium* keratitis is low—1.6% in our center and 1.4%–1.6% in other centers in India[13,15]—it explains the low predictive values. However, these four specific signs had higher likelihood ratios, and probabilities (post-test odds) in diagnosing *Pythium* keratitis, and therefore they still play a useful clinical role.

None of the above signs can be considered as a *sine qua non* of *Pythium* keratitis due to their presence in patients with fungal keratitis. Therefore, we devised a scoring system to make clinical diagnosis more robust. In a previous study, a similar tool was used to differentiate fungal keratitis from bacterial keratitis.[24] We derived two clinical scores by including signs that had a significant area in the ROC curve [Fig. 2]. In clinical score 2, a dry ulcer surface was excluded as 50% of patients with fungal keratitis in the present study were positive for this sign, and it has traditionally been associated with fungal keratitis. The AUC of clinical score 2 was higher [Fig. 2b], and a score of 2 or more had a sensitivity of 55.6% and a false-positive rate of only 1.4% [Table 3].

The findings of our study have multiple applications in the clinic. In a situation where direct microscopy is inconclusive or the culture results are delayed, or the keratitis is worsening despite adequate anti-fungal treatment and *Pythium* keratitis is suspected, the clinical scoring system may guide treatment choices. The probability of erroneously diagnosing *Pythium* keratitis in the presence of one sign (score 1+) is approximately 11.6% (clinical score 2), and reduces to 1.4% if two signs (score 2+) are present. This has sufficient power to guide treating ophthalmologists to change treatment course or re-investigate. In a community-based eye care facility lacking a microbiology laboratory, a significant score will alert the primary eye care worker to timely refer the patient to a higher center for a microbiology work-up, and thus avoid any delay in diagnosis and treatment. Ophthalmologists will be able to alert microbiologists, and forewarned, the latter may specifically look for signs[9,17] suggestive of *Pythium insidiosum* during direct microscopic examination of slides from corneal scrapings. Special stains like calcofluor white[20,21] or iodine–potassium iodide–sulfuric acid,[18] or other diagnostic techniques like nested polymerase chain reaction, immunoassays or matrix-assisted laser desorption ionization-time of flight mass spectrometry can be used to identify the organism.[29]

A limitation in our study was its retrospective study design. We mitigated the effect of this by including patients with complete medical records and clinical photographs, and positive culture with identification of species. While a prospective study is a better option, the low prevalence of *Pythium* keratitis is a limiting factor. In our study, only hyaline filamentous fungi were included. Hence the findings cannot be generalized to dematiaceous fungi or *Candida* keratitis. However, the keratitis caused by both of these fungi have a very distinct clinical appearance[20,31] and are unlikely to be confused with *Pythium* keratitis. It is also unlikely that *Pythium* keratitis, which resembles fungal keratitis very closely, will be confused with bacterial keratitis as the morphological features of the latter[23,30] are singular and distinct. Therefore, bacterial keratitis was not included in the present study. Our clinical scoring system needs validation, but given the low prevalence of *Pythium* keratitis, multi-centric prospective studies will be required to validate them.

### Conclusion

It appears, from our findings, that *Pythium* keratitis can be clinically differentiated from fungal keratitis with moderate certainty. Tentacles, a reticular pattern, intrastromal dots and peripheral furrows are key signs in arriving at a diagnosis, and including them in a scoring system increases the reliability of the diagnosis. A clinical diagnostic approach plays an

### Table 3: Clinical scoring systems for the diagnosis of *Pythium* keratitis

| Total score | Clinical score 1 (6 signs) | 1-specificity | Total score | Clinical score 2 (5 signs) | 1-specificity |
|-------------|---------------------------|---------------|-------------|---------------------------|---------------|
| 1           | 0.963                     | 0.507         | 1           | 0.852                     | 0.116         |
| 2           | 0.741                     | 0.101         | 2           | 0.556                     | 0.014         |
| 3           | 0.519                     | 0.014         | 3           | 0.222                     | 0.000         |
| 4 or more   | ≤0.222                    | 0.000         | 4 or more   | ≤0.074                    | 0.000         |

Clinical score 1: Dry ulcer surface, tentacles, intrastromal pinhead-sized lesions, ring-shaped infiltrate, reticular pattern and furrow at ulcer periphery. Clinical score 2: Tentacles, intrastromal dots, ring-shaped infiltrate, reticular pattern, furrow at ulcer periphery.
important supportive role and does not replace a microbiology investigation of the keratitis.

