Original Article

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

Samrat Chatterjee, Deepshikha 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.

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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[3]—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 using a microbiology culture, but zoospore formation requires a difficult technique, which is not routinely practiced in most laboratories,[9–16] 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,[10–13] which is the primary target of anti-fungal drugs. Instead, a better outcome is reported with a combination of antibiotics.[18,19]

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 filaments of hyaline 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 pinhead-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

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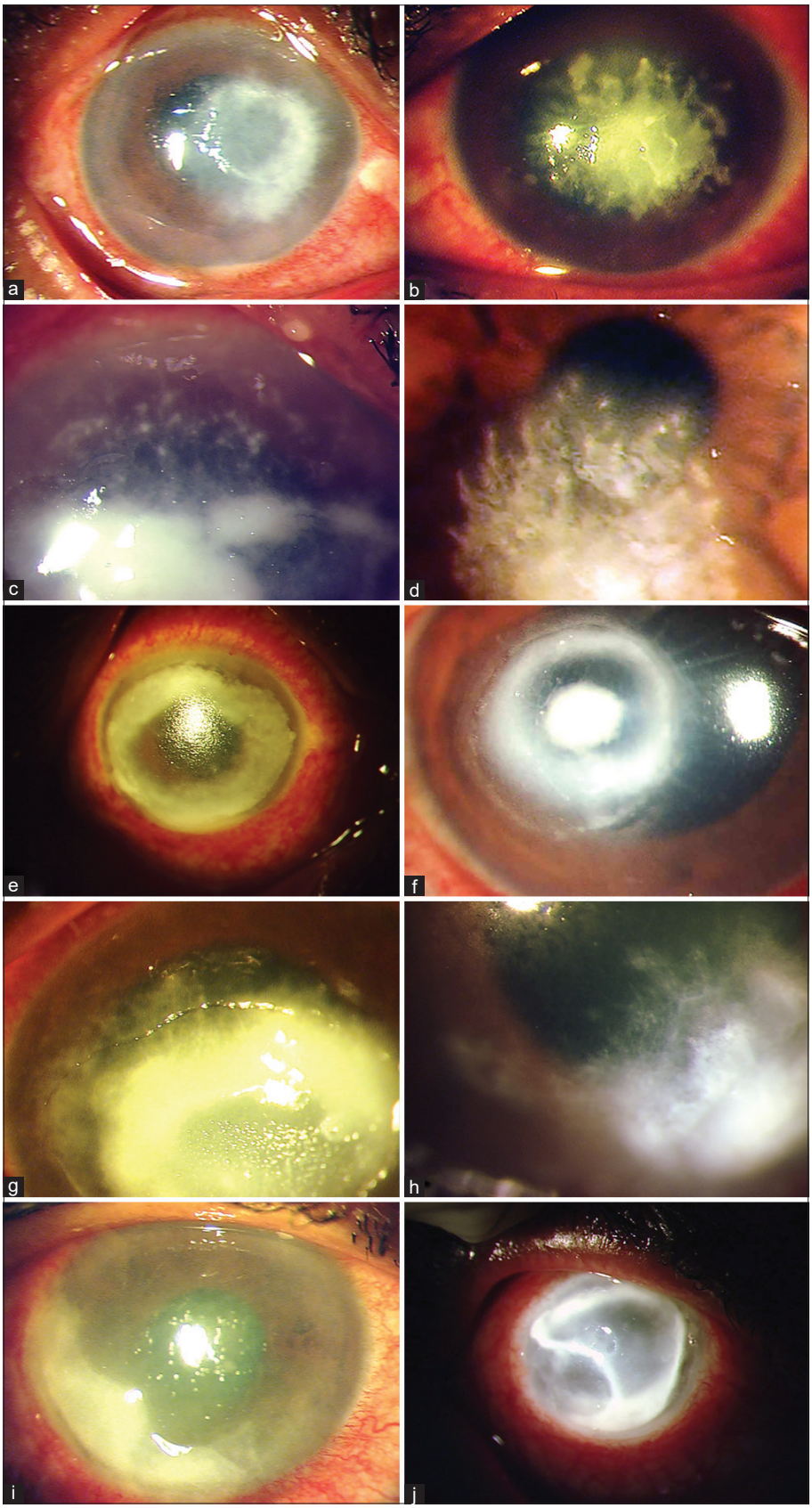

