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Fungal keratitis:

an overview of 15 years of clinical and laboratory aspects

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Fungal keratitis: an overview of 15 years of clinical and laboratory aspects

TRABALHO FINAL DO 6º ANO MÉDICO TENDO EM VISTA A ATRIBUIÇÃO DO GRAU DE MESTRE NO ÂMBITO DO CICLO DE ESTUDOS DE MESTRADO INTEGRADO EM MEDICINA

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Acronyms

CHUC – Centro Hospitalar e Universitário de Coimbra

LogMAR – Logarithm of the Minimum Angle of Resolution

VA – Visual acuity

AV – Acuidade Visual

CA – *Candida albicans*

FK – Fungal keratitis

DM – Diabetes mellitus

FI – Fungal infections

PK – Penetrating keratoplasty

HSV – Herpes simplex vírus

BCVA – Best corrected visual acuity

SPSS – Statistical Package for the Social Sciences

Abstract

Purpose: Evaluate the microbiological profile, risk factors, epidemiological characteristics, and the therapeutic approach in patients with fungal keratitis diagnosed at Centro Hospitalar e Universitário de Coimbra (CHUC). We believe that by understanding the characteristics of these patients, assessing their predisposition to a specific fungus and, consequently, to a specific therapy, we will be able to minimize the time from the onset of the clinical picture to the institution of adequate treatment, improving the patient's prognosis.

Methods: We identified all cases of fungal keratitis from the microbiologic records between 2005 and 2020 at CHUC. Demographic data, manifested symptoms, clinical signs, risk factors, visual acuity (VA), therapeutic management and functional outcomes were noted. We compared epidemiological context, clinical characteristics, risk factors, treatment and outcomes between filamentous (group 1) and yeast (group 2) fungi.

Results: In a total of 49 eyes of 49 patients (mean age 59.24 ± 17.8) with fungal keratitis, 33 had a filamentous fungus (group 1) and 16 had a yeast fungus (group 2). At presentation, patients diagnosed with filamentous-type fungi more often present with corneal abscess comparing to yeast-like fungi (75.8% *vs*. 43.8%; p=0.027). Furthermore, eyes with filamentous fungi had significantly better initial VA (mean 1.53 ± 1.03 logMAR versus 2.16 ± 0.60 logMAR, p=0.042) and patients with yeast fungi had a greater improvement in VA (group 2: -0.32 ± 0.73 logMAR versus group 1: -1.33 ± 1.32 logMAR, p=0.007). Regarding risk factors, the group of filamentous fungi were more prone to present with a traumatic injury (33.3% *vs.* 0.00%; p=0.022) and yeast fungi had a statistically significant higher prevalence in patients with a history of previous ocular surgery (62.5% *vs* 30.3%; p=0.032). No correlations were found between the type of fungi and contact lens use, ocular surface disease and previous keratitis. Initiation of antifungal therapy within 72 hours of onset was predictive of a greater VA improvement (p=0.048).

Conclusion: In our department, filamentous fungi are the most prevalent agents causing fungal keratitis, by a ratio of 2:1. We identified traumatic ocular injury as a risk factor for filamentous keratitis and previous ocular surgery associated with infection with yeast agents. No other risk factors exhibited correlation with fungal infection, namely contact lens wear. Our study clearly demonstrated that early initiation of antifungal therapy is critical for better prognosis.

Keywords: fungal keratitis, risk factors, medical treatment, visual outcomes.

Resumo

Objetivo: Avaliar o perfil microbiológico, fatores de risco, características epidemiológicas e a melhor abordagem terapêutica nos doentes com queratite fúngica diagnosticados no CHUC. O melhor conhecimento das características destes doentes, avaliando a sua predisposição para o tipo de infeção fúngica, vai permitir minimizar o tempo entre o início do quadro clínico e a instituição da terapêutica especifica, melhorando assim o prognóstico do doente.

Métodos: Análise retrospetiva dos registos clínicos dos doentes com diagnóstico de queratite fúngica no CHUC entre 2005-2020. Foram avaliados: dados demográficos, sintomatologia, sinais clínicos, fatores de risco, acuidade visual (AV), gestão terapêutica e resultados funcionais. Comparamos o contexto epidemiológico, características clínicas, fatores de risco, tratamento e resultado anatómico final entre fungos filamentosos (grupo 1) e leveduras (grupo 2).