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Conflicts of interest
There are no conflicts of interest.

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Appendix

Appendix 1: Protocol of the institute in the management of microbial keratitis. This is a brief description of the clinical examination, documentation of clinical findings, and microbial evaluation of patients presenting with microbial keratitis.

Eye Institute

Patients presenting with microbial keratitis at our institute are evaluated according to a fixed protocol. This includes detailed history of risk factors and symptoms, measurement of visual acuity with logMAR visual acuity charts, slit-lamp evaluation, fundus examination, B-scan evaluation of the posterior segment in eyes with dense media opacity, syringing of the nasolacrimal duct and slit-lamp photography. The details of the ulcer that are noted are location (central, paracentral, peripheral, total), shape, color of the infiltrate (whitish-gray, yellowish or any pigmentation), appearance of the surface (dry, wet, or raised), measurement of the ulcer dimensions with the help of the slit-lamp reticle (two greatest opposite diameters), ulcer margins (well-defined, feathery or hyphate), stromal involvement (anterior, posterior or full-thickness), presence of corneal stromal thickness thinning, descemetocele or perforation, anterior chamber reaction (hypopyon, endothelial exudates) and iris neovascularization. Other examinations include pupillary reaction, lens status (clear, cataract, pseudophakia or aphakia), fundus evaluation, and if the view is obscured, then a B-scan ultrasonography is ordered. Intraocular pressure (digitally or tonopen) and syringing to assess patency of the nasolacrimal duct are done routinely. Clinical photography at a digital slit-lamp imaging system is done for all patients. This includes photographs taken with diffuse illumination and optic section illumination at 5 × and 8 × magnification. This is followed by corneal scrapings under topical anesthesia (4% proparacaine) with a no. 15 surgical blade on a Bard–Parker handle at the slit-lamp. The material from the corneal scrapings are smeared on the sterile glass slides for 10% potassium hydroxide wet mount preparation, Gram and Giemsa stained, and also directly inoculated in 5% sheep blood agar, chocolate agar, Sabouraud dextrose agar, potato dextrose agar and brain-heart infusion. The smears are examined under direct light microscopy, and initial treatment is based on the findings which are modified according to culture and antibiotic susceptibility reports and clinical progress. The media are incubated at appropriate temperatures and atmospheric conditions and reviewed daily for growth for a maximum period of 10–14 days. Any positive growth is identified on the basis of colony characteristic, staining pattern and biochemical tests. A diagnosis of fungal keratitis is made if fungal filaments or spores are detected on direct light microscopy and/or there is a growth in any media. Identification of fungal species is done based on colony characteristics and microscopic examination of spores. A presumptive diagnosis of *Pythium* keratitis is made by the presence of aseptate or sparsely septate broad ribbon-like filaments with folding or terminal bends in direct microscopy of 10% potassium hydroxide wet mount or Gram and Giemsa staining; and flat, feathery, light-brown colonies with filiform margins on blood agar and/or adherent flat, smooth, opaque, and yellowish-white colonies, with filiform margins on Sabouraud dextrose agar.
Appendix 2: Description of clinical signs of *Pythium* and fungal keratitis. Detailed description of clinical signs of *Pythium* and fungal keratitis with appropriate references and a clinical photograph depicting some typical signs of fungal keratitis

1. Feathery margins: A feathery margin consisted of distinct, fine, linear lines in the corneal stroma radiating from the ulcer margins giving it a typical fuzzy appearance [See below in Fig. (a)]\(^{[21-23]}\)

2. Tentacles: Tentacles were thicker and longer than hyphae, that radiated from the ulcer margins [Fig. 1a and 1b].\(^{[10,13-15]}\)

3. Plaques: In this study, plaques were dry-raised slough, which were non-pigmented [See below in Fig. (b)].\(^{[21-23]}\)

4. Intrastromal dots: Intrastromal dots were pinhead-sized stromal lesions [Fig. 1c and 1d].\(^{[10,13-15]}\)

5. Ring infiltrates: Ring infiltrates, as the name suggests, were ring-like stromal infiltrates [Fig. 1e and 1f].\(^{[21-23,27]}\)

