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Clinicopathological correlation in fungal keratitis and a possible evidence of opportunistic infection: a report of two cases

Correlação clínico-patológica em ceratites fúngicas e uma possível evidência de infecção oportunista: relato de dois casos

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ABSTRACT

The authors describe two cases of fungal keratitis which, upon histopathological examination, are found to have in common the absence of epithelium, without ulceration and the lack of integrity of the Bowman's layer with the presence of fungal forms in their interior. Through them the authors suggest probable mechanisms of recurrence of fungal keratitis, highlighting the possible existence of an "unusual external route" that would occur by the fungus penetration through the full Bowman layer. Although these findings appear not yet reported or recovered in the ophthalmic literature, the authors suggest that they could be possible opportunistic infection signals which, however, require more evidence to be considered as such.

Keywords*: keratitis; Eye infections, fungal; Cornea/histopathology, Cornea/microbiology; Opportunistic infections; Case reports*

RESUMO

Os autores relatam dois casos de ceratite fúngica, que apresentam em comum no exame histopatológico: a ausência de epitélio, sem ulceração e a integridade da camada de Bowman com a presença de formas fúngicas no seu interior. São sugeridos prováveis mecanismos de recidiva das ceratites fúngicas, ressaltando uma possível "via externa não usual", por meio da penetração do fungo através da camada de Bowman íntegra. A existência desta infecção oportunista não está relatada na literatura oftalmológica e são necessárias mais evidências para que seja considerada como tal.

Descritores: Ceratite; Infecções oculares fúngicas, Córnea/histopatologia, Córnea/microbiologia; Infecções oportunistas; Relatos de casos

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Introduction

Fungi are organisms that can cause various diseases in the human body, and the mycotic infections may happen and affect all the structures of the eye[1]. Fungal infections of the eye have been observed and reported with greater frequency during the last decades. This seems to be the result of better diagnostic methods and undestanding that some species of fungus generally considered non pathogenic or saprophytic are not able to infect the eye. Some fungi are called opportunistic pathogens. Indeed, as the eye is under study, pathogenic opportunistic fungi play a much more important role than normal pathogens, and the infection most commonly caused by opportunistic fungi are mycotic keratitis[2].

Keratomycosis was described for the first time by Leber in 1879[3], and an increase in their frequency was also described in recent decades[4-6], and this disease is still a challenge to ophthalmologists in terms of diagnosis and treatment[7-9]. Most studies about the etiology of fungal keratitis in Brazil are based on the analysis of material from corneal scrapings through direct research and/or culture[7,10,11]. Even abroad there are few works about the histopathologic features of mycotic keratitis[12]. It is important, however, to submit surgical samples of microbial keratitis cases to histopathological examination, especially if the microbiological diagnosis is unknown[13]. The histopathological examination of corneal buttons can reveal the presence of fungal elements in 75% of patients[5]. Sato et al. have shown that of 63 patients subjected to hot penetrating keratoplasty for mycotic corneal ulcer, whose corneas were received in the Brazilian Transplantation Registry between 1982 and 1988, in 24 (38.1%) the diagnosis of mycotic keratitis was only possible by histopathological examination due to negative microbiological tests[14]. Histopathological studies offer some advantages over culture the in the diagnosis of mycotic keratitis, since the contamination is avoided, the penetration of the tissue can be assessed, and the results of surgical procedures can be anticipated[15].

So, we describe two cases of fungal keratitis with recurrence after treatment with penetrating keratoplasty, and whose corneal buttons had been sent for histopathological examination to the Eye Bank of Hospital Geral de Fortaleza. We highlight the discovery of an intact Bowman'a layer with fungi inside. We also try to correlate the meaning of the findings in the histopathological examination to the clinical diagnosis and the results of microbiological analysis. By studing the possible meanings of these findings we hope to provide evidence to support the diagnosis and treatment of fungal keratitis, as well as prevent the recurrence of these infections when treated with corneal transplant for therapeutic purposes.