Resultados: Num total de 49 pacientes (idade média 59,24 ± 17,8) com queratite fúngica, 33 tinham fungo filamentoso (grupo 1) e 16 fungos leveduriformes (grupo 2). Os doentes com diagnóstico de fungo do tipo filamentoso manifestaram mais frequentemente abcesso corneano quando comparados com os fungos do tipo levedura (75,8% *vs* 43,8%; p=0,027). Além disso, os doentes com queratite por fungo filamentoso apresentavam uma AV inicial significativamente maior (média 1,53 ± 1,03 logMAR *vs* 2,16 ± 0,60 logMAR, p=0,042) e os doentes com fungos de tipo levedura tinham uma melhoria mais significativa da AV após tratamento (grupo 2: -0,32 ± 0,73 logMAR versus grupo 1: -1,33 ± 1,32 logMAR, p=0,007). Em relação aos fatores de risco, o grupo dos fungos filamentosos mostrou-se mais propenso a apresentar lesão traumática (33,3% *vs.* 0,00%; p=0,022) e os fungos leveduriformes apresentam prevalência mais elevada em pacientes com história de cirurgia ocular prévia (62,5% vs 30,3%; p=0,032). Não foram encontradas correlações entre o tipo de fungo e o uso de lentes de contato, doença da superfície ocular e queratite prévia. O início da terapêutica antifúngica até 72 horas após o aparecimento do quadro clínico foi preditivo de um maior aumento da AV (p=0,048).

Conclusão: No nosso centro os fungos filamentosos são os mais prevalentes, com um rácio de 2:1. Identificámos o traumatismo ocular como fator de risco para infeção por fungo filamentoso e a história de cirurgia ocular prévia associada à infeção por leveduras. Nenhum outro fator de risco em análise demonstrou correlação com a infeção fúngica, nomeadamente o uso de lentes de contacto. O nosso estudo demonstrou, de forma clara, que a precocidade na instituição de terapêutica dirigida é fundamental para um melhor prognóstico.

Palavras-Chave: queratite fúngica, fatores de risco, tratamento médico, resultados visuais.

Introduction

Infectious keratitis can be caused by multiple infectious agents like viruses, fungi, bacteria and protozoa. More than 100 species of fungi can cause keratitis, the most commonly associated being *Fusarium*, *Aspergillus* and *Candida Albicans*. Different fungal species have different susceptibilities to external factors such as temperature, climate and urbanization, so its geographical distribution is quite variable. For example in tropical areas, filamentous fungi are predominant while it is believed that in temperate climates, yeasts are more common.[1]

Epidemiologically, fungal keratitis (FK) represents 20-60% of infectious keratitis in tropical and subtropical areas.[2] In Europe, it is more unusual, especially in its temperate regions, however an increasing number of FK has been recently documented.[2,3] This increase is thought to be associated with the use of contact lenses, among other factors.[1]

The main associated risk factor is corneal trauma, primarily with vegetable matter. However, other predisposing factors are known, such as: diabetes mellitus (DM), long-term use of steroids and topical and/or systemic antibiotics, ocular surface disorders, previous eye surgery, particularly penetrating keratoplasty (PK), and pre-existing Herpes Simplex Virus (HSV) keratitis.[1] In addition, it is important to understand how the risk factors correlate to the type of pathogenic fungus. For example, *Fusarium* is more often associated with vegetable trauma or the use of contact lenses, while *Candida* is more frequent in patients with previous eye disease and chronic use of topical corticosteroids.[2,3]

Although the pathogenesis of keratomicosis has not been fully elucidated, it is believed to result from an imbalance between the host's defense capacity and the fungus virulence. Thus, a break in the homeostasis of the ocular surface caused, for example, by an eye trauma causes epithelial damage to the cornea, which is the main barrier against infection.[3] This damage to the corneal epithelium is a gateway for fungi. When fungi contact the corneal stroma, they must find a way to survive, penetrate the internal tissues and resist the host's immune system. If these conditions are fulfilled, the fungus proliferates and releases mycotoxins and proteolytic enzymes that promote an inflammatory reaction. Then, the fungi can damage Descemet membrane and reach the anterior chamber and the posterior segment.

Patients with FK usually report a sudden onset of pain, blurred vision, red eye, photophobia and tearing. At the slit-lamp, patient presents with an inflamed eye and a corneal ulcer with associated opacity.[2,4] Although some signs are more suggestive of fungal infection – such as corneal ulcer, presence of irregular edges/feathers and satellite lesions – or in the particular case of filamentous fungi, the association with hypopion, no sign is pathognomonic of FK, which can lead to a delay in its diagnosis.[1,2,5]

A definitive diagnosis is one of the most difficult problems for the ophthalmologist. Suspicion arises from clinical manifestations, the unresponsiveness to antibiotic treatment and the presence of risk factors. Confirmation is supported by laboratory diagnostic methods.[6] For laboratory diagnosis, collection and culture of ulcer exudate must be performed using sabouraud/chloramphenicol agar plates that are incubated at 37ºC. After the isolation of the fungus, in culture, its morphological characterization is carried out at a macro and microscopic level in order to obtain its identification (genus/species) and

for the latter it is almost always necessary to resort to molecular biology techniques.[7] Unfortunately, fungi, especially filamentous fungi, grow slowly, which makes diagnostic confirmation often time-consuming.[8] The use of other modalities like molecular techniques such as real-time polymerase chain reaction and sequencing offers a significant reduction in time required for accurate diagnosis of such infections.[2,6,9]

The treatment also poses some difficulties, namely in choosing the antifungal and the best route for its administration, considering the ocular biodistribution and bioavailability. Currently, antifungals can be divided into three classes: polyenes, azoles and echinocandins.

