Confocal microscopy as an aid in the diagnosis of keratitis due to *Candida parapsilosis*

Microscopia confocal como auxílio no diagnóstico de ceratite por *Candida parapsilosis*

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ABSTRACT

To describe a case of treatment-resistant corneal infiltrate in a patient 4 months after penetrating keratoplasty. The etiological diagnosis was established through microbiology and in vivo corneal confocal microscopy. A 80-year-old female patient presented with stromal infiltrate in the donor corneal bud 4 months following penetrating keratoplasty. Initially, the corneal culture was positive for *Candida parapsilosis*. In vivo corneal confocal microscopy demonstrated approximately 4 µm hyperreflective spherical microorganisms with a suggestive *Candida spp*. A second corneal culture was positive for *Candida sp.*, and hyphae presence in the analyzed material was observed. The patient remained refractory to therapy. Confocal microscopy of the cornea continues to grow as a useful, non-invasive diagnostic method and in monitoring response to therapy. This report contributes by detecting *Candida sp*. in in vivo corneal confocal microscopy to aid in the diagnosis of keratitis.

RESUMO

Relatar caso de infiltração corneana resistente ao tratamento em um paciente 4 meses após o retransplante da córnea. O diagnóstico etiológico foi estabelecido por microbiologia e microscopia confocal da córnea. Uma paciente de 80 anos de idade apresentou infiltrado estromal no botão corneano doador 4 meses após o retransplante da córnea. Inicialmente, a cultura da córnea era positiva para *Candida parapsilosis*. A microscopia confocal da córnea demonstrou micro-organismos de aproximadamente 4 µm esféricos e hiper-refletivos sugestivos de *Candida spp*. Uma segunda cultura de córnea foi positiva para *Candida sp.*, e foi observada a presença de hifas no material analisado. A paciente permaneceu refratária à terapia. A microscopia confocal da córnea continua a crescer como um método de diagnóstico útil e não invasivo e no monitoramento da resposta à terapia. Este relato contribui por meio da detecção da *Candida sp*. na microscopia confocal da córnea para auxiliar no diagnóstico das ceratites.

INTRODUCTION

Corneal fungal infections are predominantly caused by filamentous fungi, specifically *Fusarium spp.* and *Aspergillus spp.*, accounting for approximately 95% of cases. Yeasts, particularly *Candida spp.*, also contribute to these infections. *Fusarium spp.* and *Aspergillus spp.* species are more prevalent in tropical and subtropical regions, whereas Candida species are more commonly encountered in temperate climates.[1]

Fungal keratitis typically presents with a prolonged and subacute clinical course. Symptoms may include pain, visual blurring, hyperemia, tearing, and photophobia. If left untreated, the infection can progress, leading to complications such as corneal ulceration, opacity, and potentially endophthalmitis.[2]

The incidence of fungal keratitis (FK) surpasses a million eyes affected each year worldwide, probably 1.4 million.[3] Although FK is a rare complication after corneal transplantation 0.16% incidence,[4] its effects can be devastating.

There are several risk factors for FK in corneal transplant recipients, such as altered normal ocular surface flora, chronic use of antibiotics and corticosteroids, eyelid abnormalities, sutures and malnourished or immunocompromised receptor.[5]

Traditional methods of diagnosis include culture and invasive corneal biopsy.[6] Delayed or false negative cultures can lead to a delay in the diagnosis of FK.[7]

The high rate of vision loss following corneal fungal infections, and the limitations associated with traditional diagnostic methods reveal the need for quicker and less invasive diagnostic options.[8] In this scenario, in vivo confocal microscopy (IVCM) is becoming a more valuable diagnostic tool.

We aimed to describe a case of *Candida parapsilosis* diagnosed by IVCM whereas treatment-resistant after penetrating keratoplasty.

CASE REPORT

A 43-year-old female patient, admitted to the service on February 5, 1980.

The patient underwent the following surgical procedures on the left eye: topoplasty in 1983, corneal hypertrophic membrane peel in 1994; non-penetrating profound sclerectomy in 1999; cataract surgery associated with keratoplasty in 2003.

The transplanted cornea developed cloudiness and best corrected visual acuity (BCVA) from 20/200 according to Snellen's chart, resulting in the patient undergoing a new corneal penetrating keratoplasty on November 12, 2016.