Description of the Cases

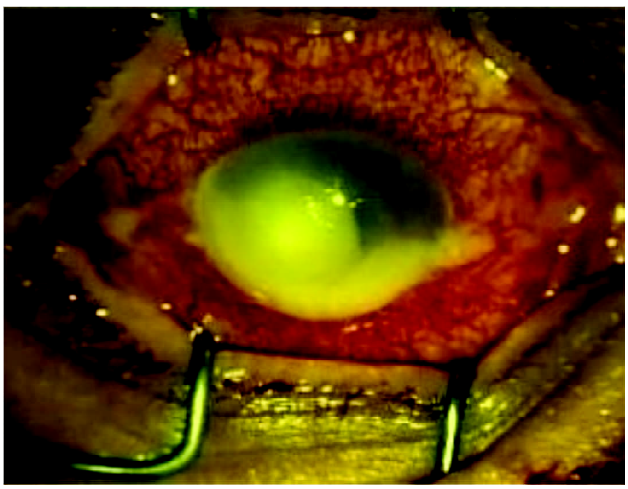
CASE 1

Male patient aged 55 years old was referred to the ambulatory of external eye diseases of Fundação de Ciência e Pesquisa Maria Ione Xerez Vasconcelos (Fortaleza – Ceará) with a history of infectious keratitis of probalbe fungal clinic etiology and complaint of pain, low visual acuity (LVA) and hyperemia in the right eye (RE) for about 30 days. He was in use of moxifloxacin eyedrops (5 mg/ml) every 2 hours, natamycin 5% eyedrops every 2 hours and oral ketoconazole 400 mg/day.

During the first appointment the lesion scraping was collected, and due to a slower clinical evolution, the hypothesis of fungal infection was kept, being prescribed moxifloxacin eyedrops (5mg/ml) every 3 hours, amphotericin B 0.15% eyedrops every hour, and oral ketoconazole 400mg/day. The patient evolved with progressive worsening (Figure 1a), being subjected to therapeutic penetrating keratoplasty. At surgery, all the visibly infected tissue was removed (Figure 1b), and then the anterior chamber was washed with 0.5% povidone-iodine. Like any urgent transplant, the tissue removed was sent for histopathological examination at the Eye Bank of Hospital Geral de Fortaleza, and subjected to routine preparation. Once fixed in 10% neutral formalin in the surgical center, the corneanos buttons are sent to the Pathological Anatomy department and cut. The inclusion is made in paraffin, with subsequent cuts of 2 µm, and the staining is made with hematoxylin-eosin. In case of doubt when confirming the diagnosis of fungal keratitis, PAS (*Periodic Acid of Schiff reaction*), methanamine silver and Gomori reaction stainings are made. After preparation, the tissues are examined with optical microscope by the authors.

For the immediate postoperative period the prescription was gatifloxacin eyedrops 0.3% every 2 hours, amphotericin B 0.15% every hour, prednisolone acetate eyedrops 1% every 3 hours, oral ketoconazole 400 mg/day and oral prednisone 40 mg/day.

In the first postoperative day (1st POD) the patient was fine and with no signs of recurrence. Meantime, he brought the result of direct research showing septate hyphae. However, in the 3rd POD we observed at biomicroscopy of the right eye an infiltrate in the temporal region superior to the graft also involving the recipient cornea. At that time, the oral corticosteroid was discontinued, and the prednisolone acetate 1% eyedrops was reduced to 2 times/day. The 10th POD showed progressive worsening (Figure 1c), so the anterior chamber was washed with 0.5% povidone-iodine. At that moment, the result of the culture was already available, showing the growth of *Fusarium sp*.. Despite the anterior chamber wash, the clinical profile continued getting worse, and the patient was submitted to a new urgent therapeutic keratoplasty 16 days after the first one. Once again, the infected tissue removed was sent for histopathological examination. The graft remained transparent until about two months of monitoring, and with no signs of recurrence of infection, although the corectopia and cataract were visible (Figure 1d).