Figure 1: Slit-lamp images of the cornea in diffuse illumination in patients with *Pythium* keratitis (left column) and fungal keratitis (right column) showing the different clinical signs. Tentacles in *Pythium* keratitis (a) and in *Fusarium solani* keratitis (b). Intrastromal dots present in *Pythium* 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 (C), 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.mede.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: Comparison of demographic characteristics, risk factors and clinical features of *Pythium* and fungal keratitis

Variable	Pythium keratitis *n*=27	Fungal keratitis *n*=69	χ^2	*P*
Sex				
Male	17 (63.0)	46 (66.7)		
Female	10 (37)	23 (33.3)	0.118	0.731
Place of residence				
Rural	21 (77.8)	48 (69.6)		
Urban	6 (22.2)	21 (30.4)	0.647	0.421
Occupation				
Farmer	9 (33.3)	32 (46.4)		
Laborer	1 (3.7)	3 (4.3)		
Desk-worker	3 (11.1)	9 (13.0)		
Home-maker	8 (29.6)	19 (27.5)		
Unclassified	6 (22.2)	6 (8.7)	3.721	0.445
History of trauma				
Present	13 (48.1)	48 (69.6)		
Absent	14 (51.9)	21 (30.4)	3.842	0.050
Laterality				
Right eye	19 (70.4)	33 (47.8)		
Left eye	8 (29.6)	36 (52.2)	3.976	0.046
Location of the ulcer				
Central	10 (37.0)	33 (47.8)		
Paracentral	4 (14.8)	13 (18.8)		
Peripheral	3 (11.1)	11 (15.9)		
Total cornea	10 (37.0)	12 (17.4)	4.261	0.235
Epithelial defect				
Present	27 (100)	64 (92.8)		
Absent	0 (0)	5 (7.2)	2.064	0.151
Stromal depth involvement by ulcer				
Partial thickness	9 (33.3)	38 (55.1)		
Full thickness	18 (66.7)	31 (44.9)	3.670	0.055
Surface of the ulcer				
Dry	22 (81.5)	34 (49.3)		
Wet	5 (18.5)	26 (37.7)		
Indeterminate	0 (0)	9 (13.0)	9.179	0.010
Color of the stromal infiltrate				
Whitish-gray	20 (74.1)	46 (66.7)		
Yellow	7 (25.9)	23 (33.3)	0.496	0.481
Raised surface of the ulcer (plaque)				
Present	1 (3.7)	14 (20.3)		
Absent	26 (96.3)	55 (79.7)	4.049	0.044
Margin of the ulcer				
Well demarcated	4 (14.8)	11 (15.9)		
Hyphate	7 (25.9)	55 (79.7)		
Tentacles	16 (59.3)	3 (4.4)	38.273	<0.0001
Intrastromal dots				
Present	7 (25.9)	1 (1.4)		

Contd...

Table 1: Contd...

Variable	Pythium keratitis *n*=27	Fungal keratitis *n*=69	χ^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/ Descemetocele	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

were culture-positive for *Pythium* keratitis. The diagnosis of the infection was by both positive smears and culture in 14 patients (51.9%), only culture in 11 patients (40.7%), and culture of the corneal button in 2 patients (7.4%).

For comparison, 69 patients with culture-positive hyaline fungal keratitis, in whom the fungal species had been identified, were selected [Appendix 3]. The identified fungal species were *Aspergillus flavus* in 34 patients (49.3%), *Fusarium solani* in 29 patients (42.0%) and *Acremonium spp*. in 6 patients (8.7%).

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 ($P = 0.088$). A multivariate regression analysis [Appendix 4] identified tentacles in the ulcer margins (odds ratio (OR): 24.1, 95% CI: 3.8–158.1, $P = 0.001$) and peripheral furrows (OR: 60.6, 95%, CI: 5.1–712.3, $P = 0.001$) as independent prognosticators of *Pythium* keratitis.

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.

