Case Report: Disseminated *Sporothrix brasiliensis* Infection with Endocardial and Ocular Involvement in an HIV-Infected Patient

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Abstract. Disseminated sporotrichosis occurs in individuals with impaired cellular immunity, such as in cases of neoplasia, transplantation, diabetes, and especially, acquired immunodeficiency syndrome. This report presents a 32-year-old Brazilian human immunodeficiency virus (HIV)-infected patient who developed a protracted condition of disseminated sporotrichosis with endocarditis, bilateral endophthalmitis, and lymphatic involvement. He needed cardiac surgery to replace the mitral valve. *Sporothrix brasiliensis* isolates were recovered from cultures of subcutaneous nodules and mitral valve fragments. Species identification was based on classical and molecular methods. The patient received amphotericin B for 52 days and subsequently, oral itraconazole. He remains asymptomatic, and he is on maintenance therapy with itraconazole. Despite his positive clinical outcome, he developed bilateral blindness. To our knowledge, this case is the first report of endocarditis and endophthalmitis caused by *S. brasiliensis*.

INTRODUCTION

Sporotrichosis has worldwide distribution, although most human and animal cases reported have been in Latin America, the United States, and Japan.^{1–3} *Sporothrix schenckii* is considered to be the classical agent for this mycosis. *S. brasiliensis*, *S. globosa*, and *S. mexicana* have recently been defined as separate species through molecular and phenotypic features and different geographical distribution. Only the first two are considered pathogenic. *S. brasiliensis* is commonly found in South America, especially in Brazil.^{4,5}

Most infections occur through accidental implantation of infectious agents after traumatic skin contact with colonized material.³ Transmission also occurs through inhalation of infectious propagules and bites or scratches from several mammals like dogs, armadillos, and cats.^{6–10}

The clinical picture depends on inoculum size, depth of inoculation, thermal tolerance of the infecting strain, and host immunity. Several conditions like alcoholism, diabetes, neoplasia, and acquired immunodeficiency syndrome are associated with disseminated cases and greater severity.^{11–13}

Most human immunodeficiency virus (HIV)-infected patients with disseminated sporotrichosis show protracted conditions, late diagnosis, and poor outcomes, despite antifungal treatment. Meningeal, ocular, respiratory, osteoarticular, and lymphatic involvement have been described as associated with fungemia.^{14–17}

This report aimed to present the clinical and evolutive features of an HIV-infected patient with sporotrichosis involving the mitral valve, eyes, and lymph nodes.

CASE REPORT

A 32-year-old male farmer was admitted to the Teaching Hospital in Uberaba, Minas Gerais, Brazil, in August of 2010. He complained of progressive bilateral visual deficit that had begun 2 months earlier. He had lost 14 kg but had not had fever or associated symptoms. There was no history of trips, skin injuries, or contact with animals, and he did not smoke

or drink. At clinical examination, he looked eutrophic but presented disseminated subcutaneous small nodules that were invisible but evident to the touch. He had previously undergone ophthalmological outpatient examination showing exudative granulomatous-like yellow lesions in right eye choriorretinal structures, whereas left eye evaluation was hindered by pupillary occlusion. *Toxoplasma gondii*, Epstein-Barr, and *Treponema pallidum* antibody tests were negative, cytomegalovirus immunoglobulin G (IgG) antibody test was reactive, and enzyme-linked immunosorbent assay (ELISA) anti-HIV test was positive (confirmed using Western blot). Chest radiography was normal. The Mantoux test and lymph bacterioscopy for *Mycobacterium leprae* were both negative. CD4⁺ T-cell baseline and viral load levels were 560 cells/mm³ and 92,158 RNA copies/mL, respectively.

The severity and granulomatous appearance of the ocular lesions led to instituting tuberculosis therapy. Histopathological examination of subcutaneous nodule fragment tissue showed granulomatous reaction with suppurative focus, but neither mycobacteria nor fungal structures were found. Three weeks later, culturing of subcutaneous nodule fragment tissue yielded fungal structures resembling *Sporothrix* sp. Oral itraconazole (200 mg bid) was prescribed, and antituberculosis drugs were withdrawn, with a presumptive diagnosis of disseminated sporotrichosis.