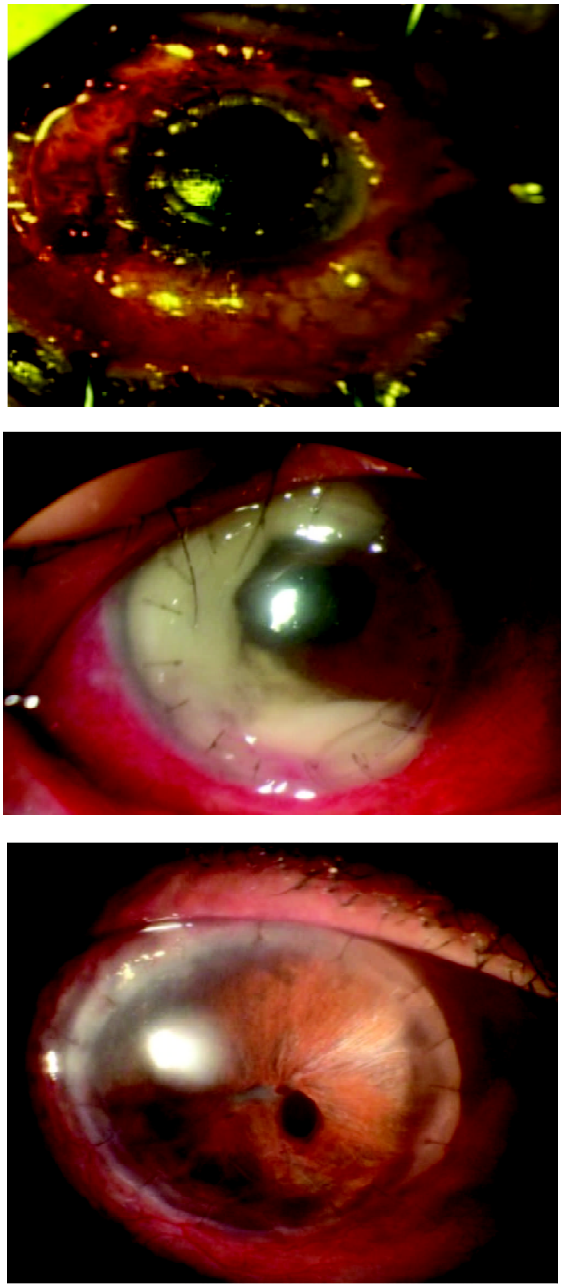


Figure 1: Clinical development of fungal keratitis and its recurrence after therapeutic penetrating keratoplasty in case 1. a) Clinical aspect of fungal keratitis that developed with progressive worsening despite clinical treatment. b) Appearance in the immediate postoperative period of therapeutic penetrating keratoplasty showing that all visibly infected tissue had been removed. c) Recurrence of infection in the postoperative period, after progressive deterioration of the clinical profile. d.) Transparent graft with no signs of recurrence of the infection in about two months of follow-up after the second therapeutic penetrating keratoplasty.

The histopathological examination of the tissue from the first keratoplasty showed fungi only in the form of yeast (Figure 2a). Ulceration was shown, with a large number of fungi on the surface. Still in the same tissue, yeasts were seen inside the Descemet, as well as yeasts, spores and inflammatory cells in the anterior chamber (Figure 2b).

