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Mucormycosis in Mato Grosso, Brazil: A Case Reports, Caused by *Rhizopus microsporus* var. *oligosporus* and *Rhizopus microsporus* var. *rhizopodiformis*

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Abstract We identified the etiological agents responsible for two fatal cases of rhinocerebral mucormycosis with the classical risk factor for uncontrolled type II diabetes mellitus. Their initial symptoms did not point immediately to the suspicion of mucormycosis. Case 1, caused by *Rhizopus microsporus* var. *oligosporus*, was a 52-year-old man who presented with a painful pimple on his nose, which evolved with swelling, erythema, and a central pustule on his right hemiface suspected to be cellulitis. After 7 days of antibiotic treatment, the patient worsened with signs of sepsis and the lesion evolved to necrosis involving all his right face. Case 2, caused by *Rhizopus microsporus* var. *rhizopodiformis*, was a 57-year-old woman placed on continuous therapy with azathioprine and corticoids after a renal transplant due to chronic arterial hypertension and uncontrolled type II diabetes mellitus. Because she was suspected to have sepsis, the patient was treated with broad-spectrum antibiotics and mechanical ventilation, yet she deteriorated. Because *Candida* spp. were isolated from urine and a BAL, she was treated with fluconazole for 10 days, then substituted by caspofungin. Two weeks later, she presented with exophthalmus of the left eye that was surrounded by a large inflammatory and necrotic area. Both patients were the diagnosed with mucormycosis via direct microscopy of necrotic material prior to their death.

Keywords Mucormycosis · Brazil · *Rhizopus microsporus* var. *oligosporus* · *Rhizopus microsporus* var. *rhizopodiformis*

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Introduction

Mucormycosis is a rare but usually very aggressive, life-threatening, opportunistic fungal infection caused by a group of ubiquitous saprophytic organisms of the order Mucorales. Species of the genera *Rhizopus*, *Rhizomucor*, *Mucor,* and *Absidia* are the most frequently isolated agents from human lesions. These

fungi are distributed in the environment worldwide, and the infection can be acquired by the deposition of spores on wounds or on fragile mucous membranes, by ingestion or inhalation. Immunodepressed patients, mainly those with ketoacidosis, neutropenia, and users of corticoids are at highest risk. After germining, the fungus can cause pathogenic lesions as a result of invasion and growth within the walls and the lumen of the major blood vessels, ensuing thromboembolism, resulting in ischemia and tissue necrosis [1–5].

Classically, the following major clinical forms are described: rhino-orbital-cerebral, pulmonary, cutaneous, gastrointestinal, and disseminated. The most common presentations are the rhino-orbital-cerebral and the pulmonary forms and, together with disseminated infection, they are associated with the highest mortality, ranging from 78 to 100% [3]. On the other hand, the cutaneous form comprises less than 10% of all cases and shows the best prognosis, with less than 10% mortality. Metabolic ketoacidosis and severe neutropenia are the major risk factors for, respectively, the rhino-orbital-cerebral form and the pulmonary form [2–8].

Diabetes mellitus is a critical predisposing factor in 36–88% of all mucormycosis cases [2–10]. All types of diabetes mellitus have been reported as risk factors for patients with mucormycosis. Nevertheless, mucormycosis was found to be the first clinical manifestation of some patients who had undiagnosed diabetes mellitus [3]. The most common clinical feature found in mucormycosis with diabetes mellitus is sinus disease (66%), followed by pulmonary infection (16%) [3, 4]. Usually, these lesions evolve very rapidly and present high mortality rates; therefore, the prognosis depends on early diagnosis and proper treatment.

A definitive mycological diagnosis of mucormycosis has to be properly made as follows: (a) visualization of typical mucoralean hyphae by direct microscopy of wet mounts in a 10% KOH solution; (b) isolation of the agent in culture, which usually requires a few days; (c) definitive identification of species and varieties of an isolate may require a few weeks and include morphological and physiological tests [3–7]. More recently, molecular identification tools have been described, but they are not yet available in the lab routine [11–13]. Even when a correct and early diagnosis is achieved and appropriate treatment is started, the overall survival rate of diabetic patients with mucormycosis only reaches approximately 60% [3]. The following two cases represent the first well-documented rhino-orbital-cerebral form of mucormycosis recently diagnosed in Cuiabá, capital of the Brazilian Center-Western state of Mato Grosso (MT), with complete identification of the etiological agents.