Four months after the surgery, the patient sought consultation complaining of ocular discomfort and conjunctival hyperemia. Upon examination, a corneal infiltrate was observed affecting the donor rim stroma and 0.3% gatifloxacin (Zymar®) was started. Fifteen days after beginning the treatment, the infiltrate decreased, did not blush, and pigmented keratic precipitates were noted on the endothelium posterior to the affected area (Figure 1A). On April 11, 2017, superficial corneal scraping was performed on the area of cellular infiltrate for microbiological analysis and culture. Although culture results were positive for *C. parapsilosis*, microbiological analysis was negative. On April 27, 2017, the patient returned with eyelid edema, 4 + / 4 + conjunctival hyperemia, and ulcer deepening. Topical pimaricin 2%, gatifloxacin 0.3% (Zymar®), and prednisolone 1% were started, along with oral ciprofloxacin at 500 mg orally 2 times daily. On July 27, 2017, increased cellular infiltration was noticed axially along with endothelial folds (Figure 1B). On this occasion, 0.3% ciprofloxacin and 0.1% dexamethasone (Maxiflox D®) were started and the pimaricin concentration was increased to 4%. The stromal lesion progressed to a whitish lesion with spiculated edges (Figure 1C).

On October 26, 2017, Confocal Microscopy was performed (Heidelberg Engineering, Spectralis OCT) in which hyperreflective spherical microorganisms of approximately 4 µm were observed, suggestive of yeast (Figures 2A and 2B). On December 2, 2017, a second corneal scrape was performed and confirmed the diagnosis of *C. parapsilosis*, followed by an intrastromal injection of voriconazole 2 mg/0.1 mL. The second superficial culture was positive for *Candida* sp. with growth on blood agar and chocolate agar associated with a microbiological plaque and the presence of yeast (Figure 3).

Four months after the voriconazole intrastromal injection, the patient had persistent infiltrate and a 20/100 BCVA. Glaucoma therapy, 1% amphotericin eye drops four times a day, and ketoconazole 400 mg (oral intake) daily were being administered. The patient did not respond to clinical treatment, and intra-stromal voriconazole injection were needed and had to have a new penetrating keratoplasty.

Approved by the following research ethics committee: *Instituto Suel Abujamra*, São Paulo, Brazil (CAAE # 68297822.7.0000.5477).

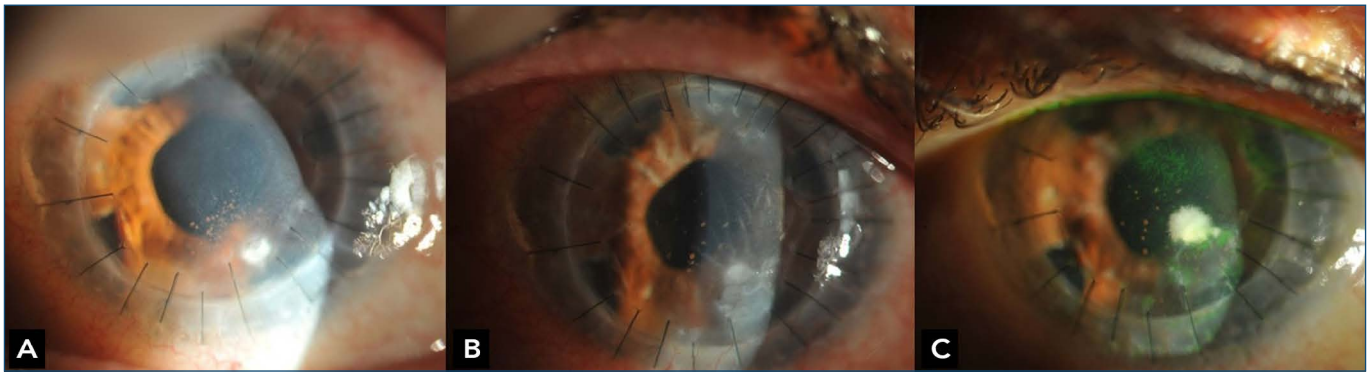

Figure 1. Slit lamp biomicroscopy of the patient's cornea at different stages of presentation. (A) Peripheral corneal infiltrate associated with pigmented keratic precipitates 10 days following corneal penetrating keratoplasty performed on November 12, 2016. (B) Corneal infiltrate associated with endothelial folds and pigmented keratic precipitates on July 7, 2017. (C) A white stromal corneal lesion with spiculated edges associated with pigmented keratic precipitates on October 19, 2017.