Polyenes bind to the fungus's ergosterol in the plasma membrane, compromising its integrity and determining the loss of cytoplasmic constituents. In this group, amphotericin B stands out as a macrocyclic active capable of fighting *Aspergillus* and *Candida* species. Given their broad spectrum of action, they play an important role in treating systemic fungal infections (FI) in hospitals. Although it is most often used as fortified eye drops, intracameral injections are useful and are associated with a faster resolution of hypopyon. In practice, amphotericin B is usually the antifungal of choice in the treatment of FK, despite its spectrum not including fungi of the *Fusarium* species and being a potent dose-dependent nephrotoxic.[4] Natamycin is also part of this class, however it is not marketed in Europe.

The azoles (ketoconazole, fluconazole and itraconazole) and their derivatives act by disturbing the synthesis of ergosterol with a consequent change in the permeability of the fungal cell membrane. Ketoconazole was the first of this group, but later a new subgroup emerged, the triazoles (voriconazole, posoconazole) whose advantage is its broader spectrum of action and less severe adverse reactions. Voriconazole has been shown to have a wide therapeutic window covering not only filamentous fungi but also *Candida* and other species such as *Fusarium, Paecilomyces* and *Scesdosporium* and most importantly, has the ability to achieve good concentrations in various eye tissues (cornea, aqueous, and vitreous).[10,11] Fluconazole has the advantage of having fewer side effects and good intraocular penetration, in addition, it can be effective in patients who do not respond to conventional antifungal medical treatment for Candida and Alternaria keratitis.[12] However, has the disadvantage of having low coverage for filamentous fungi.

Echinocandins are synthetic derivatives of lipopeptides and inhibit the enzyme responsible to produce 1,3-β-D-glucan, essential for the synthesis of the fungal cell wall. Caspofungin, micafungin and anidulafungin are examples of echinocandins. These compounds are known for anti-*Candida* activity, being indicated in *Candida* and *Aspergillus* infections, including those resistant to azoles. However, they have some limitations in terms of spectrum of action and lack of oral bioavailability and corneal penetration.[10]

Antibiotics have also been studied for their in vitro fungicidal ability. Chloramphenicol had some activity against species such as *Fusarium* and *Aspergillus*, and moxifloxacin and tobramycin presented activity against *Fusarium*.[13]

Surgical intervention is currently an option for patients with disease refractory to medical treatment to control deep and severe FK. The most common surgical intervention is penetrating keratoplasty (PK), besides antifungal intraocular injections.

Conventional treatment with antifungals often does not achieve the best results, probably because corneal penetration and the bioavailability of many preparations are suboptimal, making it difficult to treat cases of invasive mycotic keratitis. For those cases where the infection may have penetrated the Descemet's membrane and spread to the anterior chamber, alternative routes have been created such as: intracameral, intrastromal and intravitreal injections.[14]

PK is a procedure in which trephines are used to excise diseased cornea and a donor corneal graft is sutured in place.[15] This procedure is intended to terminate or reduce an actively infectious corneal disease, as well as repair anatomical corneal defects such as perforations. Thus, it can be performed in the acute phase of the disease, as an attempt to control the infection and restore the integrity of the globe, or later, when the infection is under control, to improve vision.[5,16] In these cases of FK, graft rejection, recurrence of fungal infection and endophthalmitis are the most frequent and feared complications.

Enucleation or evisceration may eventually be needed in cases of blind and painful eye with uncontrollable inflammation, where recovery is deemed impossible.

For all this, prognosis depends on the causative fungus, depth and extension of the infection, timing of initiation of treatment and development of complications like corneal perforation, endophthalmitis, scleritis, corneal scarring or melting.[15]

With this study, we aim to evaluate the microbiological profile, risk factors, epidemiological characteristics, and the best medical and/or surgical approach in patients with FK diagnosed at the CHUC in the last 15 years.

Materials and Methods

A retrospective review of all cases with laboratorial diagnosis of fungal keratitis established between 2005 and 2020 in CHUC was performed.

Patients were selected from a database from CHUC Clinical Pathology Laboratory, and their clinical records reviewed. Data referring to these cases were collected using clinical files, both electronic and paper.

The following data was collected: isolated fungi, month of diagnosis, age, sex, VA and clinical signs at presentation, presence of risk factors, time between presentation and laboratorial confirmation, type of treatment (medical and/or surgical) and its timing. The result was evaluated according to functional (visual acuity) and anatomical outcomes (enucleation vs globe preservation).