Case Reports

Case 1

A 52-year-old man, from in Cuiabá (MT), with a several year history of poorly controlled type II diabetes mellitus, presented with a 10-day history of a painful pimple on his nose, which evolved with swelling, erythema, and a central pustule on his right face diagnosed as cellulitis. After treatment with broad-spectrum antibiotics for 7 days, he worsened the lesion evolved to dry necrosis on his right face and the patient presented signs of severe sepsis with fever, tachycardia, tachypnea, and oliguria. Moreover, laboratory analyses showed that the patient had hyperglycemia of 520 mg/dL and metabolic ketoacidosis, and the necrosis expanded over his right face (Fig. 1). Soon he suffered heart failure and after revival, he underwent a new antibiotic schedule with ceftriaxone + clindamicin. However, 2 days later, he presented with shock, anuria, and multiorganic failure leading to death. As no autopsy was performed, it was presumed that death was most likely due to intracranial extension of the infection.

Mycology—Direct microscopy of a scraping of the necrotic border revealed thick non-septate hyaline hyphae. The macroscopic and microscopic morphology of the culture recovered from this scraping after inoculation on MA 2% (Malt Extract Agar 2%) and incubation at 37, 40, 45 and 50°C for 3–5 days was examined. Macroscopic observation revealed a pale brown to grey colony, up to 10 mm in height, within 3 days at 37 and 40°C, but restricted growth at 45°C and no growth at 50°C. Microscopically the rhizoids were simple. Microscopy of cultures grown at 40°C for 5 days revealed brownish sporangiophores up to 400 μm in length and up to 13 μm in width, in groups of 1–3; sporangia were spherical grayish, up to 80(–100) μm, predominantly

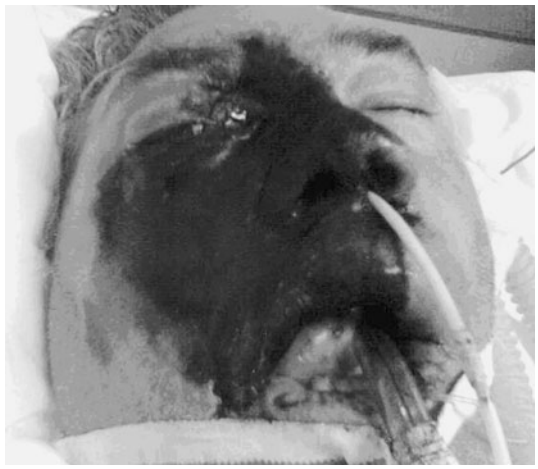

Fig. 1 Right facial necrosis of case 1 patient extending to the orbit

50–60 μm in diameter; columellae were globose to subglobose; sporangiospores were subglobose to ellipsoid, up to 9 μm, smooth. These morphological and physiological features indicate the mold was *Rhizopus microsporus* v. Tiegh. var. *oligosporus* (Saito) Schipper & Stalpers (Schipper & Stalpers 1984; de Hoog et al., 2000) which was deposited in the Reference Culture Collection of INCQS (Fiocruz) as *Rhizopus microsporus* var. *oligosporus* INCQS 40289 (Fig. 2).

Case 2

A 57-year-old woman from Cuiaba (MT), undergoind continuous therapy with azatioprina and corticoids after a renal transplant due to chronic arterial hypertension and uncontrolled type II diabetes mellitus, was admitted with chronic diarrhea and arterial hypotension. The initial diagnosis was sepsis due to an abdominal focus. She was treated with broad-spectrum antibiotics and mechanical ventilation, but failed to respond and deteriorated, due to a disseminated fungal infection. Subsequently, due to *Candida* spp. in her urine and a BAL culture, she was treated with fluconazole for 10 days and then substituted by caspofungin. Due to renal failure, she underwent hemodialysis. Two weeks later she presented with exophthalmus of the left eye that was surrounded by a large inflammatory and necrotic area (Fig. 3). Necrotic material was biopsed and sent to the Laboratory of Mycology.