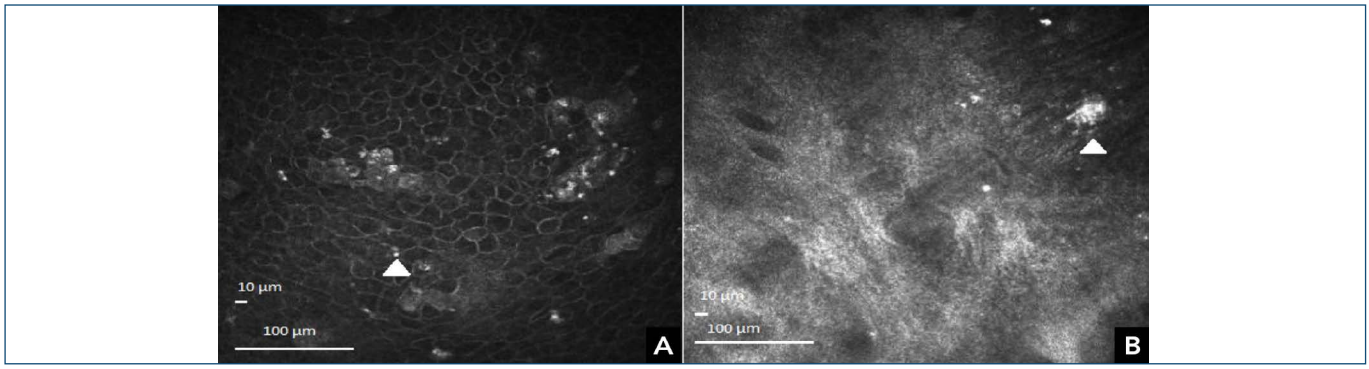

Figure 2. Confocal microscopy of keratitis caused by yeast. (A) A monolayer of corneal epithelial basal cells with an approximately 4 µm hyperreflexive spherical microorganism suggestive of yeast (arrowhead). (B) The anterior stromal region with fibrosis and collagen fibril disorganization, showing a cluster of hyperreflective spherical microorganisms measuring approximately 4 µm (arrowhead), suggestive of yeast.

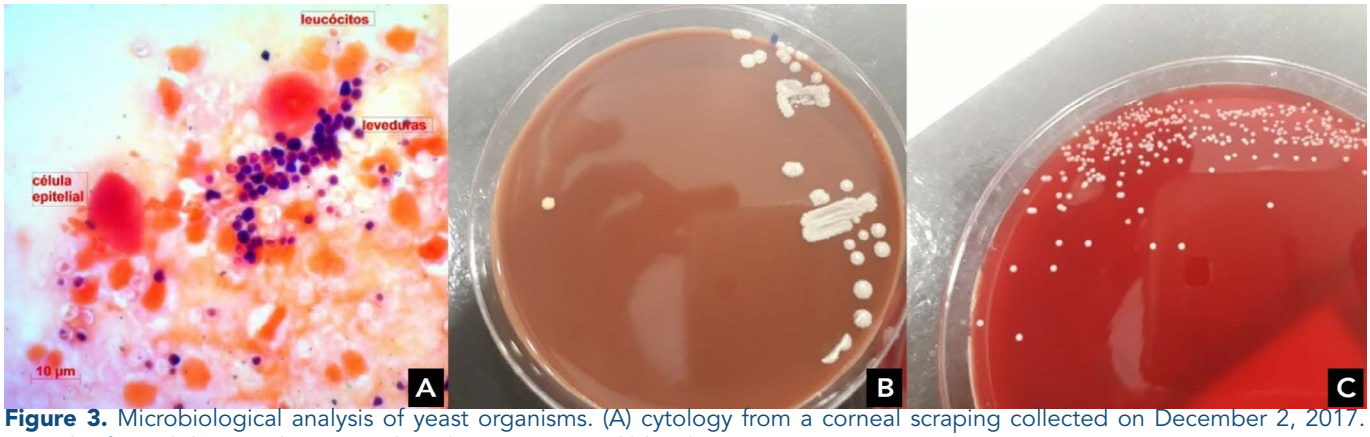

Figure 3. Microbiological analysis of yeast organisms. (A) cytology from a corneal scraping collected on December 2, 2017. Growth of Candida sp. colonies on chocolate agar (B) and blood agar (C).

