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# Infectious keratitis in southern Brazil: a comparison culture negative and culture positive patients

## *Ceratite infecciosa no sul do Brasil: comparação entre pacientes com cultura negativa e positiva*

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### *Abstract*

**Purpose:** *To compare clinical-epidemiological profile and treatment outcome between culture negative and culture positive keratitis patients.* ***Methods:*** *Patients with suspected infectious keratitis seen at two ophthalmic hospitals in Curitiba, Brazil, between June 2014 and April 2016, were prospectively studied. Ophthalmological exam with corneal scraping and microbiological tests were performed. Data regarding follow up, surgical interventions and treatment outcome were collected after 12 weeks of the first visit trough medical chart review. From the results of the culture, two groups were formed: culture negative keratitis (CNK) and culture positive keratitis (CPK).* ***Results:*** *According to inclusion criteria 21 patients were classified as culture negative keratitis and 20 patients as culture positive keratitis. The number of patients on antibiotic drops at the first visit was greater in CNK group (90.5% versus 60%; p=0.032). Surgical procedures were necessary in 3 patients (15%) in CNK group and in 7 patients (36,8%) in CPK group (p=0.155). Treatment success was achieved by 85% (17/20) of the patients in CNK group and by 61% (11/18) of the patients in CPK group (p=0.144). There was no significant difference between groups regarding age, gender, place of residence, presence of comorbidities, risk factors for infectious keratitis, duration of symptoms and characteristics of corneal ulcer.* ***Conclusions:*** *Previous treatment with antibiotics correlates with negative culture results. There was no significant difference in treatment outcome between culture negative and culture positive keratitis patients.*

***Keywords:*** *Infectious keratitis; Microbial keratitis; Corneal ulcer; Microbial culture; Antibiotic drops*

### Resumo

**Objetivo:** Comparar os perfis clinico-epidemiológicos e os desfechos entre pacientes com ceratite com cultura positiva e pacientes com ceratite com cultura negativa. **Métodos:** Pacientes com ceratite infecciosa, atendidos em dois hospitais oftalmológicos em Curitiba, Brasil, entre junho de 2014 e abril de 2016, foram estudados prospectivamente. Exame oftalmológico, raspado de córnea e exames microbiológicos foram realizados no primeiro atendimento. Os dados quanto a seguimento e desfecho foram coletados após 12 semanas do primeiro atendimento através de revisão de prontuário. A partir dos resultados das culturas, dois grupos foram formados: ceratite com cultura negativa e ceratite com cultura positiva. **Resultados:** Vinte e um pacientes foram classificados como ceratite com cultura negativa e 20 como ceratite com cultura positiva. O número de pacientes em uso de colírio antibiótico no primeiro atendimento foi maior no grupo de cultura negativa (90,5% versus 60%; p=0,032). Sete pacientes (37%) no grupo cultura positiva precisaram de procedimentos cirúrgicos no manejo da ceratite, versus 3 pacientes (15%) do grupo cultura negativa (p=0,155). Oitenta e cinco por cento (17/20) dos pacientes do grupo cultura negativa alcançaram sucesso no tratamento, contra 61% (11/18) dos pacientes no grupo cultura positiva (p=0,144). Não houve diferença entre os grupos quanto a idade, gênero, local de procedência, presença de comorbidades, fatores de risco, duração dos sintomas e características da úlcera de córnea. **Conclusão:** Tratamento prévio com colírio de antibiótico correlaciona-se com resultados negativos de cultura. Não houve diferença no desfecho após tratamento entre os pacientes com cultura negativa e cultura positiva.