During hospitalization, systolic mitral murmur was detected. Echocardiograms showed increased left atrial diameter and thickened mitral valve with posterior disruption and anterior perforation. Aneurysm formation and moderate regurgitation were also observed. There were no clinical or echocardiographic findings of heart failure (Figure 1). Thus, endocarditis was suspected, and itraconazole was replaced by intravenous amphotericin B. The patient underwent cardiac surgery to replace his mitral valve with a mechanical prosthesis. Histopathological examination on the mitral valve showed a granulomatous and suppurative process with caseous necrosis but without mycobacteria or fungal structures. Three weeks later, mitral valve fragment culturing yielded fungal structures resembling *Sporothrix* sp.

Amphotericin B was administered for 52 days, including 10 days on liposomal amphotericin. Control echocardiograms were normal. One year later, he is asymptomatic and receiving

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FIGURE 1. Echocardiographic assessment showing increased diameter of the left atrium and lesioned mitral valve with aneurysmatic formation and regurgitation. (A, B, and E) Transversal view showing mitral valve vegetation. (C and F) Apical view showing mitral valve vegetation. (D) M-mode echocardiogram showing mitral valve lesion. IVS = interventricular sept; LA = left atrium; LV = left ventricle; MV = mitral valve; RA = right atrium; RV = right ventricle.

oral itraconazole and antiretroviral therapy. Despite this positive outcome, he developed bilateral blindness.

PHENOTYPIC CHARACTERIZATION OF *SPOROTHRIX* SP. ISOLATES

Sporothrix isolates were grown at four temperatures (30°C, 35°C, 37°C, and 40°C) in potato dextrose agar (PDA, Becton, Dickinson and Company, Sparks, MD) medium. Fungus specimens (approximate diameter = 1 mm) were centrally inoculated in Petri dishes (diameter = 9 cm), and the plates were placed upside down. Colony diameter (in millimeters) was measured in two orthogonal directions after 21 days of incubation. Carbohydrate assimilation was tested using conidia on four sugars: dextrose, sucrose, raffinose, and ribitol. The microplates were read after 10 days of incubation at 25°C.⁵

To study macroscopic and microscopic features, isolates were subcultured on PDA plates and cornmeal agar (CMA; 30 g corn, 15 g agar, 1 L tap water) for 10–12 days and incubated at 30°C in the dark. All experiments were run in triplicate. These data together were used as taxonomic characters applied to dichotomous keys for *S. schenckii* complex species as previously described.¹⁸ To compare phenotypic characteristics, the Brazilian *S. brasiliensis* type strain CBS 120339 was used.⁵

MOLECULAR CHARACTERIZATION

DNA was extracted and purified directly from fungal mycelial colonies using the Fast DNA kit protocol (MP Biochemicals LLC, Solon, OH). Using the primers CL1 and CL2A,¹⁹

an 800-bp calmodulin amplicon was amplified from genomic DNA and followed by DNA sequencing in both strands.

The macroscopic morphological characteristics of *Sporothrix* isolates resembled type strain CBS 120339. After 21 days of incubation at 30°C, colonies on PDA attained a diameter of 48.7 ± 0.06 mm; they were moist, glabrous, and creamy to dark brown. At 35°C and 37°C, after 21 days of incubation, the isolates had grown to colony diameters of 17.12 ± 2.10 and 8.96 ± 0.72 mm, respectively. They did not grow at 40°C. Microscopically, the isolates presented thin, sporulated, and septated hyaline hyphae on PDA after 7–12 days at 30°C and developed intercalary or terminal conidial clusters on sympodial conidiophores. The blastoconidia were round and dark (Figure 2).

Thermal dimorphism switching was achieved after several subcultures in Brain Heart Infusion (BHI, Becton, Dickinson and Company) agar and incubation at 37°C, thereby developing a creamy yeast-like colony. Carbohydrate assimilation tests were run in triplicate. The isolates were able to assimilate glucose and ribitol as the only source of carbon, and they did not assimilate raffinose or sucrose. Using morphological and physiological techniques, they were classified as *S. brasiliensis*.