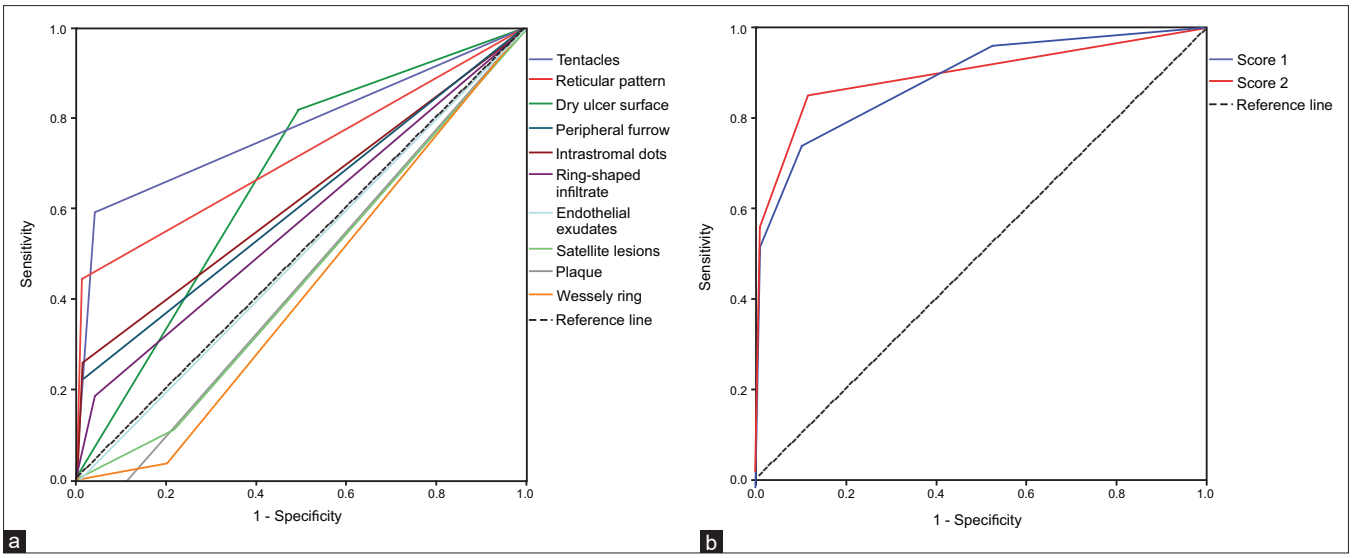

Figure 2: Receiver operative characteristic curve of clinical signs diagnostic of *Pythium* keratitis. Panel on the left (a) shows the various clinical signs (a) and panel on the right (b) shows the two different clinical scoring systems to clinically diagnose *Pythium* keratitis

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 0 67.1)	2.4 (1.6-3.4)	99.0 (8.3-99.4)	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 (98.5-99.0)	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.[9,11,13,16] 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%.[12] 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

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

Clinical score 1 (6 signs)			Clinical score 2 (5 signs)		
Total score	Sensitivity	1-specificity	Total score	Sensitivity	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

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.[28] 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[9,17] or iodine–potassium iodide–sulfuric acid,[16] 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[30,31] 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[23,24] 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