**Descritores:** Ceratite infecciosa; Ceratite microbiana; Úlcera de córnea; Cultura microbiana; Colírios antibióticos

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## Introduction

Infectious keratitis is a serious disease of the cornea that demands an urgent attention. It is one of the most important causes of preventable unilateral blindness in the world.[1] The incidence of this condition varies from 5.3 per 100,000 persons-year in Olmsted County, Minnesota, USA to 113 per 100,000 persons-year in Madurai District, South India.[2,3] In addition, factors such as climate characteristics, occupational activity, and contact lens wear affect the type of causative agents that are more prevalent in each world region.[4] In southern Brazil, where the climate is subtropical, and the population is predominantly urban, studies indicate that bacteria is the most prevalent etiological agent and Acanthamoeba keratitis is increasing over time.[5–7]

Active corneal infection triggers inflammatory and immune responses to preserve ocular integrity, which may lead to loss of transparency and regularity of cornea. In view of this, visual acuity decreases in a large percentage of cases due to corneal scars.[8] Prompt diagnosis and specific treatment according to the etiological agent is the best path to a better visual prognosis.[9]

History of infectious exposure and clinical exam is important to determine the causative agent; yet, because there are no unequivocal characteristics of a specific pathogen, the recovery of microorganisms by culture is the gold standard of diagnosis. [10,11] Cultures have variable positivity rates among published series, usually 40 to 60%.[4,12–17]

Otri et al. and Badhange et al., in UK and India respectively, studied infectious keratitis regarding the differences on clinical features and outcomes between positive and negative culture patients.[15,18] Because of the distinct characteristics of the population, the scenario of infectious keratitis is quite different in both countries. In our pioneer study, we aimed to find out if, in tertiary centers in Curitiba, there are significative differences between culture negative and culture positive keratitis patients regarding clinical-epidemiological profile and treatment outcome.

## Methods

This prospective study followed the statements of the Declaration of Helsinki and was approved by the local research ethics committee. Patients with presumed infectious keratitis who present themselves to one of two tertiary centers of care (Hospital Evangélico de Curitiba or Hospital de Olhos do Paraná) in Curitiba, Brazil, between June 2014 and April 2016 were recruited. In the first part of the study patients were examined under the care of cornea experts; a study form was completed with data regarding clinical-epidemiological aspects and a standardized corneal scraping procedure performed for microbiological analysis. In the second part of the study, data regarding follow up, surgical interventions and treatment outcome, were collected after 12 weeks of the first visit trough medical chart review. Patients signed the informed consent agreement to participate.

Infectious keratitis was defined as a suppurative corneal infiltrate associated with an overlying epithelia defect of at least 1mm. Patients with suspected herpes virus keratitis, neurotrophic ulcers, shield ulcers, autoimmune ulcers and marginal catarrhal ulcers were excluded. Detailed information from each patient was obtained; including age, gender, place of residence, history of previous systemic and ocular conditions; history of recent ocular disease, presence of risk factors for infectious keratitis; duration of symptoms and topical treatment on course. Characteristics of the corneal ulcer, including size and location and the presence of hypopyon, were registered. The formula of the area of the ellipse was used to calculate the ulcer size (A=semi-major axis x semi-minor axis x $\varpi$); semi-major axis was defined as the half of the ulcer longest dimension and semi-minor axis was defined as the half of the ulcer smallest dimension. The location of the ulcer at the cornea was defined as central (up to 3mm from de center), peripheric (up to 3mm from the limbus) and paracentral (between central and periphery).

Corneal ulcer scraping procedure was performed as follows: after the instillation of anesthetic drops, the edges of the ulcer were scraped with a number 15 blade, and the material was smeared onto glass slides for Gram stain and directly inoculated in culture media. For each culture media a new cornea scrape was performed. The sequence of inoculation was: blood agar, chocolate agar, tioglicolate broth, Sabourad-dextrose agar, and non-nutrient agar plates seeded with Escherichia coli. All the microbiology analysis was performed in the same laboratory: laboratory of microbiology of the Hospital Evangélico de Curitiba. The blood agar, chocolate agar and tioglicolate broth were incubated at 35°C for 2 days. The Sabourad-dextrose agar was incubated at 25°C for 30 days. The non-nutrient agar plates seeded with Escherichia coli were incubated at 25°C for 21 days. The significance of the culture was based on either confluent growth (10 or more colonies) on the inoculum of one solid medium, and/or growth in more than one medium and/or growth in one medium consistent with direct smear result and/or for Acanthamoeba, the presence of trophozoite trails or cysts observed on the non-nutrient agar plates under x10 objective lens. All bacterial and fungal isolates were identified by using standard laboratory techniques (colony characteristics, Gram and KOH stain, morphology, rate of growth, color, consistency, texture, microscopic features and results of biochemical tests).