For this analysis, the following risk factors were recorded: contact lenses wear, recent intraocular surgery (defined as surgery up to one month before the onset of symptoms), previous PK (performed more than six months since the onset of symptoms), non-penetrating ocular trauma (vegetable matter or other material), ocular surface diseases (dry eye syndrome, topical glaucoma medication...), history of intraocular surgery (performed at least six months from the onset of symptoms), pre-existing HSV keratitis and systemic disease (DM and other metabolic diseases, autoimmune diseases, infectious diseases etc.). Furthermore, data included best corrected visual acuity (BCVA) at presentation and at end of follow-up.

For statistical purposes, data was categorized in two groups, according to the isolated fungal agent: filamentous-type (group 1) and yeast (group 2). Descriptive statistics was performed for the whole group and for the two subgroups, and data from subgroups were compared. Correlations between epidemiological context, risk factors, clinical course and final anatomic outcome were made.

Categorical variables were analyzed using relative valid frequency (Valid %), absolute frequency (Count N), and valid total count (Valid N). Quantitative variables were described using mean, standard deviation, minimum and maximum. Mann-Whitney, Chi-square and Fisher's exact tests were used to determine significant differences between fungi types. Whenever needed, Snellen VA was converted to logMAR, for lower visual acuities a review by *Schulze-Bonse et al* was used.[17] All statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS), version 27. A p value less than 0.05 was considered statistically significant.

This study was designed and conducted according to the tenets of the Declaration of Helsinki for medical research involving human subjects and approved by the CHUC ethics committee.

Results

A total of 64 eyes of 64 patients with laboratorial diagnosis of FK were identified in the database. Of those, 15 were excluded from this study because of incomplete records. Data from 49 patients is presented, 22 female (44.9%) and 27 male (55.1%). The mean age at diagnosis was 59.2 ± 17.8 years (minimum 19 – maximum 86). No statistical differences were identified between subgroups (filamentous fungi vs yeasts). Mean follow-up time was 87 days (minimum 1 - maximum 476).

The identified pathogens are detailed in Table 1. Filamentous agents were more frequent than yeast, by the ratio of 2:1. The most frequently identified filamentous agents were *Fusarium* and *Aspergillus Flavus*. Among yeast, *Candida Albicans* and *Candida Parapsilosis* were the most common.

Regarding the seasonal distribution, the incidence of these infections was most notorious in Autumn/Winter (Figure 1). However, no association between the season of the year and the type of fungus involved was found.

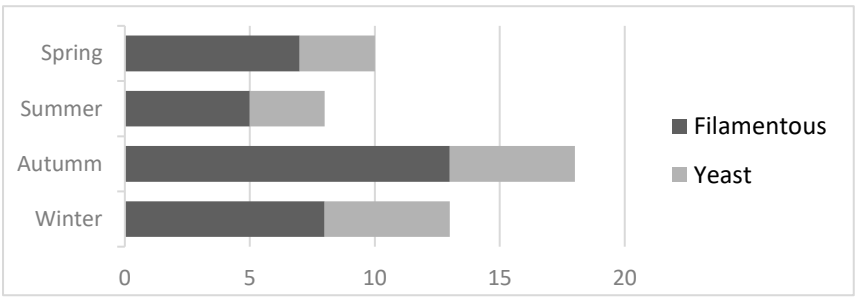

Figure 1: Seasonal distribution

As presented in Table 2, patients present more often with red eye (77.8%) and ocular pain (75.5%). Patients diagnosed with filamentous-type fungi more often present with corneal abscess comparing to yeast-like fungi (75.8% *vs* 43.8%; p=0.027). As for VA, 33 patients presented with an initial VA of less than 1/10 in the Snellen scale (>1logMAR) (67%) and 10 patients had a VA greater than 4/10 (<0.4logMAR) (20.4%).

Patients in group 1 had significantly better VA at presentation (mean VA 1.53±1.03logMAR *vs* 2.16±0.60logMAR; p=0.042) and eyes with yeast fungi had a greater VA improvement after treatment (-0.32±0.73logMAR *vs* -1.33±1.32logMAR; p=0.007). (Table 2)

Predisposing risk factors were identified in all 49 patients (100%). As shown in Table 3, the most common risk factor was the presence of systemic diseases (n=29; 61.7%) followed by surgical causes (n=20; 40.8%), that includes eyes with recent and/or past intraocular surgery. One third of the patients have history of previous PK (34.0%) and 14 (29.8%) suffered recent non penetrating ocular trauma, of which six (12.8%) with vegetable matter. Ocular surface diseases were present in 12 patients. DM was the systemic disease more commonly found (n=10, 21.3%). Seven patients (14.9%) had a history of previous keratitis, six due to HSV (12.8%). A minority were contact lenses wearers (n=5; 10,6%). Patients diagnosed with filamentous-type fungi were more prone to present with a traumatic injury comparing to yeast-like fungi (33.3% *vs* 0.00%; p=0.022). Patients with history of surgical intervention were more likely to have yeast fungus (62.5% *vs* 30.3%; p=0.032). There were no significant differences concerning other risk factors.