important supportive role and does not replace a microbiology investigation of the keratitis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Virgile R, Perry HD, Pardanani B, Szabo K, Rahn EK, Stone J, *et al*. Human infectious corneal ulcer caused by Pythium insidiosum. Cornea 1993;12:81-3.
2. Murdoch D, Parr D. Pythium insidiosum keratitis. Aust N Z J Ophthalmol 1997;25:177-9.
3. Krajaejun T, Pracharktam R, Wongwaisayawan S, Rochanawutinon M, Kunakorn M, Kunavisarut S. Ocular pythiosis: Is it underdiagnosed? Am J Ophthalmol 2004;137:370-2.
4. Badenoch PR, Mills RA, Chang JH, Sadlon TA, Klebe S, Coster DJ. Pythium insidiosum keratitis in an Australian child. Clin Exp Ophthalmol 2009;37:806-9.
5. Lekhanont K, Chuckpaiwong V, Chongtrakool P, Aroonroch R, Vongthongsri A. Pythium insidiosum keratitis in a contact lens wearer. Cornea 2009;28:1173-7.
6. Tanheco TY, Stacy RC, Mendoza L, Durand ML, Jakobiec FA, Colby KA. Pythium insidiosum keratitis in Israel. Eye Contact Lens 2011;37:96-8.
7. Barequet IS, Lavinsky F, Rosner M. Long-term follow-up after successful treatment of Pythium insidiosum keratitis in Israel. Semin Ophthalmol 2013;28:247-50.
8. Thanathanee O, Enkvetchakul O, Rangsin R, Waraasawapati S, Samerpitak K, Suwan-apichon O. Outbreak of Pythium keratitis in rainy season: A case series. Cornea 2013;32:199-204.
9. Sharma S, Balne PK, Motukupally SR, Das S, Garg P, Sahu SK, *et al*. Pythium insidiosum keratitis: Clinical profile and role of DNA sequencing and zoospore formation in diagnosis. Cornea 2015;34:438-42.
10. Bagga B, Sharma S, Guda SJM, Nagpal R, Joseph J, Manjulatha K, *et al*. Leap forward in the treatment of Pythium insidiosum keratitis. Br J Ophthalmol 2018;102:1629-33.
11. Agarwal S, Iyer G, Srinivasan B, Agarwal M, Kumar SPS, Therese LK. Clinical profile of Pythium keratitis: Perioperative measures to reduce risk of recurrence. Br J Ophthalmol 2018;102:153-7.
12. Agarwal S, Iyer G, Srinivasan B, Benurwar S, Agarwal M, Narayanan N, *et al*. Clinical profile, risk factors and outcome of medical, surgical and adjunct interventions in patients with Pythium insidiosum keratitis. Br J Ophthalmol 2019;103:296-300.
13. Ravula H, Lalitha P, Radhakrishnan N, Rameshkumar G, Prajna NV, Srinivasan M. Pythium keratitis in South India: Incidence, clinical profile, management, and treatment recommendation. Indian J Ophthalmol 2019;67:42-7.
14. Gurnani B, Christy J, Narayana S, Rajkumar P, Kaur K, Gubert J. Retrospective multifactorial analysis of Pythium keratitis and review of literature. Indian J Ophthalmol 2021;69:1095-101.
15. Vishwakarma P, Mohanty A, Kaur A, Das S, Priyadarshini SR, Mitra S, *et al*. Pythium keratitis: Clinical profile, laboratory diagnosis, treatment, and histopathology features post-treatment at a tertiary eye care center in Eastern India. Indian J Ophthalmol 2021;69:1544-52.
16. Mittal R, Jena SK, Desai A, Agarwal S. Pythium Insidiosum Keratitis: Histopathology and rapid novel diagnostic staining technique. Cornea 2017;36:1124-32.
17. Bagga B, Vishwakarma P, Sharma S, Jospeh J, Mitra S, Mohamed A. Sensitivity and specificity of potassium hydroxide and calcofluor white stain to differentiate between fungal and Pythium filaments in corneal scrapings from patients of Pythium keratitis. Indian J Ophthalmol 2022;70:542-5.
18. Ramappa M, Nagpal R, Sharma S, Chaurasia S. Successful medical management of presumed Pythium insidiosum keratitis. Cornea 2017:36:511-4.
19. Chatterjee S, Agrawal D. Azithromycin in the management of Pythium insidiosum keratitis. Cornea 2018;37:e8-9.
20. Saini JS, Jain AK, Kumar S, Vikal S, Pankaj S, Singh S. Neural network approach to classify infective keratitis. Curr Eye Res 2003;27:111-6.
21. Kauffman HE, Wood RM. Mycotic keratitis. Am J Ophthalmol 1965;59:993-1000.
22. Jones BR. Principles in the management of oculomycosis. Am J Ophthalmol 1975;79:719-51.
23. Dunlop AA, Wright ED, Howlader SA, Nazrul I, Hussain R, McClellan K, *et al*. Suppurative corneal ulceration in Bangladesh. A study of 142 cases examining the microbiological diagnosis, clinical and epidemiological features of bacterial and fungal keratitis. Aust N Z J Ophthalmol 1994;22:105-10.
24. Thomas P, Leck AK, Myatt M. Characteristic clinical features as an aid to diagnosis of suppurative keratitis caused by filamentous fungi. Br J Ophthalmol 2005;89:1554-8.
25. Dahlgren MA, Lingappan A, Wilhelmus KR. The clinical diagnosis of microbial keratitis. Am J Ophthalmol 2007;143:940-4.
26. Dalmon C, Porco TC, Lietman TM, Prajna NV, Prajna L, Das MR, *et al*. The clinical differentiation of bacterial and fungal keratitis. A photographic survey. Invest Ophthalmol Vis Sci 2012;53:1787-91.
27. Theodore FH, Jakobiec FA, Juechter KB, Ma P, Troutman RC, Pang PM, *et al*. The diagnostic value of ring infiltrate in acanthamoebic keratitis. Ophthalmology 1985:92:1471-9.
28. Altman DG, Bland JM. Diagnostic tests 2: Predictive values. BMJ 1994;309:102.
29. Chitasombat MN, Jongkhajornpong P, Lekhanont K, Krajaejun T. Recent update in diagnosis and treatment of human pythiosis. Peer J 2020;8:e8555.
30. Garg P, Vemuganti GK, Chatterjee S, Gopinathan U, Rao GN. Pigmented plaque presentation of dematiaceous fungal keratitis: A clinicopathologic correlation. Cornea 2004;23:571-6.
31. Sun RL, Jones DB, Wilhelmus KR. Clinical characteristics and outcome of candida keratitis. Am J Ophthalmol 2007;143:1043-5.