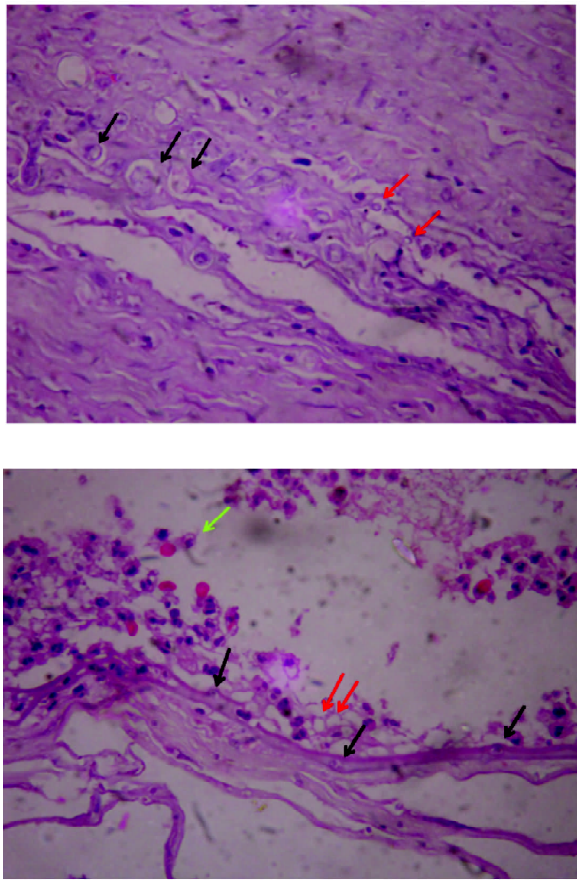
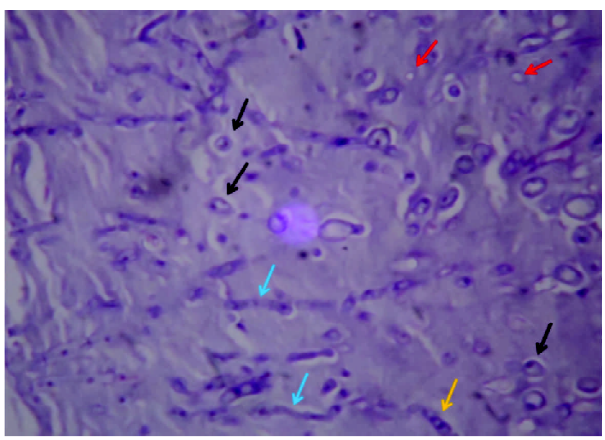


Figure 2: Histopathological examination findings in the tissue from the first keratoplasty of case 1. a) Presence of fungi only in the form of yeast. Yeasts (black arrows). Spores (red arrows). (paraffin, H.E. – 400x). b) Yeasts within the Descemet's membrane (black arrows). Translucent yeast (green arrow) and spores (red arrows) in the anterior chamber. (paraffin, H.E. – 400x).

The histopathological examination of tissue from the second keratoplasty showed fungi both in the form of hyphae and yeast, and in a much larger amount than that found in the tissue from the first surgery (Figure 3a). This second tissue also showed almost total absence of epithelium, and intact Bowman's layer and Descemet's membrane with yeasts and spores inside it (Figures 3b and 3c).



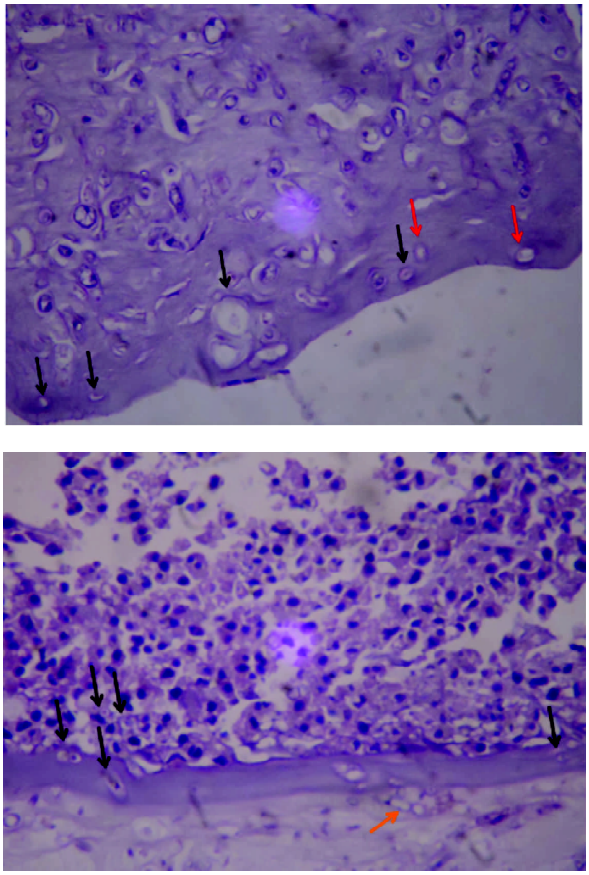


Figure 3: Histopathological examination findings in the tissue from the second keratoplasty of case 1. a) Presence of fungi only in the form of hyphae and yeast. Yeasts (black arrows). Spores (red arrows). hyphae (blue arrows). Pseudo-hyphae (orange arrow). (paraffin, H.E. –500x). b) Intact Bowman's layer with yeasts (black arrows) and spores (red arrows) inside. (paraffin, H.E. – 500x). c) Intact Descemet's membrane with yeasts inside (black arrows). Spores inside the Descemet are not marked. In the deep stroma there is a pseudo-hyphae (orange arrow). In the anterior chamber yeasts are seen (black arrows). (paraffin, H.E. – 500x).