Mycology—Direct microscopic exam of the necrotic material showed many thick non-septate hyaline hyphae suggestive of a mucoralean fungus. After inoculating necrotic material onto Sabouraud 2% dextrose agar a mucularean fungus was obtained, which was cultured on MA 2% (Malt Extract Agar 2%) and incubated at 37, 40, 45 and 50°C for 3–5 days for macroscopical and microscopic examination. The exam revealed a dark grayish brown colony, up to 10 mm in height within 3 days at 37 and 40 °C; good growth and sporulation at 45°C and growth at 50°C. Microscopic observation of the cultures grown at 40°C for 5 days revealed: simple rhizoids; brownish sporangiophores up to 500 μm in length and up to 10 μm in width, in groups of 1–4; sporangia were spherical grayish, black, up to 100 μm, predominately 60–80 μm

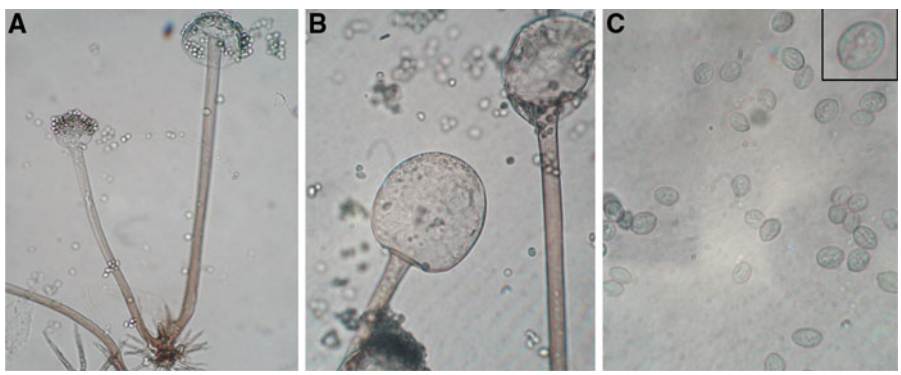

Fig. 2 *Rhizopus microsporus* var. *oligosporus:* **a** Sporangiophores, columellae, and rhizoids (Lactophenol cotton blue, ×200); **b** Columellae (Lactophenol cotton blue, ×400); **c** Sporangiospores (Lactophenol cotton blue, ×1,000)

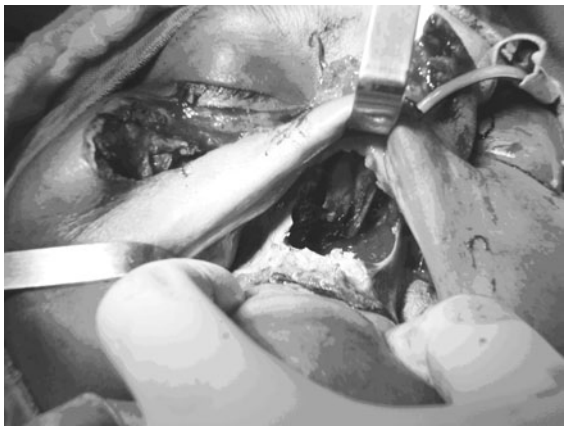

Fig. 3 Extensive necrosis of right cheek, reaching right nasal cavity and adjacent bones

diameter; columellae were pyriform-ellipsoidal, 80% of the sporangium diameter; and sporangiospores minutely spinulose, sub-globose to globose, up to 5(–6) µm. These morphological and physiological characteristics indicate the mold was *Rhizopus microsporus* v. Tiegh. var. *rhizopodiformis* (Cohn) Schipper & Stalpers (Schipper & Stalpers 1984; de Hoog et al., 2000), which was deposited in the Reference Culture Collection of INCQS (Fiocruz) as *Rhizopus microsporus* v. var. *rhizopodiformis* INCQS 40289 (Fig. 4).

Immediately upon obtaining the microbiological data, the patient was treated with high doses of intravenous amphotericin B together with broad-spectrum antibiotics and resection of the necrotic tissues. Despite this aggressive medical and surgical management, she died within 6 days.