Patients with suspected bacterial keratitis were treated with fourth generation fluoroquinolones or a combination of fortified antibiotics (cefazolin 50 mg/mL and gentamicin14 mg/mL or vancomycin 50mg/ml, and amikacin 33mg/ml,) hourly. Natamycin 5% or amphotericin b 0.15% were added when fungal keratitis was considered. If Acanthamoeba keratitis was suspected Polyhexamethylene biguanide 0.02% was started hourly. Fungal and acanthamoeba corneal ulcers were scraped regularly to help with healing.

Culture negative keratitis group (CNK) was formed by patients with clinical features of infectious keratitis who had none micro-organism either in smear or culture. Culture positive keratitis group (CPK) was formed by patients with clinical features of infectious keratitis who fulfilled at least one criterion of significant growth. The number and type of surgical procedures needed were registered. Treatment success was defined as complete healing of the ulcer within 12 weeks of the first visit. Treatment failure was defined as persistent corneal ulcer, perforation, need for emergency penetrating keratoplasty (ePK) or globe evisceration.

The comparison of the groups defined by culture results was performed using Student's t test or the non-parametric Mann-Whitney test. Fisher's exact test or Qui-square test was used for the analysis of categorical data. The normality condition of continuous quantitative variables was evaluated by Shapiro-Wilk test. $P \leq 0.05$ was considered significant. The data was analyzed using the Software Stata/SE v. 14.1. StataCorpLP, USA.

## Results

Sixty-three patients were studied. All cases were unilateral. Twenty-one patients fulfilled the criteria for the negative culture keratitis group (CNK) and 20 fulfilled the criteria for the positive culture keratitis group (CPK). A total of 10 patients were excluded because although the culture did not recovery any microorganism, the smear was positive; and 12 patients were excluded because culture recovered microorganisms but did not fulfilled the criteria of significance.

In CNK group the mean age was 45.8 years (SD=19.3) (15 to 89 years) and in CPK group 51.2 years (SD=12.7) (31 to 79 years) (p=0.3). The male to female ratio was 1,1:1 in CNK group and 4:1 in CPK group (p=0.1). The majority of patients in both groups lives in urban areas (76,2% in CNK and 65% in CPK; p=0.5). Systemic diseases were reported by 2 patients in CNK group and by 6 patients in CPK group (p=0.12). Ocular comorbidities were present in 9 patients with CNK (42%) and in 6 patients (30%) with CPK. Risk factors for infectious keratitis were present in 12 patients (57%) in CNK group and in 15 patients (75%) in CPK group (p=0.32); table 1.

**Table 1**
**Comparison of different comorbidities and risk factors between culture negative and culture positive patients**

| Systemic comorbidities | Culture negative | Culture positive |
|---|---|---|
| **Arterial hypertension (AHT)** | 1 | 3 |
| **Diabetes Melitus (DM)** | 1 | 1 |
| **AHT + DM** | 0 | 2 |
| **Ocular comorbidities** | | |
| Ocular allergy | 2 | 0 |
| Keratoconus | 1 | 0 |
| Penetrating keratoplasty | 1 | 1 |
| Aniridia/aphacia | 1 | 0 |
| Glaucoma | 1 | 2 |
| Glaucoma + amaurosis | 0 | 2 |
| Macular scar | 0 | 1 |
| High myopia | 1 | 0 |
| Facial paralysis | 1 | 0 |
| **Risk factors** | | |
| Contact lens | 6 | 6 |
| Ocular trauma | 3 | 5 |
| Contact lens + trauma | 1 | 0 |
| Bullous keratopathy + contact lens | 1 | 2 |
| Exposure keratopathy | 1 | 0 |
| Chronic use of corticosteroids | 0 | 2 |