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 reticule (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 microcopy 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]

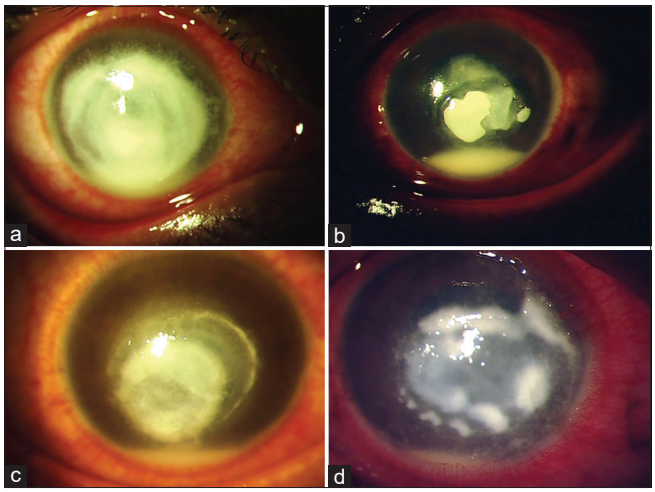
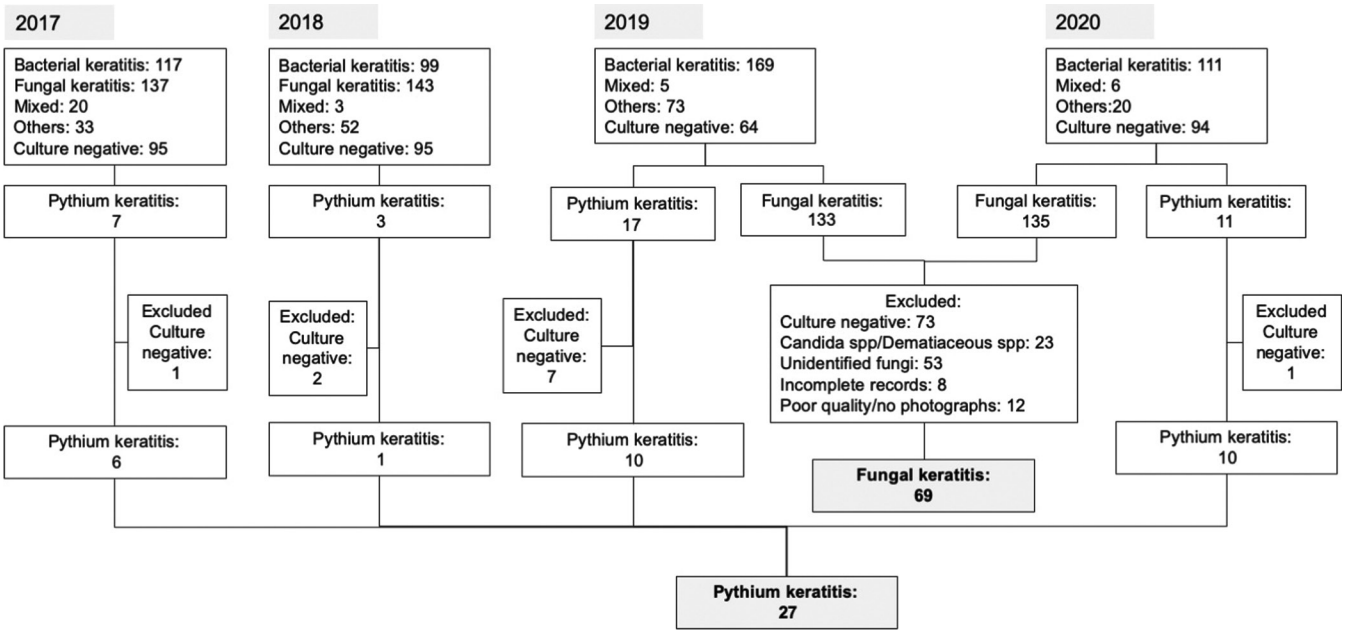