CASE 2

Male patient aged 44 years old was referred to the ambulatory of external eye diseases of Fundação de Ciência e Pesquisa Maria Ione Xerez Vasconcelos (Fortaleza – Ceará) with a history of infectious keratitis of probalbe fungal clinic etiology for about 20 days. He was in use of natamycin 5% eyedrops every 3 hours and moxifloxacin eyedrops (5mg/ml) every 3, and showed persistent condition. Due to the lack of response to clinical treatment, he soon started therapeutic penetrating keratoplasty. At surgery, as in the previous case, all the visibly infected tissue was also removed, and then the anterior chamber was washed with 0.5% povidone-iodine. Once again, like any urgent transplant, the infected tissue removed was sent for histopathological examination at the Eye Bank of Hospital Geral de Fortaleza, and subjected to routine preparation. In the immediate postoperative period, the prescription was gentamicin eyedrops 5% every hour, moxifloxacin eyedrops (5mg/ml) every 2 hours, and oral ketoconazole 400mg/day.

However, in the 7th POD, the patient was already showing signs of recurrence of the infection, which was progressively worsening despite the maintenance of medication, having been referred to new therapeutic penetrating keratoplasty twenty-eight days after the first one. The infected corneal button removed during surgery was also sent for histopathological examination.

The histopathological examination of tissue from the first keratoplasty showed spores and yeasts predominant especially in the anterior stroma (Figures 4a, 4b and 4c). The epithelium was almost entirely absent, and the Bowman's layer was intact to its full extent (Figures 4a and 4b). No inflammatory reaction or fungi were found in the anterior chamber (Figure 4a).

The histopathological examination of tissue from the second keratoplasty also showed spores and yeast predominatly in the anterior corneal stroma (Figure 5a). Here, the Bowman's layer was also intact to its full extent, and spores could be seen inside it (Figure 5b).