DISCUSSION

Candida albicans is also the most common fungi associated with keratitis or endophthalmitis following corneal transplantation.[9] Candidoses caused by other *Candida sp.* (*Candida glabrata, Candida tropicalis, and C. parapsilosis*) have been increasing in prevalence due to higher antifungal resistance and the constant improvement in molecular diagnostic methods.[10]

C. albicans can be found as normal flora on the ocular surface (cultured at a rate of approximately 28% from the conjunctival surface).[11] It can be associated with ophthalmic disease, likely opportunistically, and there are a few

case reports of *C. parapsilosis* keratitis occurring primarily in patients of greater risk including newborns, intensive care patients, and transplant patients.[10]

Although FK after corneal keratoplasty is an uncommon complication, it can be challenging to determine the cause of fungal infection. In the present case, the origin of the infection was not possible to be determined, and the possibility of a previously contaminated donor graft cannot be ruled out.

In a series of six cases of FK after keratoplasty, presented between 1 and 9 months after surgery, all had positive cultures and IVCM detected *Candida* sp. in five of six patients. This study described *Candida* as round hyperreflective deposits, about 2 to 5 µm in diameter, distributed in clusters.[12]

The clinical presentation with a lesion on the periphery of the transplanted bud and close to the sutures is suggestive of late fungal inoculation. As we did not observe any significant eyelid changes, epithelial defects, or loose sutures, it is possible that the patient's immune status was important to the development of FK.

A systemic review and meta-analysis of the accuracy of IVCM for diagnosing *Acanthamoeba sp.* keratitis and FK showed a sensitivity of 94 and 88%, respectively. Despite the limited analysis of published retrospective studies using IVCM for FK, he considered the method to be acceptable.[13] Differential diagnosis using IVCM is well established for larger agents such as *Acanthamoeba sp.* Keratitis, yeast-like fungi and filamentous. In the case of bacteria, IVCM would help us to assess the inflammation of corneal tissue and monitor the response to treatment.[13]

Failure to achieve resolution through topical drug therapy of FK, whether due to inefficacy, refractoriness, or slow response, contributes to the need for surgical treatment. In vivo confocal microscopy has broadened our diagnostic arsenal, as exemplified in the present case. It is rapid, non-invasive, and can be used without sedation.

A previous study characterized *C. parapsilosis* infections in oral mucosa in vitro and described that spherical fluorescent red microorganisms of approximately 4 µm can form pseudohyphae. *Candida glabrata* has been described as hyper-reflective, granular, 3 to 5 µm round to oval microorganisms without pseudohyphae formation.[14] *C. albicans* can be differentiated by the presence of chlamydospores and pseudomycelia with a diameter of 7 to 13 µm.[15]

A recent review discussed the standard for evaluating images in categories by studying 21 original articles and 39 case reports on the use of IVCM and FK. The limitations of IVCM were shown to be the patient, who may experience pain during the procedure, the examiner, and the evaluator. The sensitivity of the test increases with the patient's clinical history and physical examination at the slit lamp, and with the trained and experienced performer and evaluator of the images. Cost is also another limiting factor in the use of IVCM. The benefits of using IVCM in FK are indisputable.[11]

The influence of topical steroids on the assessment of IVCM images has been documented in clinical studies. For instance, research involving patients with dry eye syndrome treated with loteprednol demonstrated significant reductions in the presence of subbasal dendritic cells and hyperreflective stromal keratocytes following the administration of the steroid. This indicates that topical steroids like loteprednol can substantially modulate corneal cellular activity, highlighting the need for careful interpretation of IVCM images post-treatment.[16]

Overall, a prolonged case of intractable and progressive FK caused by *C. parapsilosis* is described. The difficulty in treating the present case was likely in part due to the diagnostic delay of the fungal species, and the election to start with lower concentrations of topical medications that have fewer potential side effects. The use of IVCM confirmed to be a very valuable diagnostic tool as it allows early diagnosis of the etiology and rapid treatment with better visual responses for the patient. There is need for more reports of IVCM images, as they may aid in the training process of evaluators, as well as for enriching the database and possibly using artificial intelligence to evaluate images and improve sensitivity.

AUTHORS CONTRIBUTIONS

Bianca Luiza Valduga Guareschi: contributed to reviewing articles, submitting them to the Ethics Committee, collecting data, describing case reports and writing the article.

Clainijane Ramalho Borges: contributed to the literature review, drafting of the article, and data collection.

Paula Kataguiri: contributed to the literature review, data collection, confocal microscopy and critical review of the written work.

Tadeu Cvintal: contributed to the literature review, data collection, and critical review of the written work.

Victor Cvintal: contributed to the literature review, data collection, and critical review of the written work.

Bret Alan Moore: contributed to translating and correcting spelling into English, as well as critically reviewing the work.

Fabiano Montiani Ferreira: contributed to the literature review, data handling, and critical revision of the written work.

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