Figure: Some typical signs of fungal keratitis; (a) feathery margins, (b) plaque, (c) Wessely ring, and (d) satellite lesions

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

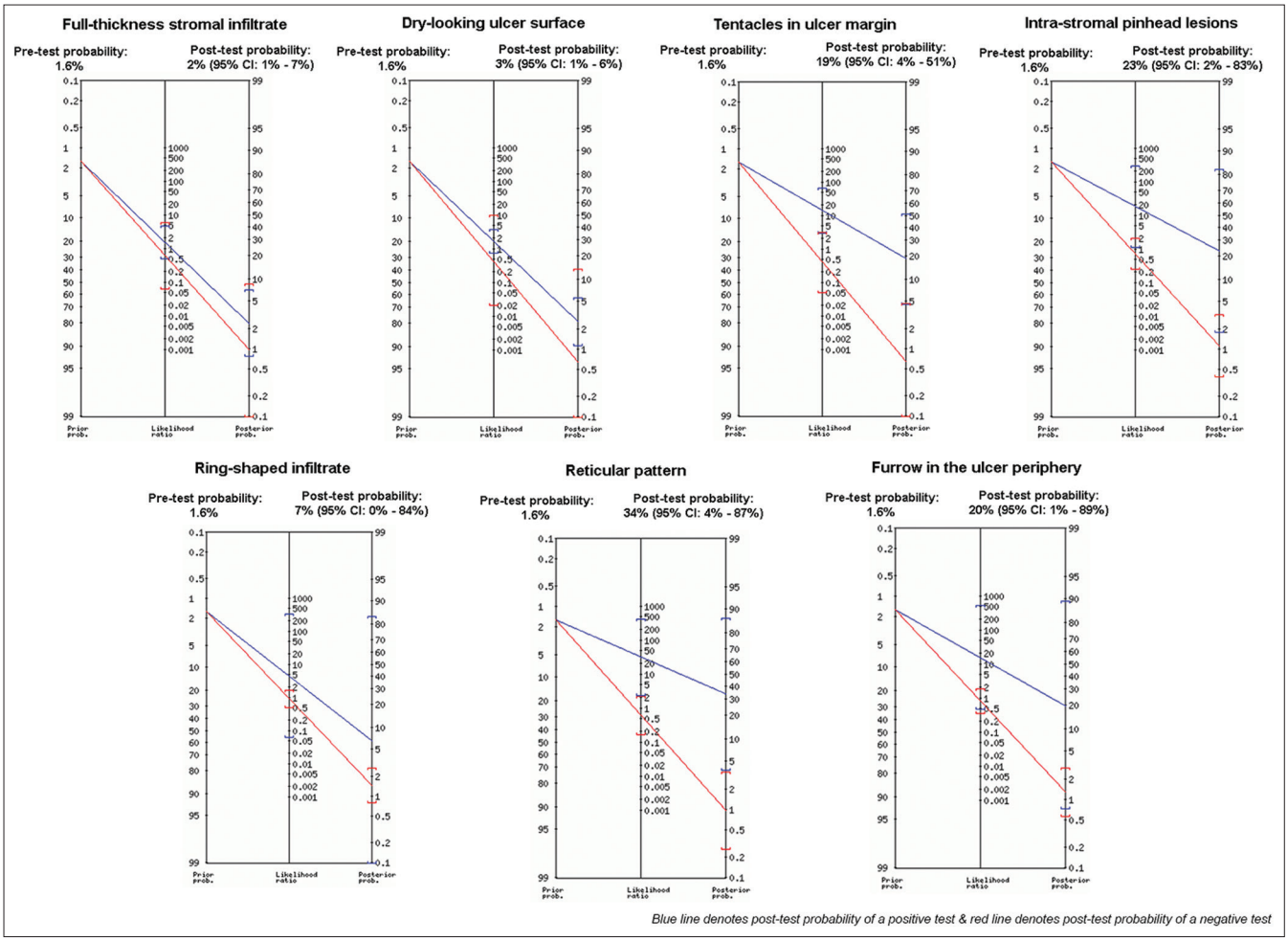
Association of clinical signs in *Pythium keratitis*. Univariate analysis (Chi-square test)

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



Blue line denotes post-test probability of a positive test & red line denotes post-test probability of a negative test

Appendix 6: Table showing the area under the curve ulcer of various ulcer characteristics

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