The mean time between the onset of symptoms and the start of antifungal treatment was eight days (SD = 9). However, on average, it took four days between the onset of symptoms and the collection of microbiological material (SD = 3) and seven days between the collection and the release of the respective result (SD = 4).

Antifungal therapy was administered within the first 72 hours in 18 patients (43.9%), of which 15 were empirically administered. Of the remaining patients, 23 received antifungal treatment only after the microbiological result and eight of the patients did not receive antifungal therapy at all. Those who received antifungal therapy within the first 72 hours of clinic onset are associated with a greater VA improvement (p=0.048).

Table 4 describes the type of medical treatment prescribed. Amphotericin B (n=22; 48.3%) was the most prescribed antifungal, followed by Clotrimazole (n=21; 45.7%) and Voriconazole (n=17; 37.0%). Antibiotic therapy was used in 91.5% of cases (n=43) and corticosteroids in 68.8% (n=33). The use of corticosteroids did not negatively affect the outcome (evisceration/enucleation p=0.210 and endophthalmitis p=0.393). Medical treatment was administered topically and/or systemically. (Figure 2) Almost half of the patients required the addition of another antifungal drug to the initial regimen (n=19; 40.4%).

In the group of filamentous forms, 16 were treated with Voriconazole, 15 with Amphotericin B, 15 with Clotrimazole, nine with Fluconazole, two with Posaconazole, and one with Chlorhexidine. Three of these patients did not receive antifungal treatment. Patients diagnosed with filamentous type were more likely to be treated with voriconazole (48.6% *vs* 7.7%; p=0.016) (Table 4).

On the other hand, In the group of the yeast forms, seven patients were treated with Amphotericin B, six with Clotrimazole, four with Fluconazole, two with Itraconazole and one with Voriconazole. Five of these patients did not receive antifungal treatment.

Figure 2 shows the route of administration of the chosen antifungal according to the type of fungus involved.

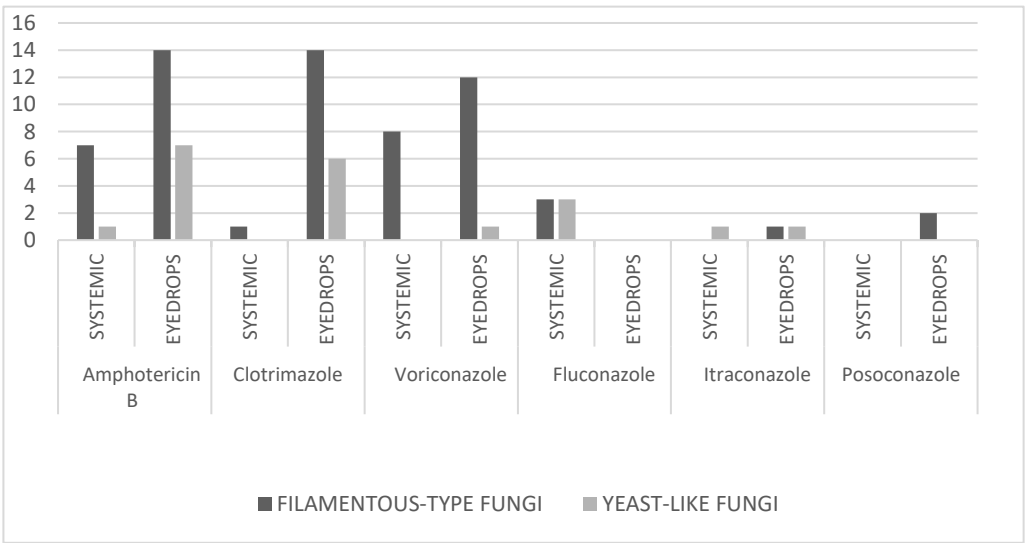

Figure 2: Route of administration of the chosen antifungal

After obtaining the microbiological result, 40.4% of the patients had their medical therapy modified, adding and/or changing the initial antifungal. Of the 15 patients whose medical therapy was empirically instituted within the first 72 hours, only five of them had to change the antifungal, and the rest evolved favorably with the therapy initially instituted.

Regarding surgical treatment, 32 (66.7%) underwent surgical intervention. Penetrating keratoplasty was performed in 21 eyes (43.8%). The need for PK did not differ significantly between subgroups (p=0.327). Anterior chamber injection (39.6%) was performed in 19 patients, of which eight were antibiotic injection, six Voriconazole and five Amphotericin B, with no statistical differences between groups (filamentous fungi *vs* yeast).

