Bone sporotrichosis: 41 cases from a reference hospital in Rio de Janeiro, Brazil

Vanessa Ramos¹, Guis S-M. Astacio², Antonio C. F. do Valle², Priscila M. de Macedo², Marcelo R. Lyra², Rodrigo Almeida-Paes², Manoel M. E. Oliveira³, Rosely M. Zancopé-Oliveira², Luciana G. P. Brandão², Marcel S. B. Quintana², Maria Clara Gutierrez-Galhardo², Dayvison F. S. Freitas¹,²*

¹ Pós-Graduação em Medicina Tropical, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil,
² Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil,
³ Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

* dayvison.freitas@ini.fiocruz.br

Abstract

Background

Bone sporotrichosis is rare. The metropolitan region of Rio de Janeiro is hyperendemic for zoonotic sporotrichosis and the bone presentations are increasing.

Methods

We studied a retrospective cohort of 41 cases of bone sporotrichosis, diagnosed from 1999–2016. The inclusion criteria was fungal culture isolation from any clinical specimen associated to bone involvement (radiography and/or computed tomography) compatible with fungal osteomyelitis or histopathological findings of bone material compatible with sporotrichosis. Molecular identification was performed when possible.

Results

Male patients represented 58.5% of the cases, with a cohort median age of 43 years. Immunosuppressive conditions were present in 68.3% of the patients, mostly HIV coinfection (51.2%). Multifocal bone involvement (more than one anatomical segment) was diagnosed in 61% of the patients, while 39% presented unifocal involvement. The bones of the hands were the most affected (58.5%), followed by the feet (41.5%) and tibia (26.8%). Multifocal group was characterized by a higher proportion of males (p = 0.0045) with immunosuppressive conditions (p = 0.0014). Amphotericin B followed by oral itraconazole was the main treatment, with a median time of 16.7 months (1.5 to 99.2 months), and cure of 53.7% of the patients (84.6% of immunocompetent and 39.3% of immunocompromised patients). Sequelae occurred in 12.2% of the patients—amputations (7.3%) and ankylosis (4.9%), while 22% died in the course of the disease. Sporothrix brasiliensis was the causative agent in all the 9 (22%) performed cases.
Conclusions
Bone sporotrichosis is a chronic, challenging condition with prolonged treatment, often with poor results and sequelae.

Author summary
Sporotrichosis is a subcutaneous mycosis, more common in tropical and subtropical countries, and the involvement of bones is rare. The metropolitan region of Rio de Janeiro is hyperendemic for zoonotic sporotrichosis and the bone presentations are increasing. We evaluated 41 cases of bone sporotrichosis at a reference center in infectious diseases. There was a predominance of men with immunosuppressive conditions, notably HIV infection and alcoholism. Immunosuppression was related to multifocal bone involvement and more severe disease. This form of the disease is a chronic, challenging condition with prolonged treatment, often with poor results and sequelae.

Introduction
Sporotrichosis is a worldwide subcutaneous mycosis, especially in tropical and subtropical regions [1]. The causative fungi, *Sporothrix* spp., live associated with plants, soil or decomposing organic material. The classic transmission is through traumatic inoculation of the fungus in the skin [2] and the metropolitan region of Rio de Janeiro is hyperendemic for cat-transmitted zoonotic sporotrichosis caused, in most cases, by *Sporothrix brasiliensis* [3–4]. The cutaneous forms usually account for more than 90% of the cases [2,3,5]. Osteoarticular involvement is the most common extracutaneous manifestation of sporotrichosis [2], nevertheless, it is considered rare [6–14].

Bone sporotrichosis occurs by contiguity or hematogenous spread, presents with an indolent course of pain and limitation of articular movement [15], alone or as part of a disseminated infection [6]. Its treatment is longer and requires a higher daily dose of antifungals, compared to cutaneous sporotrichosis. Itraconazole 400 mg/day is recommended for at least 12 months and, in more severe cases, the use of intravenous amphotericin B (AMB) may be necessary [16].

We evaluated the socio-demographic and epidemiological characteristics, and the clinical evolution of the patients with bone sporotrichosis, in a reference hospital, aiming to describe the cases and to find explanatory variables.

Methods
Ethics statement
All procedures performed were in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments, as well as the Brazilian ethical standards—Resolution (CNS 466/12). The Instituto Nacional de Infectologia Evandro Chagas (INI) Review Board approved the study under the number 64068717.4.0000.5262. Written consent was waived, justified by the difficulty in obtaining it from most of the patients, and based on the compromise and responsibility of the principal investigator in anonymizing and protecting the patients' personal data.
Place of study, patients, and study design

INI, Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil, is a reference center for the treatment of sporotrichosis in the state of Rio de Janeiro and, since the beginning of the increase in the number of cases of human sporotrichosis, in the late 90s, patients are followed up in a cohort. From this primary cohort, we selected patients with culture-proven sporotrichosis from any clinical specimen and associated bone involvement, from 1999 to 2016. Patients with other culture-proven causes of osteomyelitis were excluded from the study.