6. Wessley ring: Wessley rings or immune rings were halo-like stromal infiltrates surrounding the main ulcer [See below in Fig. (c)].\(^{[21-23]}\)

7. Satellite lesions: Satellite lesions were round, irregular lesions adjacent to the primary ulcer [See below in Fig. (d)].\(^{[21-23]}\)

8. Reticular pattern: A reticular pattern consisted of a network of linear and dot-like stromal opacities in the subepithelial or superficial corneal stroma radiating from the ulcer margin [Fig. 1g and 1h].\(^{[5,10]}\)

9. Peripheral furrow: A furrow was a groove in the corneal stroma present at the periphery of the ulcer [Fig. 1i and 1j].\(^{[10,13-15]}\)

Figure: Some typical signs of fungal keratitis; (a) feathery margins, (b) plaque, (c) Wessely ring, and (d) satellite lesions
Appendix 3: Patient flowchart. This flowchart gives the inclusion and exclusion of patients in this study.
Appendix 4: Table showing univariate and multivariate analyses of various clinical signs for diagnosis of *Pythium* keratitis

### Association of clinical signs in *Pythium keratitis*. Univariate analysis (Chi-square test)

| Signs                     | Odds ratio (95% CI) | P    |
|---------------------------|---------------------|------|
| Dry surface               | 4.529 (1.539-13.333)| 0.008|
| Full-thickness stromal infiltrate | 2.452 (0.967-6.215) | 0.093|
| Plaque                    | 0.151 (0.019-1.212) | 0.091|
| Intrastromal dots         | 23.0 (2.762-205.095)| 0.001|
| Satellite lesions         | 0.450 (0.119-1.701) | 0.366|
| Ring ulcer                | 5.0 (1.104-22.654)  | 0.066|
| Wessely ring              | 0.693 (0.603-0.797) | 0.153|
| Reticular pattern         | 54.40 (6.561-451.054)| <0.001|
| Peripheral furrow         | 19.429 (2.212-170.640)| 0.002|
| Tentacles                 | 32.0 (7.982-128.286) | <0.001|
| Endothelial exudates      | 0.917 (0.265-3.175) | 0.861|

### Multivariate analysis (multiple logistic regression)

| Signs                      | Odds ratio (95% CI) | P    |
|----------------------------|---------------------|------|
| Dry surface                | 7.981 (0.806-79.069)| 0.076|
| Plaque                     | 0.071 (0.003-1.758) | 0.106|
| Intrastromal dots          | 9.659 (0.396-235.852)| 0.164|
| Ring ulcer                 | 6.445 (0.522-79.549)| 0.146|
| Reticular pattern          | 3.797 (0.252-57.100)| 0.335|
| Peripheral furrow          | 142.733 (6.910-2948.242)| 0.001|
| Tentacles                  | 35.220 (5.490-225.929)| <0.001|
Appendix 5: Fagan’s nomogram showing the post-test odds of various clinical signs to correctly predict *Pythium* keratitis.
### Appendix 6: Area under the curve ulcer of different ulcer characteristics in the diagnosis of *Pythium* Keratitis

| Clinical feature       | Area under the curve with 95% confidence interval | $P$   |
|------------------------|--------------------------------------------------|------|
| Tentacles              | 0.78 (0.65-0.90)                                 | <0.001|
| Reticular pattern      | 0.71 (0.58-0.84)                                 | 0.001|
| Dry ulcer surface      | 0.66 (0.55-0.78)                                 | 0.014|
| Peripheral furrow      | 0.64 (0.47-0.74)                                 | 0.115|
| Intrastromal dots      | 0.62 (0.49-0.76)                                 | 0.063|
| Ring-shaped infiltrate | 0.57 (0.44-0.70)                                 | 0.282|
| Endothelial exudates   | 0.49 (0.37-0.62)                                 | 0.932|
| Satellite lesions      | 0.45 (0.32-0.57)                                 | 0.420|
| Wessely ring           | 0.42 (0.32-0.56)                                 | 0.379|
| Plaque                 | 0.42 (0.30-0.54)                                 | 0.208|