Table 4 shows that 18 patients (36.7%) developed endophthalmitis. Five of these patients required enucleation/evisceration of the eye and all of them were caused by filamentous fungus. Among enucleated eyes (n=8; 16.3%), seven were in group 1 and one in group 2 (21.2% *vs* 6.3%), and no statistical differences were found between groups (p=0.184). No significant differences were found among groups in endophthalmitis rate as well (p=0.938).

Discussion

FK of the cornea has emerged as a major eye disease worldwide. While in Europe several studies about this pathology have been published, Portugal has scarce literature. So, our goal is to evaluate the microbiological profile, risk factors, epidemiological characteristics, and the best medical and/or surgical approach in patients with fungal keratitis diagnosed at CHUC.

Corneal infections have been declared a silent epidemic. Although it's incidence has never been accurately estimated, a review by *Brown et al* estimates that more than a million eyes are affected each year by FK, most commonly in tropical areas where it accounts for 67% of the entire corneal infections.[2,18] In addition, some studies report an increase in the incidence rate in temperate countries, such as Portugal.[19]

As previously mentioned, the type of fungus involved depends on a variety set of factors.[15] Regarding external factors, according to current literature, yeast fungi are more associated with temperate climates.[2] On the contrary, in our Center, filamentous fungi were the most frequently identified (ratio 2:1). In comparison with another study carried out in Portugal, at Centro Hospitalar Universitário S. João - Porto, whose sample is similar to ours (n=43), we can see that the fungal distribution is not similar across the country. While in our center, filamentous fungi prevail, in the study carried out in Porto, filamentous fungi have the same prevalence as yeasts.[3] This demonstrates the importance of knowing the fungal flora of the region to adapt our therapeutic approach.

The average age reported in studies from other European countries, such as France, Ireland and Switzerland, varies between 47.4-63.7 years, which is quite similar to that presented in our study.[19]

Clinically, there is no pathognomonic sign of FK, which is one of the reasons why this pathology is an important clinical challenge. In our study, corneal abscess was more often reported with filamentous fungi (75.8% *vs* 43.8%) and this association was statistically significant (p=0.027). However, this may represent a description bias and, therefore, a larger sample is needed to draw conclusions. Most importantly, eyes with filamentous fungi presented *ab initium* with a significantly better VA (mean 1.53 ± 1.03 logMAR *vs* 2.16 ± 0.60 logMAR, p=0.042), showing that early VA can be predictive of the fungus involved.

We reported that besides systemic risk factor (61.7%), surgical causes (40.8%) and, particularly an history of previous PK (34.0%) were the most prevalent risk factors. These conditions are well known predisposing factors to these infections, along with others such as the use of contact lenses and ocular surface disease, factors that were not so prevalent in our Center. In fact, there are divergences between the studies, while some refer to ocular trauma as a preponderant factor in developed countries, others report that in European countries, the use of contact lenses is predominant, followed by ocular surface disorder and ocular trauma at the end of the list.[19] In a review carried out in another portuguese hospital, contact lenses, history of keratitis and previous keratoplasties were the main risk factors.[3] On the other hand, ocular trauma was much less incident compared to our Center, probably due to less agricultural activity in that region.

Moreover, in temperate regions, ocular surface diseases and pre-existing HSV keratitis are associated with predisposition to candida and candida-like keratitis, which was not observed in this review.[2]

PK was the second most frequent risk factor, present in 34.0% of our patients, this incidence is more evident than in other studies in Europe that report 7.1%.[18] However, non-European studies report 24% incidence and a review from another portuguese Center reported 32.6%.[3] This procedure can be a risk factor not only because it creates a gateway for microorganisms but also because of the need for corticosteroids in the postoperative period. In our study PK seems to be more associated with yeast fungus FK (p=0.056), but more studies are needed to substantiate this claim.

Antifungal therapy took an average of eight days to be prescribed, but in 30.6% of patients it was administered empirically within the first 72 hours of clinical onset. The 18 patients whose therapy was started within the first 72 hours after symptom onset showed a greater VA improvement (p=0.048). The other patients saw the start of therapy delayed, probably because of the difficulty in laboratory diagnosis, making it imperative to create faster fungal identification techniques.

Some patients did not receive antifungal treatment (n=8), three because they had small abscesses that healed with antibiotic administration alone and one was eviscerated due to deep infection prior to antifungal administration. Another one had co-infection with a bacterial agent whose result was much earlier than the result of the fungal agent, probably for this reason it went unnoticed. Once again, we highlight the importance of rapid microbiological detection of these agents and also the need to confirm the results daily. For the remaining three patients, it was not possible to determine why antifungals were not prescribed.

Corticosteroid therapy was used in most patients (68.8%), but in 85% of cases (n=29) a gradual reduction was started immediately after the disclosure of laboratory results. This attitude may justify why its use is not associated with a worse outcome.