Patient management

Patients were submitted to clinical evaluation, mycological examination (direct microscopy and culture) of clinical specimens and blood tests (blood count, biochemistry, liver function, erythrocyte sedimentation rate (ESR), and high sensitivity C-reactive protein (hs-CRP)).

Investigation of bone lesions was performed in patients with exuberant cutaneous lesions adjacent to bone surfaces (usually associated with pain, edema, and limitation of movement) and in those with disseminated cutaneous lesions or disseminated disease. In the first indication, local bone radiography was done and, in the second, a bone screening (total skeletal radiographs or bone scintigraphy) was performed to search for asymptomatic lesions. Regarding treatment, at the INI, patients with cutaneous forms receive oral itraconazole, whereas patients with disseminated sporotrichosis receive AMB up to clinical improvement, complemented by oral itraconazole, until clinical cure [16].

Molecular identification

Nine of the patients’ Sporothrix clinical isolates could be recovered at the institutional Laboratory of Mycology and the species was identified by the T3B PCR fingerprinting, as previously described [17].

Definition of bone sporotrichosis

Bone involvement was defined by imaging (primarily radiography and computed tomography) compatible with fungal osteomyelitis, analyzed by two independent radiologists and/or histopathological findings of bone material compatible with sporotrichosis. The healing of bone lesions was determined by normalization of the bone images or estimated by stabilization of the lesions through comparative images every three-six months.

Data collection and statistical analysis

We reviewed the medical charts of the selected patients for the collection of socio-demographic, epidemiological, clinical and laboratory data. The data were entered into a database in the program FormSUS (service for the creation of forms of public access of the Brazilian National Health System) and analyzed with the assistance of the library of the R program, version 3.3.0. Contingency tables and association tests (qui-square and Fisher exact) were used to compare groups.

Univariate and multivariate analyses were performed using the Cox model of proportional risks, considering the time the patient was under treatment until the time of cure, and skin color, sex, immunosuppression, form of bone involvement, cat bite and alcoholism as predictors. For those patients who did not cure, such as those who died, those who lost follow-up or those still in treatment, the curve was censored at such moments (date of death, date of loss of follow-up and date of analysis).
Results

Forty-one cases of bone sporotrichosis were included and represented 0.9% of the 4,617 cases of sporotrichosis treated at the INI-Fiocruz during the studied period, mostly concentrated in the last decade (Graph 1).

Socio-demographic and epidemiological characteristics

Male patients represented 58.5% (n = 24) of the cases, while 41.5% (n = 17) were female. Non-white patients were 73.2% (n = 30, 23 brown and 7 black) and white patients, 26.8% (n = 11). The median age was 43 years (range: 16–79 years). Regarding the schooling: 58.5% (n = 24) studied up to 9 school years and 34.1% (n = 14) had more than 9 school years, including 7.3% (n = 3) with a bachelor’s degree. In 7.3% (n = 3), the schooling was unknown. Most of the patients came from the city of Rio de Janeiro (n = 17, 41.5%), mainly from the north and west zones of the city, followed by municipalities in the capital’s metropolitan region (Duque de Caxias: 17.1%, n = 7 and Belford Roxo: 9.8%, n = 4). The most prevalent occupations (48.8%, n = 20) were those related to household activities, such as housewife, housemaid, unemployed, retired and student. Contact with domestic cats was reported by 70.7% (n = 29) of the patients and, within this group, bites and/or scratches were reported in 41.4% (n = 12).

Clinical characteristics

Table 1 presents clinical data of the patients. In brief, the initial clinical presentation of sporotrichosis was the disseminated cutaneous form in 70.7% of the cases (n = 29), followed by lymphocutaneous (22%, n = 9) and fixed cutaneous (3%, n = 3). Immunosuppressive conditions, present in 68.3% (n = 28) of the patients, included human immunodeficiency virus (HIV) infection (51.2%, n = 21), alcoholism (22.0%, n = 9), a combination of both (23.8%, n = 5), malnutrition, and chronic use of corticosteroids (4.9%, n = 2, each). Diabetes mellitus, although a potential immunosuppressive condition, was not considered so in this study since patients were stable for this comorbidity.
Table 1. Description of the initial clinical form, comorbidities and bones affected, of the patients with bone sporotrichosis, treated at the INI-Fiocruz, from 1999 to 2016.