The phenotypic data were confirmed through molecular analysis on nucleotide sequences of the calmodulin amplicon. Basic Local Alignment Search Tool (BLAST) search revealed that this isolate (GenBank accession number JN204360) showed 99% similarity with a Brazilian type strain (CBS 120339) of *S. brasiliensis*.

DISCUSSION

Disseminated sporotrichosis cases have shown meningeal, respiratory, osteoarticular, lymphatic, and ocular involvement

FIGURE 2. Micromorphology of the simpodial and sessil conidia of the *S. brasiliensis* strain Ss 265 developed on CMA media after 10 days of incubation at 25°C. Hyaline conidia simpodially arranged in a bouquet-like fashion at the ends of the conidiophores are shown in A. Globose dark pigmented sessile conidia are shown in details. (Scale bar: 3 µm).

in both HIV-infected and non-HIV patients^{14,20–23}; however, endocarditis involvement was not reported to date.

Our patient presented ocular symptoms as the first feature of systemic disease. Granulomatous uveitis was found in the first ophthalmological examination, and given the high prevalence of tuberculosis in Brazil, empirical therapy was started. On admission, after obtaining the patient's complete history, clinical examination showed disseminated subcutaneous nodules and mitral valve murmur. Culturing of subcutaneous nodule tissue yielded *Sporothrix* sp. isolates. Hence, ocular and cardiac lesions were presumptively correlated with this finding, and oral antifungal therapy was replaced by intravenous amphotericin B.

Granulomatous uveitis caused by *S. schenckii* infection has previously been reported. It is a protracted condition with poor outcomes, leading to total blindness in most cases.^{24–26} Our patient's evolution was similar, with bilateral endophthalmitis despite surgical procedures and systemic antifungal therapy, thereby confirming previously reported severity of ocular sporotrichosis and poor prognosis for eyesight. Our patient's ocular infection probably originated from hematogenous spread, although no fungus was recovered from blood cultures.^{23,27}

Fungal endocarditis accounts for 1.3–6% of all infectious endocarditis cases. These are commonly severe, leading to high morbidity and mortality.²⁸ *Candida* species are the most prevalent agents and are present in one-half to two-thirds of fungal endocarditis patients.^{28,29} This infection can occur through contaminated devices or liquids during continuous catheterization.³⁰ Neutropenia, neoplasia, intravenous drug use, immunosuppressive therapy, and total parenteral nutrition are the most common risk factors.^{31,32} The second most common agent is *Aspergillus* sp. This agent is associated with heart valve surgery and disseminated aspergillosis, usually evolving to acute conditions of greater severity than *Candida* cases.³³ Occasionally, endocarditis caused by rare fungal species has also been reported.^{34,35}

Our patient received 2 weeks of oral itraconazole before fungal endocarditis was suspected. Cardiac surgery was immediately performed to replace the severely damaged mitral valve observed on echocardiograms. It could not be established whether the patient had any previous mitral valve lesion as a pre-disposing factor, but together with the ocular and cutaneous lesions, it is highly likely that persistent fungemia had occurred, despite negative blood cultures.

The guidelines and recommendations from different authors and centers dealing with fungal endocarditis patients indicate that the best outcomes are obtained through combining medical and surgical therapies.^{28,36,37} Although both ocular and cardiac surgery was performed, only mitral valve tissue could be cultured, again yielding *Sporothrix* sp. isolates and confirming cardiac involvement. In contrast, histopathological examination of subcutaneous nodules and mitral valve fragments did not show *Sporothrix* sp. infection. This finding is in line with previous descriptions from HIV patients and can be correlated with reduced numbers of microorganisms in the inflammatory process.¹²

To our knowledge, this case is the first report of endocarditis and endophthalmitis caused by *S. brasiliensis*. This species was recently recognized through molecular characteristics within the *S. schenckii* complex and is also considered pathogenic for humans and animals.⁵ According to molecular and epidemiological data, *S. brasiliensis* is mainly distributed in South America, especially in Brazil.

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