Surgical intervention was required in two thirds of cases, of which 68.8% were filamentous fungi and 31.2% yeasts. PK was performed in almost half of our patients (n=21), being necessary in 48.5% of patients with filamentous fungi and in 33.5% of those with yeast-like fungus (p=0.327). These data are similar to those presented by *Nowik et al* in Poland which reported that 42% of patients with FK underwent PK. Also, a tertiary referral center in the southeastern US described a 32% rate of need for PK. In Portugal, another center showed a therapeutic PK rate of 53.5%, also being more frequently necessary in filamentous fungi (63.6%) than yeast (42.9%) (p=0.086).[3]

Intracameral injection was required in about 40% of patients (n=19). Voriconazole was the most used antifungal in this technique (13%) followed by Amphotericin B (10.9%). Both reported to be successful in treating refractory infections and endophthalmitis, but several shortcomings of intraocular amphotericin B injection have been reported, such as retinal necrosis at low concentrations, which makes the use of Voriconazole more beneficial.[20]

Endophthalmitis is present in practically the same proportion of patients with filamentous fungus and yeast fungus (36.4% *vs* 37.5%; p=0.938). Among them, five had to undergo evisceration or

enucleation and all were caused by filamentous fungi. This highlights the severity of the infection by filamentous agents, however a larger sample is needed to assess this association.

Overall, 16.3% of our patients underwent enucleation/evisceration, and no statistical differences were identified between groups (p=0.184). These values are relatively higher compared to those reported in other countries, like Germany where a multiyear series reported a 9% rate of globe removal, in a sample of 116 patients from various centers across the country.[21] Our sample compares to that from another Portuguese Center, that presented higher evisceration rate (27.9%).[3] Actually, *Brown et al* predicted that almost 100 000 eyes are removed every year due to FK worldwide.

The time of follow-up for this pathology is long, reinforcing the difficulty of treating these patients, this is not only deleterious for the patient, but also entails significant direct and indirect costs.

As a transversal limitation to retrospective studies, it should be noted that it is possible that we have not identified all possible cases. In addition, our sample is limited to those cases where microbiological exam was performed and positive for fungi. Therefore, it does not represent the whole population with fungal keratitis. On the other hand, a standardized data recording process is needed, both in the hospital and in the pre-hospital setting, because CHUC is a reference patient center from other hospitals, where it is possible that some therapy was administered prior to the one described. Furthermore, it is not possible to investigate specific risk factors without a control group, however, we were able to draw some important conclusions for daily clinical practice. Finally, although it is a study covering 15 years of medical records, it only provides a review of only one center, contrasting with several articles carried out in other countries. It is imperative to continue to study off this pathology in order to improve its prognosis.

Conclusion

With this study, we were able to draw important conclusions. First, in our region, filamentous fungi are the most prevalent (ratio 2:1). Patients with this type of fungus more often have a better initial AV and present with corneal abscess. Patients with keratitis in a traumatic context are more likely to have a filamentous fungus as well. On the other hand, patients with previous ocular surgery are more likely to have FK caused by yeast-like fungi. No other risk factors exhibited correlation with fungal infection, namely contact lens wear.

Early initiation of antifungal therapy (within the first 72 hours) is associated with greater VA improvement, being critical for better prognosis. This data reinforces the need to identify and diagnose these patients as soon as possible. Microbiology constitutes the diagnostic pillar, therefore, the use of molecular methods becomes increasingly important, compared to traditional culture, due to the advantage of obtaining results earlier.

FK remains one of the greatest challenges for the ophthalmologist. It is important that similar studies continue to be carried out to make the approach to these patients more effective, improving their prognosis.

Attachments

TABLE 1: Microorganism identified in eyes with fungal keratitis between 2005-2020 at CHUC

VARIABLES	COUNT N	VALID %
FILAMENTOUS SPECIES	33	67.3
Fusarium spp	10	20.4
Aspergillus flavus	6	12.2
Alternaria spp	3	6.12
Aspergillus spp	3	6.12
Paecilomyces lilacinus	3	6.12
Scedosporium apiospermum	3	6.12
Aspergillus fumigatus	2	4.81
Aspergillus penicilloides	1	2.04
Fusarium dimerum	1	2.04
Penicillium spp	1	2.04
YEAST SPECIES	16	32.7
Candida albicans	9	18.4
Candida parapsilosis	4	2.04
Candida famata	1	2.04
Candida tropicalis	1	2.04
Saccharomyces cerevisiae	1	2.04

TABLE 2: Clinical features and statistical analysis in patients with fungal keratitis. Comparison of filamentous fungi and yeast

PK, penetrating keratoplasty; BCVA, best corrected visual acuity; logMAR, logarithm of the minimum angle of resolution