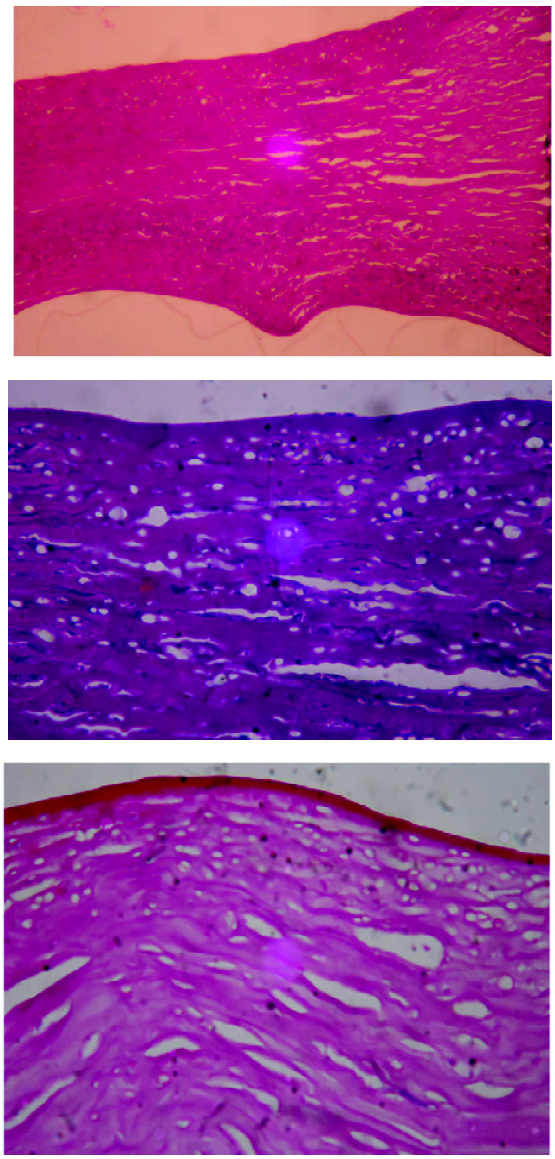


Figure 4: Histopathological examination findings in the tissue from the first keratoplasty of case 2. a) Bowman's layer intact to its full extent. Presence of spores and yeast predominatly in the anterior corneal stroma. Absence of inflammatory reaction and fungi in the anterior chamber. (paraffin, H.E. – 125x). b) Bowman's layer intact to its full extent. Presence of spores and yeast predominatly in the anterior corneal stroma. (paraffin, H.E. – 400x). c) Bowman's layer intact to its full extent. Presence of spores and yeast predominatly in the anterior corneal stroma. (paraffin, H.E. – 500x).

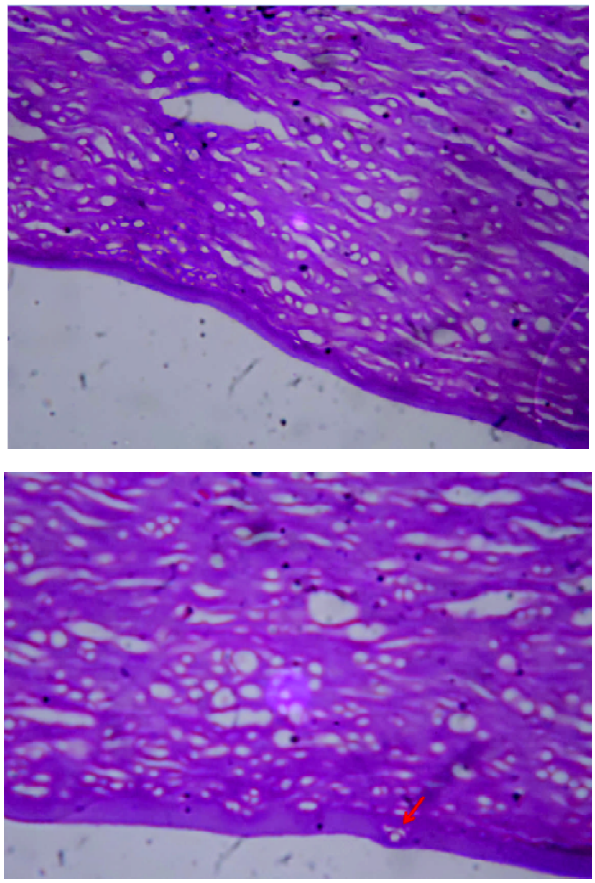


Figure 5: Histopathological examination findings in the tissue from the second keratoplasty of case 2. a) Presence of spores and yeast predominatly in the anterior corneal stroma. (paraffin, H.E. – 600x). b) Bowman's layer intact to its full extent with spores inside. Spores inside the Bowman's layer (red arrow). (paraffin, H.E. – 500x).

Discussion

In case 1, we emphasize three points: (1) the positive results of the corneal scrapings, despite the clinical treatment established, (2) the discrepancy between the results of microbiological tests (direct research and culture) of the corneal scrapings and the histopathological examination findings, and (3) how the recurrence of fungal infection happened. In case 2, we also researched how the recurrence of the infection happened.

So, in case 1, we see positive results of direct research and culture from the corneal scrapings collected before the first surgery when the patient was already under treatment with topical and systemic antifungal for some time. We proposed as an explanation for this fact the findings of the histopathological examination of fungi on the ulcerated surface of tissue injury from the first surgery. We also suggest that if the corneal ulcer scrapings had been collected from the recurrent injury, the result would be negative, since in the histopathological examination of tissue from the second surgery there were no fungi on the surface of the lesion. That fact had been previously noted. Monte e Stadtherr, studying fungal keratitis by histopathological examination findings in cases from therapeutic penetrating keratoplasty, had observed fungi on the superficial part of the piece in 45% of cases. Since those cases were from therapeutic keratoplasty, they were mostly cases treated and refractory to clinical treatment, thus suggesting that if the scrapings for microbiological studies had been collected from the lesion almost half of them could have shown positive results. Monte and Stadtherr, however, had limited clinical data from patients to provide a more accurate evidence on this fact[16].