The mean duration of symptoms at the first visit was 19.1 days (SD=35.2) (2 to 150 days) in CNK group and 20.1 days (SD=35.4) (1 to 150 days) in CPK group (p=0.98). Of the 21 patients in CNK group only 2 were not on topical ocular medication at the first visit; 18 were on antibiotic drops and 1 was on a combination of corticosteroid and antibiotic drops. The number of patients on antibiotic drops at the first visit was significantly greater in CNK group (90.5% versus 60%; p=0.032). Of the 20 patients in CPK group 7 were only on antibiotic drops, 3 were only on corticosteroid drops, 3 were on a combination of corticosteroid and antibiotic drops, 1 were on a combination of antibiotic drops and acyclovir ointment and one on a combination of antibiotic drops, acyclovir ointment and corticosteroid. The microbiology results of the 12 patients previous treated with antibiotics in the culture positive keratitis group are show in table 2. The number of patients on corticosteroid drops at the first visit was significantly greater in CPK group (35% versus 4.76%; p=0.02).

**Table 2**
**Microbiology results of the 12 patients previous treated with antibiotics in the culture positive keratitis group**

| Duration of symptoms * | Previous treatment (n=12) | Smear result | Culture result |
|---|---|---|---|
| 150 | Moxifloxacin, pdn1% | Negative | Acanthamoeba sp. |
| 10 | Moxifloxacin, acyclovir, pdn1% | Amoeba cysts | Acanthamoeba sp. |
| 14 | Moxifloxacin, 0.1% dexamethasone | Negative | Non-identified yeasts |
| 21 | Moxifloxacin | Negative | Coagulase negative Staphylococci |
| 2 | Moxifloxacin | Gram-negative bacilli | Pseudomonas sp. |
| 10 | Moxifloxacin | Negative | Aspergillus fumigatus |
| 60 | Ofloxacin, PHMB | Negative | Acanthamoeba sp. |
| 10 | Ciprofloxacin, acyclovir | Hyphae | Acremonium sp |
| 30 | Moxifloxacin | Hyphae | Phoma spp. |
| 9 | Gatifloxacin 0.5%, 0.1% dexamethasone | Hyphae | Fusarium sp. |
| 10 | Vancomycin, gentamicin | Hyphae | Aspergillus niger |
| 20 | Gatifloxacin 0.5% | Hyphae | Fusarium sp. |

The results of the smear examination report were: Gram-positive cocci identified in 2 of 20 (10%); Gram-negative bacilli in 5/20 (25%); fungi hyphae in 6 of 20 (30%), and amoeba cysts in 1 patient (5%). Based on culture reports and criteria of significance 9/20 (45%) were diagnosed as pure bacterial keratitis, 8/20 (40%) were diagnosed as pure fungal keratitis and 3/20 (15%) were diagnosed as pure acanthamoeba keratitis. The most frequent bacterial isolate identified were Pseudomonas sp.

**Table 3**
**Microbial isolates from 20 cultures**

| Bacteria n=9 | Number of patients |
|---|---|
| Streptococcus pneumoniae | 2 |
| Staphylococcus coagulase-negative | 1 |
| Staphylococcus aureus | 1 |
| Pseudomonas sp. | 3 |
| Moraxella sp. | 1 |
| Serratia marcescens | 1 |
| **Fungi n=8** | |
| Fusarium sp. | 3 |
| Aspergillus fumigatus | 1 |
| Aspergillus niger | 1 |
| Phoma sp | 1 |
| Acremonium sp. | 1 |
| Non-identified yeast | 1 |
| **Amoeba** | **3** |

and Streptococcus pneumoniae (Table 3). In CPK group topical antimicrobials were prescribed according to culture results. Topical medication prescribed for patients with CNK can be seen in table 4.