Discussion

The number of reported cases of mucormycosis has continuously increased in recent years, in part as the result of increasing use of immunosuppressive therapy but also as a consequence of increased awareness of clinicians and improved laboratory diagnosis [4–6]. Although many species of several genera of the Mucorales are known to cause angioinvasive infections, the most commonly isolated species belong to the genera *Rhizopus*, *Mucor*, *Absidia,* and *Rhizomucor*. About half of all cases were caused by *Rhizopus* species, mainly by *R. arrhizus* (*R. oryzae*), followed by *R. microsporus* var. *rhizopodiformis* [3].

Although many immunodeppressive conditions might represent a risk factor for an angioinvasive infection by members of the Mucorales, two predisposing factors are most important: presence of metabolic acidosis and altered and deficient function of neutrophils and/or monocytes, which hinder their capacity to control fungal growth in diabetic patients [4, 8].

Both of our cases, caused by different varieties of *Rhizopus microsporus*, presented the classical rhinocerebral invasive form of mucormycosis. They had also the most typical and important underlying risk factor for this clinical presentation, namely an uncontrolled or poorly controlled type II diabetes mellitus, a condition that commonly evolved to ketoacidosis and other associated complications. In most cases with this

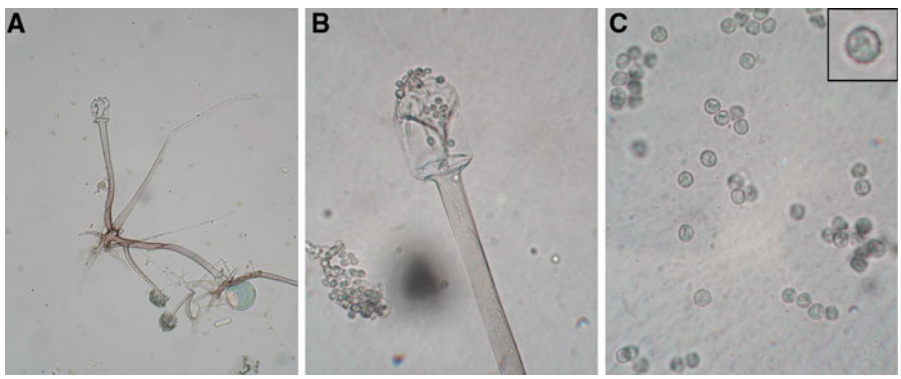

Fig. 4 *Rhizopus microsporus* var. *rhizopodiformis*: **a** Sporangiophores and rhizoids (Lactophenol cotton blue, ×100); **b** Columellae (Lactophenol cotton blue, ×400); **c** Sporangiospores (Lactophenol cotton blue, ×1,000)

clinical presentation, the onset manifests in the nasal mucosa or paranasal sinuses, from where the fungus invades the ethmoid, the maxillary sinuses and the nasolachrymal duct, and finally the cranium through the orbital vessels [10, 11].

However, the initial manifestations of our cases pointed strongly to bacterial infection, namely a face pimple that rapidly evolved to a clinical picture of cellulitis in case 1 and chronic diarrhea which evolved to sepsis in case 2, which probably postponed the recognition of the severe life-threatening mucormycosis.

Case 1 was caused by *R. microsporus* var. *oligosporus*, a rare agent of mucormycosis. His first symptom was a painful pimple on his nose that evolved to a necrotizing pustule on his right face, which was initially diagnosed as cellulitis.

It is worth mentioning that in some parts of Asia this fungus is used as a starter culture for the home production of *tempeh*. This indicates a continuous but harmless contact of the fungus with healthy populations [14]. Although it can't be corroborated by published cases, this finding deserves to be reported.

Case 2 was caused by *R. microsporus* var. *rhizopodiformis*, the second most common agent of mucormycosis, a variety commonly associated with to cutaneous and gastrointestinal forms [3]. Curiously, she presented initially with chronic diarrhea and the first suspected diagnosis was sepsis due to an abdominal focus, which did not respond to broad-spectrum antibiotics and evolved to a disseminated infection, including the rhinocerebral manifestation.