Discrepancy between the results of microbiological tests (direct research and culture) from the corneal scrapings and the findings of histopathological examination was also evidenced in case 1. We noted that the result of the injury scraping collected before the first surgery showed filamentous fungi in the direct research (septate hyphae) and in the culture (*Fusarium sp.*); however, the histopathological examination of the tissue from the first keratoplasty showed fungi only in the form of yeast. Previous studies had already suggested possible discrepancies between microbiological tests and histopathological findings in yeast infections[16,17]. The preponderance of yeast forms in its histopathology from the literature in Brazil showing preponderance of filamentary form in study of material collected from the surface (lesion scrapings), the presence of various forms of fungi (hyphae, pseudo-hyphae and yeasts) in one tissue, and the presence of the mycelia in the ulcerated corneal surface led Monte e Stadtherr to propose that fungi take various forms during their penetration into the cornea, so that hyphae would be located more superficial and yeasts would be deeper[16]. Guarner et al. suggest that the discrepancy between the results of culture and findings in the histopathological examination could be due to morphological characteristics of fungi that have been altered by the use of antifungal medication or host responses, or due to the existence of a double infection and only a fungus be growing in the culture[17]. We are also aware that fungi are also classified according to their morphology in dimorphic, as well as yeast and filamentous[1]. The dimorphic group may have yeast or filamentous form depending on the temperature to which it is exposed[18]. However, we do not believe case 1 is a dimorphic fungus, as Fusarium sp, an admittedly filamentous fungus, grew in the culture[19]. We emphasize, however, that our objective at the moment would not be trying to justify this discrepancy, but rather to demonstrate that possibility. Based on this possibility, we then studied the current role of the corneal scraping as a guide in the treatment of fungal keratitis. The corneal scraping has been used for laboratory confirmation of fungal keratitis[7,10,11], which is suggested prior the onset of treatment[20]. The fungal form found in the scraping has also been used to guide the choice of antifungal medication to be used in the treatment. If the scraping reveals the presence of septate hyphae suggesting filamentous fungi, then there is a general agreement that natamycin 5% is the drug of choice. When yeasts or pseudo hyphae are present on the scraping, treatment with topical amphotericin 0.15% is indicated[20]. We faced a possible difference between the fungal form found in the results of microbiological tests from the corneal scraping and that found in the histopathological examination; however, we suggest that the value of the scraping was only to confirm the existence of fungal infection, and not a guide in choosing the drug to be used.

In both cases, we studied how the recurrence of fungal infection may have happened after the therapeutic keratoplasty. We suggest the existence of three routes which fungi could have traveled within the cornea and that would have contributed to the recurrence of keratitis: (1) internal route, (2) usual external route, (3) unusual external route. As the internal route (1) we

consider the penetration of the fungus in the cornea through the Descemet's membrane, thus following the posterior-to-anterior direction in the corneal stroma. We called external route (2) the route of the fungus by ulceration, corneal stroma, Descemet's membrane and endothelium to the anterior Chamber, a well recognized route and therefore called by us usual. The penetration of the fungus through the Bowman's layer, following the anterior-posterior direction in the corneal stroma, in turn, would be the unusual external route (3); a route which we haven't found description yet and which we tried to illustrate in the two cases in question. So, in case 1, we suggest the possibility of recurrence of the infection in three ways: internal route, unusual external route, or an association between internal route + unusual external route. The internal route would then be strengthened by the presence of yeasts and spores in the anterior chamber in the histopathological examination of the tissue from the first surgery. Despite washing the anterior chamber with 0.5% povidone-iodine, the spores could have remained in the anterior chamber, and the fungus could have penetrated the cornea through the Descemet's membrane. The unusual external route, in turn, would be strengthened by the discovery of an intact Bowman's layer with yeasts and spores inside in the histopathological examination of the second surgery. Fungi would have penetrated through the Bowman's layer and followed until the Descemet through this unusual route. We consider a possible association of the two routes in this case, because it would be unlikely that fungi could reach the Bowman's layer penetrating through the Descemet's membrane, since this movement direction is contrary to those normally expected in fungal penetration based on chemotaxis, thermotaxis and destruction of the extracellular matrix by fungal proteases as mentioned by Hua et al.[21]. In case 2, we suggest a probable recurrence only by the unusual external route. Once again an intact Bowman' layer with fungi (spores) inside, similar to that found in case 1, was shown now in the histopathological examination of the tissue from the second keratoplasty of case 2, strengthening the possibility of recurrence of the external route of the type taht we call unusual. The absence of fungi within the anterior chamber in the tissue of the first surgery and the presence of fungi mainly in the anterior stroma of the tissue in the second surgery also strengthen the chance of recurrence through unusual external route.