**Table 4.**
**Surgical procedures and outcomes of patients with negative culture results regarding treatment regimens employed (n=21)**

| | Previous medication | Medication prescribed after culture results | Surgical procedures and outcome |
|---|---|---|---|
| Started medication only after corneal scrapings | ‘ | (n=2) 4-generation Fluoroquinolone | Cure (n=2) |
| Continued medication on previous use | (N=13) 4-generation fluoroquinolone | (n=13) 4-generation Fluoroquinolone | Cure (n=11) BCL (n=1) Epk (n=1) |
| Change or association of medication | (N=1) Moxifloxacin + 0.1% Dexamethasone | (n=1) Moxifloxacin | Cure (n=1) |
| | (N=3) Moxifloxacin | (N=1) Moxifloxacin + Natamycin 5% | Cure (n=3) |
| | | (N=1) Cefazolin + Gentamicin + Amphotericin b | |
| | | (N=1) vancomycin | |
| | (N=2) Gatifloxacin 0.5% | (N=1) Gatifloxacin 0.5% + Amphotericin b | Cure (n=1) |
| | | (N=1) Vancomycin + Amikacin | Epk + globe evisceration (n=1) |

n=number of patients; BCL =tissue adhesive with bandage contact lens; ePK = emergency penetrating keratoplasty;

The mean size of the corneal ulcer area was 7.1 (SD=7,3) (1 to 24.7) in CNK group and 10.7 (SD=11.3) (1 to 50.2) in CPK group (p=0.165). In both groups, the location of the ulcer in the cornea was central most of the times (14/21 in CNK and 13/20 in CPK). There were 6 paracentral ulcers in each group and 1 peripheric ulcer in each group. Hypopyon was present in 4 patients (19%) in CNK group and in 8 patients (40%) in CPK group (p=0.181); table 5.

**Table 5**
**Comparison of corneal ulcer features between groups of culture negative and positive infectious keratitis**

| Corneal ulcer characteristics | Culture negative patients | Culture positive patients | p-value |
|---|---|---|---|
| Size (average area) | 7.1 ±7.3 (1-24.7) | 10.7 ±11.3 (1-50.2) | 0.165 |
| Location | | | |
| Central | 14 | 13 | |
| Paracentral | 6 | 6 | |
| Peripheric | 1 | 1 | |
| Hypopyon | 4 | 8 | 0.181 |

Surgical procedures were necessary in 3 patients (15%) in CNK group and in 7 of 19 patients (36,8%) in CPK group (p=0.155); table 6. Surgical procedures performed in CNK patients were: tissue adhesive with bandage contact lens in one patient because of a small corneal perforation; emergency penetrating keratoplasty (ePK) performed in two patients: one of them remained with a clear graft until the last follow up (12 weeks), the other patient had his graft failed and a second penetrating keratoplasty performed 8 weeks later. Unfortunately, because of an extensive ocular inflammation, excruciating pain and lack of epithelization it was opted for globe evisceration. The anatomopathological report indicated the presence of Mycobacterium kansasii in the tissue. Surgical procedures performed in CPK patients were: anterior chamber wash with amphotericin B performed in 3/20 (15%), ePK in 2/20 (10%), (one of them had undergone anterior chamber wash without success), and globe evisceration in 3/20 (15%). Two of the 3 patients who required globe evisceration were cases of fungal keratitis, one per yeast and another per acremonium sp. Both patients reported recent ocular trauma. The other was a case of moraxella sp. keratitis in a patient on chronicle use of corticosteroid drops with amaurosis because of glaucoma.