VARIABLES	COUNT N	VALID %	FILAMENTOUS TYPE FUNGI	YEAST-LIKE FUNGI	P-VALUE
Red eye	38	77.8	72.4(24)	87.5(14)	0.245
Ocular pain	37	75.5	72.3(24)	81.3(13)	0.515
Corneal abscess	32	65.3	75.8(25)	43.8(7)	**0.027**
Hyperemia	15	30.6	27.3(9)	37.5(6)	0.466
Hypopyon	11	22.4	30.3(10)	6.3(1)	0.058
Corneal ulcer	11	22.4	27.3(9)	12.5(2)	0.245
Secretions	9	18.4	21.2(7)	12.5(2)	0.460
Leucoma	8	16.3	18.2(6)	12.5(2)	0.614
Corneal perforation	3	3.0	6.1(2)	6.3(1)	>0.999
PK perforation	2	4.08	3.0(1)	6.3(1)	>0.999
Melting	2	4.08	3.0(1)	6.3(1)	>0.999
Initial BCVA (MEAN ± SD)	49	100%	1.53±1.03	2.16±0.6	**0.042**

Values are valid % (count), unless specified otherwise. SD, standard deviation.

TABLE 3: Risk factors and statistical analysis in patients with fungal keratitis. Comparison of filamentous fungi and yeast

DM, diabetes mellitus; PK, penetrating keratoplasty; HSV, herpes simplex virus

VARIABLES	COUNT N	VALID %	FILAMENTOUS-TYPE FUNGI	YEAST-LIKE FUNGI	P-VALUE
Male: Female proportion	27:22	55.1/44.9	17:16	10:6	0.469
Age(years), mean (SD)	49	100	58.9 (17.7)	59.9(18.5)	0.984
Systemic risk factors	29	61.7	53.1(17)	80.0(12)	0.077
DM	10	21.3	15.2(5)	33.3(5)	0.249
History of previous surgery	20	40.8	30.3(10)	62.5(10)	**0.032**
Previous PK	16	34.0	25.0(8)	53.3(8)	0.056
Non penetrating trauma	14	29.8	33.3(11)	0.0(0)	**0.022**
With unknow material	8	17.0	25.0(8)	0.0(0)	**0.042**
With vegetable matter	6	12.8	18.8(6)	0.0(0)	0.157
Ocular surface disease	12	25.2	15.6(6)	33.3(6)	0.119
Previous keratitis	7	14.9	9.4(3)	26.7(4)	0.188
HSV	6	12.8	6.3(2)	26.7(4)	0.072
Contact lenses	5	10.6	12.5(4)	6.7(1)	>0.999
Penetrating trauma	2	4.3	6.3(2)	0.0(0)	>0.999

Values are valid % (count), unless specified otherwise. SD, standard deviation.

TABLE 4: Statistical analysis of treatment and clinical outcomes in patients with fungal keratitis. Comparison of filamentous fungi and yeast

PK, keratoplasty penetrating; VA, visual acuity; logMAR, logarithm of the minimum angle of resolution

VARIABLES	COUNT N	VALID %	FILAMENTOUS -TYPE FUNGI	YEAST-LIKE FUNGI	P-VALUE
MEDICAL TREATMENT					
Amphotericin B	22	48.3	46.9(15)	53.8(7)	0.672
Clotrimazole	21	45.7	45.5(15)	46.2(6)	0.966
Voriconazole	17	37.0	48.6(16)	7.7(1)	**0.016**
Fluconazole	13	28.3	27.3(9)	30.8(4)	>0.999
Itraconazole	3	6.5	3.0(1)	15.4(2)	0.188
Posaconazole	2	4.3	6.1(2)	0.0(0)	>0.999
Chlorhexidine	1	2.2	3.0(1)	0.0(0)	>0.999
Addition of another antifungal to initial therapy	19	40.4	45.5(15)	28.6(4)	0.281
Antibiotic	43	91.5	90.9(30)	92.9(13)	>0.999
Corticosteroids	33	68.8	66.7(22)	73.3(11)	0.746
Acyclovir	5	10.9	12.1(4)	7.7(1)	>0.999
SURGICAL TREATMENT					
PK	21	43.8	48.5(16)	33.3(5)	0.327
Anterior Chamber injection	19	39.6			
Antibiotic	8	17.4	18.2(6)	15.4(2)	0.822
Voriconazole	6	13.0	18.2(6)	0.0(0)	0.163
Amphotericin B	5	10.9	9.1(3)	15.4(2)	0.537
OUTCOMES					
Final VA >1 logMAR	30	61.2	60.6(20)	62.5(10)	0.944
Endophthalmitis	18	36.7	36.4(12)	37.5(6)	0.938
Evisceration/Enucleation	8	16.3	21.2(7)	6.3(1)	0.184
BCVA improvement after treatment (logMAR) (MEAN±SD)	49	100%	-1.33±1.32	-0.32±0.73	**0.007**

Values are valid % (count), unless specified otherwise. SD, standard deviation.

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