Finding an intact Bowman's layer with fungi inside it in the histopathological examination of the fungal keratitis is considered unexpected. We found reports in the literature about intact Bowman's layer in the histopathological examination in cases of fungal keratitis; however, fungi were not discribed inside, and no details on these cases were provided[12]. In fact, histopathological findings typically expected in fungal keratitis would be loss of the epithelium, Bowman's layer and variable amounts of stroma or deep stromal abscess[20]. We are aware that the epithelium can be healed in cases where there is active proliferation of fungi in the deep stroma[22], but even in those cases an intact Bowman's layer would not be an expected finding. Fungi are opportunistic agents of infection which become pathogenic under conditions of impaired immune defences, and fungal infection is unusual in the absence of precipitating factors. Trauma is a recognized risk factor for fungal keratitis[5,7,10,23], and leads to the destruction of the epithelium and the Bowman's membrane, damaging the barrier to infection[20]. In both cases in question, however, we observed the recurrence of fungal infection with the recognized barrier of the Bowman's layer, where we even stated the presence of fungi inside. We then suggest the existence of other factors that would have facilitated the penetration of the fungus through this barrier. The history of the two patients showed us that they made use of topical and/or systemic medications for a long period before and/or after the first surgery. In case 1, besides the use of topical antibiotic and topical and systemic antifungal, we observed the use of systemic and topical corticosteroids after the first surgery. Case 2, made use of topical antibiotic and topical and systemic antifungal after the first surgery. We then propose that the use of medications could have facilitated the penetration of the fungus through the Bowman's layer, and finding an intact membrane could be the signal of a possible opportunistic infection facilitated by excessive use of medications. The possibility of opportunistic corneal infections facilitated by the use of medications is supported by literature. The microbiota of the conjunctiva of normal eyes was established in the early nineteenth century[24]. Studies on the microbiota of the normal conjunctiva highlight the participation of *Staphylococcus sp*. negative coagulase, *Staphylococcus sp*. positive coagulase and *Corynebacterium sp*. as the most frequent microorganisms[25]. In Brazil, fungi isolates in healthy conjunctiva range from zero[26] to 72%[27]. The conjunctival microbiota may be the source of infectious processes when the balance between the defense processes is broken[28]. Changes in normal ocular flora have been suggested by the use of topical antibiotics[29-31], and the two types of therapeutic agents most often suspected of potentiation of fungal eye infections are corticosteroids and antibiotics[32]. Increases in the incidence of fungal keratitis have also been attributed to indiscriminate use of corticosteroids[8,33,34] and broad spectrum antibiotics[8]. So, facing the possibility in the cases studied of recurrence of fungal keratitis due to opportunistic infection facilitated by the use of medications such as corticosteroids or antibiotics, we suggest that they be used with caution in post-operative of fungal keratitis when treated with therapeutic keratoplasty.

Finally, through the study of two cases of fungal keratitis with recurrence after therapeutic penetrating keratoplasty reported here, more than just describing the histopathological findings, we demonstrated that the corneal scraping for microbiological tests can be collected even after the drug treatment is established, and that microbiological tests may show fungi in different forms from those found in histopathology. Furthermore, we suggest the possibility of recurrence of fungal keratitis after therapeutic cornea transplant through opportunistic infection facilitated by excessive use of medications in the pre- and post-operative period, which brings us to warn ophthalmologists of the need of careful use of medications during the pre- and post-surgical treatments of this type of corneal infection.

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