**Table 6**
**Comparison of surgical procedures and treatment response between groups**

| | Culture negative patients | Culture positive patients | p-value |
|---|---|---|---|
| **Surgical procedure** | | | |
| BCL | 1 | 0 | |
| ePK | 1 | 1 | |
| Globe evisceration | 0 | 3 | |
| AC wash | 0 | 2 | |
| ePK and globe evisceration | 1 | 0 | |
| **AC wash + ePK** | 0 | 1 | |
| **Total** | **3** | **7** | **p=0.155** |
| **Treatment response** | | | |
| Treatment success | 17/20 (85%) | 11/18 (61%) | P= 0.144 |
| Treatment failure | 3/20 (15%) | 7/18 (39%) | |
| **Lost follow up** | 1 | 2 | |

BCL = tissue adhesive with bandage contact lens
ePK = emergency penetrating keratoplasty
AC wash = anterior chamber wash with amphotericin b

Treatment success was achieved by 85% (17/20) of the patients in CNK group and by 61% (11/18) of the patients in CPK group (p=0.144); table 6. In CNK group 15% (3/20) of the patients were classified as treatment failure (corneal perforation, emergency PK and globe evisceration) and one lost follow up right after corneal scraping procedure. In CPK group 39% (7/18) were classified as treatment failure; two of them presented persistency of the corneal ulceration until the last follow up visit (12 weeks), two patients required emergency penetrating keratoplasty because of corneal melt and three patients required globe evisceration. Two patients lost follow up, one of them after a week post-operative anterior chamber wash.

## Discussion

Infectious keratitis can be managed empirically or guided by culture results. Most community-acquired bacterial ulcers resolve with broad spectrum empiric therapy.[19] On the other hand, patients referred for evaluation in tertiary hospitals in developing countries have at least two peculiarities: they usually have the condition for a longer time, often more than a week, and they are most commonly already been treated with antibiotic drops.[9] Sixty to ninety percent of our patients were on antibiotics before corneal scrapings; our average period of symptoms before presentation was 19 to 20 days; this contrasts with the average of 4.7 days reported in UK.[15] In this scenario the role of corneal scrapings and laboratorial diagnosis cannot be overlooked. Dalgreen at al. evaluated the ability of ophthalmologists to predict laboratorial results of infectious keratitis, and they concluded that clinical judgment of the type of the infectious agent is impaired when the patient has been previously treated for some time.[10]

In our study we could observe that the culture negative keratitis group had a significant higher number of patients previous treated with antibiotics. The same finding were reported by Van der Meulen et al. and Dahlgreen et al.[10,20] Yet, McDonnell et al. found no difference in the rates of negative cultures between patients who were treated with antibiotics before culture and those who were not.[21] Other publications reported the same findings.[5,15,22] The correlation between previous use of antibiotics and higher rate of negative cultures is controversial.[10,15,18,20,21,23] The reason for this is the differences in the population studied and the methodology adopted; for instance: type of pathogens recovered, class of medication used, duration of previous treatment and time of culture incubation. Bhadange et al., reported a longer duration of symptoms and a longer duration of previous treatment in negative culture patients.[18] When we selected only patients on previous use of antibiotics, with positive culture result, the microorganisms recovered by culture were mostly fungi and Acanthamoeba. We believe that antibiotics interfere with the load of viable bacteria in the corneal ulcer, impairing culture recovery and do not interfere with culture of fungi or amoeba. Similar findings were reported by Maragon et al., although they found no statistical difference in the culture positivity rate between pretreated and non-pretreated patients, they found a delay in the recovery of pathogens and a higher frequency of fungi and Acanthamoeba in the pretreated group.[23]