Unfortunately, our patients both had a fatal outcome, mainly due to a delay in correct suspicion and subsequent mycological diagnosis of mucormycosis. The prognosis of these patients usually is poor. Therefore, attempts to make an early diagnosis in a high-risk patient are encouraged, and treatment should be initiated as fast as possible. Treatment involves a multi-mortality approach with an equally important three-point strategy that includes (a) antifungal therapy, (b) surgery, and (c) management of comorbid factors (controlling diabetes mellitus in our cases) and adjunctive treatments for improving host response [3, 4].

Blood cultures in all forms of mucormycosis are usually negative. Furthermore, even when fungal hyphae are seen in tissue specimens, fungal cultures may be negative. Therefore, it is important that clinicians attempt to collect as many clinical specimens as possible for fungal cultures, since Mucorales are sometimes difficult to distinguish from other filamentous fungi by direct microscopy and/or histopathological examination. Refrigeration of the specimens is not recommended because it may decrease the yield of positive cultures. Cultures isolated from non-sterile specimens should be interpreted with caution and will require correlation between the finding and the clinical situation [3]. Therefore, growth of a mucoralean mold does not confirm its involvement in an invasive disease. However, isolation of Mucorales from a sterile site or repeated positive cultures from a non-sterile site is considered significant in a high-risk patient with predisposing factors for the acquisition of mucormycosis [3, 4].

To reach a more rapid diagnosis of mucormycosis, more modern laboratory tools are needed, besides improved awareness by clinicians.

Treatment for mucormycosis is based on three points: (1) elimination or control of the predisposing factors, (2) surgical debridement of necrotic tissue, and (3) antifungal. Until, recently, the only available effective antifungal was amphotericin B, but fortunately important advances have been made in recent years with posaconazole, an azole with activity against zygomycete species in selected patients [15–23].

Appropriate synergy testing to determine the role of antifungal combinations and knowledge of drug–drug interactions may be extremely important to efforts to reduce breakthrough infections [24, 25]. Many institutions use fluconazole as prophylaxis for invasive fungal infections (IFI) in high-risk patients [26]. However, if more cases of zygomycosis are detected in high-risk liver transplant recipients [27], a new strategy could include drug coverage for these microorganisms [28, 29] with posaconazole, an active azole against zygomycete species [21–23]. Physicians should be aware of the potential risk of breakthrough infections caused by several resistant fungi in patients with severe immunosuppression who are receiving antifungal drugs, including caspofungine [30].

Conflict of interest None.

References

1. Ameen M, Arenas R, Martinez-Luna E, Reyes M, Zacarias R. The emergence of mucormycosis as an important opportunistic fungal infection: five cases presenting to a tertiary referral center for mycology. Int J Dermatol. 2007;46:380–4.

2. Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, et al. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clin Infect Dis. 2005;41:634–53.
3. Chayakulkeeree M, Ghannoum MA, Perfect JR. Zygomycosis: the re-emerging fungal infection. Eur J Clin Microbiol Infect Dis. 2006;25:215–29.
4. Prabhu RM, Patel R. Mucormycosis and entomophthoramycosis: a review of the clinical manifestations, diagnosis and treatment. Clin Microbiol Infect. 2004;10(Suppl. 1):31–47.
5. Eucker J, Sezer O, Graf B, Possinger K. Mucormycoses. Mycoses. 2001;44:253–60.
6. Brown J. Zygomycosis: an emerging fungal infection. Am J Health-Syst Pharm. 2005;62:2593–6.
7. Sundaram C, Mahadevan A, Laxmi V, Yasha TC, Santosh V, Murthy JMK, Purohit AK, Mohandas S, Shankar SK. Cerebral zygomycosis. Mycoses. 2005;48:396–407.
8. Karanth M, Taniere P, Barraclough J, Murray JA. A rare presentation of zygomycosis (mucormycosis) and review of the literature. J Clin Pathol. 2005;58:879–81.
9. De Shazo RD, Chapin K, Swain RE. Fungal sinusitis. New Eng J of Med. 1997;4:254–9.
10. Glockner A, Vehreschild JJ, Cornely AO. Zygomycosis-current epidemiological aspects. Mycoses. 2007;50(Suppl 1):50–5.
11. Bengel D, Susa M, Schreiber H, Ludolph AC, Tumani H. Early diagnosis of rhinocerebral mucormycosis by cerebrospinal fluid analysis and determination of 16srRNA gene sequence. Eur J Neurol. 2007;14:1067–70.
12. Cavrini F, Stanzani M, Liguori G, Sambri V. Identification of an invasive infection of *R. oryzae* in a haematological patient using a molecular technique. Mycoses. 2010;53:269–71.
13. Woo PCY, Leung SY, To KKW, Chan JFW, Ngan AHY, Cheng VCC, Lau SKP, Yuen KY. Internal transcribed spacer region sequence heterogeneity in *Rhizopus microsporus*: implications for molecular diagnosis in clinical microbiology laboratories. J Clin Microbiol. 2010;48:208–14.
14. Rusmin S, Ko SD. Rice-grown *Rhizopus oligosporus* inoculum for *Tempeh* fermentation. Appl Microbiol. 1974;28:347–50.
15. Vigouroux S, Morin O, Moreau P, Mechinaud F, Morineau N, et al. Zygomycosis after prolonged use of voriconazole in immunocompromised patients with hematologic disease: attention required. Clin Infec Dis. 2005;40:35–7.
16. Greenberg RN, Scott LJ, Vaughn HH, Ribes JA. Zygomycosis (mucormycosis): emerging clinical importance and new treatment. Curr Opin Infect Dis. 2004;17:517–25.
17. Greenberg RN, Mullane K, Van Burik AH, Raad I, Abzug MJ, Anstead G, et al. Posaconazole as salvage therapy for zygomycosis. Antimicrob Age Chemother. 2006;50: 126–33.
18. Kauffman C, Malani AN. Zygomycosis: An emerging fungal infection with new options for management. Curr Infect Dis Reports. 2007;9(6):435–40.
19. Rogers TR. Treatment of zygomycosis: current and new options. J Antimicrob Chemother. 2008;61(Suppl 1):i35–9.
20. Walsh TJ, Kontoyiannis DP. What is the role of combination therapy in management of zygomycosis ? Clin Infect Dis. 2008;47(3):372–4.
21. Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. Clin Microbiol Rev. 2000;13:236–301.
22. Van Burik JA, Hare RS, Solomon HF, et al. Posaconazole is effective as salvage therapy in zygomycosis: a retrospective summary of 91 cases. Clin Infect Dis. 2006;42:61–5.
23. Greenberg RN, Mullane K, Van Burik JA, et al. Posaconazole as salvage therapy for zygomycosis. Antimicrob Agents Chemother. 2006;50:126–33.
24. Tong SY, Peleg AY, Yoong J, et al. Breakthrough *Scedosporium prolificans* infection while receiving voriconazole prophylaxis in an allogeneic stem cell transplant recipient. Transpl Infect Dis. 2007;9:241–3.
25. Cheung C, Guo Y, Gialanella P, et al. Development of candidemia on caspofungin therapy: a case report. Infection. 2006;34:345–8.
26. Lumbreras C, Cuervas-Mons V, Jara P, et al. Randomized trial of fluconazole versus nystatin for the prophylaxis of Candida infection following liver transplantation. J Infect Dis. 1996;174:583–8.
27. Almyroudis G, Sutton DA, Linden P, et al. Zygomycosis in solid organ transplant recipients in a tertiary transplant center and review of the literature. Am J Transplant. 2006;6:2365–74.
28. Cornely OA, Maertens J, Winston DJ, et al. Posaconazole vs. fluconazole or itraconazole profilaxis in patients singh neutropenia. N Engl J Med. 2007;356:348–59.
29. Reed A, Herndon JB, Ersoz N, et al. Effect of prophylaxis on fungal infection and costs for high-risk liver transplant recipients. Liver Transpl. 2007;13:1743–50.
30. Gimenia C, Moleti ML, Micozzi A, et al. Breakthough *Candida krusei* fungemia during fluconazole prophylaxis followed by breakthrough zygomycosis during caspofungin therapy in a patient with severe aplastic anemia who underwent stem cell transplantion. J Clin Microbiol. 2005;43:5395–6.