In our culture positive group, the most frequent recovered microorganisms were bacteria, followed by fungi and amoeba. Bacteria are the most common pathogens recovered by culture in the majority of publications, but fungi exceeds bacteria in some studies which is explained by hot climate and the prevalence of agricultural activity.[4,24,25] Our recovery rate of Acanthamoeba was higher than that reported in the majority of the published series of infectious keratitis.[4,5,7,12,13,16] Many infectious keratitis studies did not include search for amoeba. Amoeba keratitis is an emerging cause of keratitis in contact lens users but have also been reported in patients with history of ocular trauma and even in patients without know risk factor.[6] It is really important to include the search for Acanthamoeba sp. in tertiary centers of care, because of the severity of the keratitis, the specificity of antimicrobial required and the long duration of treatment. We believe that the difference in the most common type of infectious keratitis pathogen in each country is one of the main reasons for the contrasts found between the published studies. In the study of Otri et al, performed in UK, the most recovered pathogens were bacteria and Acanthamoeba, they found no fungi. On the other hand, in the study of Bhadange et al.,[18] performed in India, fungi were by far the most frequent pathogens; although they included search for Acanthamoeba keratitis, they did not have any case.

We couldn't find any significant difference between groups regarding size and location of the corneal ulcer, or the presence of hypopyon. Morlet et al. investigated the risk factors for positive cultures of microbial keratitis. They found that ulcers larger than 5mm2 increase in 2,68 times the chance of a positive culture result (IC 95% 1,80 – 3,99).[26] However, other studies comparing clinical characteristics, such as infiltrate size, presence of hypopyon, localization and depth, with culture positivity found no association between them.[9,15,18]

The number of patients who needed a surgical procedure was slightly lower in CNK group, although not statistically significant. Other publications could find a correlation between culture positive patients and increased number of surgical procedures, although in one of the studies, other variables as old age (>60 years) and ulcer size were also implicated.[18,26] In concordance with previous published reports in other countries, we could not find a significant difference in the percentage of patients who achieved treatment success between culture negative and culture positive groups.[15,18] Despite the lack of identification of an infectious agent, patients in culture negative

group had no worse outcome. We believe that when the corneal scraping procedure is well performed, and microbiological analysis encompasses the most know keratitis pathogens, a negative result discards cases of fungi and amoeba, which usually have the worse prognosis in the context of developing countries. On the other hand, it seems that culture negative patients have in average less aggressive clinical cases, smaller ulcers and less hypopyon. That could indicate that these patients are mostly bacterial keratitis already in process of resolution at the time of corneal scraping, since almost all of them were being treated with antibiotics. Nevertheless, if a patient with a negative culture is not getting better, its critical to repeat corneal scrapings and/or perform corneal biopsy, to rule out fungi, Acanthamoeba and atypical mycobacteria keratitis. One of our culture negative patients required globe evisceration and the anatomopathological exam evidenced the presence of mycobacterium kansasii in the tissue. Our microbiology laboratory doesn't include the search for mycobacteria in infectious keratitis routine protocol. Mycobacterial keratitis is rare.[27] Some case series were reported relating mycobacteria to outbreaks of infective keratitis after refractive surgery.[28] When untreated, these patients may have devastating outcome as we could see here. The ophthalmologist must suspect mycobacterial infection in slowly progressive cases with no response to treatment. The corneal scraping should be repeated, and specific stains such as the Ziehl–Neelsen acid fast stain and culture on specific media such as the Lowenstein–Jensen media should be ordered. The patient mentioned above, had a previous therapeutic penetrating keratoplasty and histopathological examination of the cornea didn't evidence any microorganisms.

One of the limitations of the present study was the small sample size. Since our goal was to compare negative and positive culture keratitis patients, we were careful to exclude cases that the positivity or negativity of the culture was not certain. Yet, some presentations of herpes stromal keratitis are difficult to differentiate from bacteria, fungi or amoeba and it is possible that some patients of the culture negative keratitis group were cases of herpes, once herpes search was not performed. The ideal laboratory diagnosis protocol should include polymerase chain reaction for herpes virus.

## Conclusions

Previous treatment with antibiotics correlates with negative culture results. There was no significant difference in treatment outcome between culture negative and culture positive keratitis patients.

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