| Case | Initial clinical form | Comorbidities | Affected bones |
|------|-----------------------|---------------|----------------|
| 1    | DC / Nasal mucosa     | HIV           | Feet, R acromion, L ulna |
| 2    | DC / Oral and nasal mucosa | HIV / Alcoholism | R hand (middle phalanx of 3rd finger), R wrist, R ulna, R ankle, Knees |
| 3    | LC (R arm) / Choroiditis | HIV           | L wrist, R radius, R ulna, R humerus, R foot, R ankle |
| 4    | DC / Nasal and oral mucosa / Choroiditis | HIV           | Feet, L clavicle, R radius, L wrist |
| 5    | DC / CNS             | HIV           | Hands, R ankle, L clavicle, Feet, R wrist |
| 6    | DC / Nasal and oral mucosa / Choroiditis / CNS | HIV           | R hand (middle phalanx of 2nd, 3rd, 4th and 5th finger), R wrist, Tibias, Fibulas |
| 7    | DC / Nasal mucosa     | HIV           | Wrists, Elbows, Knees, Ankles |
| 8    | LC (L hand)           | HBP / DM      | L hand (4th Finger) |
| 9    | LC (L hand)           | HBP           | Distal phalanx of 5th L finger |
| 10   | LC (R hand)           | -             | Distal phalanx of 2nd R finger |
| 11   | DC                   | Alcoholism / Corticosteroids use | L olecranon |
| 12   | FC (R hand)           | HCV           | Distal phalanx of 1st R finger |
| 13   | LC (R hand) / Synovitis | HBP / DM      | R wrist |
| 14   | LC (L hand)           | -             | Distal phalanx of 1st L finger |
| 15   | DC / Nasal mucosa     | HBP / DM      | Proximal phalanx of 4th R finger |
| 16   | DC                   | HBP           | L metacarpal and phalanges, Middle phalanx of 4th R finger |
| 17   | DC                   | -             | R clavicle |
| 18   | DC                   | Malnutrition  | 5th L metatarsus, L tibia, L fibula |
| 19   | DC                   | HIV / Alcoholism | Feet (tarsi, L and R metatarsi, L calcaneus) |
| 20   | DC / Synovitis        | Alcoholism / Corticosteroids use | Tibias, R wrist, Hands (several phalanges), Feet |
| 21   | LC (R arm) / CNS      | HIV           | R hand (proximal phalanx of 2nd finger) |
| 22   | LC (R arm)            | HIV           | Tibias and R calcaneus |
| 23   | DC                   | HIV           | Hands (2nd R finger and 5th R metacarpus) |
| 24   | DC / Nasal mucosa     | HBP / Malnutrition | Hands (3rd R metacarpus and 2nd L metacarpus) |
| 25   | DC / Nasal and oral mucosa / Synovitis | HIV           | L foot (3rd toe), R knee |

| Case | Initial clinical form | Comorbidities | Affected bones |
|------|-----------------------|---------------|----------------|
| 26   | DC                   | HBP / DM      | L wrist, Knees |
| 27   | DC                   | Alcoholism    | L tibia and calcaneus |
| 28   | DC / Nasal and oral mucosa | HIV / HBP     | L hand (4th finger), L ankle, Knees |
| 29   | DC / Nasal and oral mucosa | DM / Lepra / Corticosteroids use | Hands (proximal and middle phalanges of 2nd to 5th R fingers and several phalanges of 2nd to 5th L fingers), R ulna, Feet, Tibias (distal extremities), Ischia, L femur |
| 30   | DC / Nasal and oral mucosa | HIV / Alcoholism | Hands (proximal phalanx of 5th R finger, middle phalanx of 4th R and L fingers, proximal phalanx of 3rd L finger), Feet (5th R metatarsus, L cuboid), Tibias |
| 31   | DC / Oral mucosa / Retinitis | -             | 2nd R finger, Feet, Skull, Costal arches, Clavicles, R ulna, L radius, L fibula and tibia |
| 32   | DC                   | Alcoholism    | R foot (distal phalanx of 2nd toe) |
| 33   | LC (R hand and arm)   | -             | Distal phalanx of 2nd R finger |
| 34   | DC / Nasal mucosa     | HIV           | Foot (1st R metatarsus) |
| 35   | DC                   | HIV / Alcoholism | L hand (proximal phalanx of 2nd finger) |
| 36   | DC                   | HIV / Alcoholism | Hands (3rd R metacarpus, 2nd L metacarpus, proximal phalanx of 4th L finger), R foot (1st metatarsus), R elbow |
| 37   | DC                   | HIV           | Hands (proximal phalanx of 3rd and 5th L fingers), Feet (5th R metatarsus, R and L calcaneus), R tibia, R and L ulnas, R and L humeri |
| 38   | DC / Nasal mucosa / CNS | HIV           | Hands (proximal phalanx of 2nd L finger), L radius and ulna |
| 39   | FC (L wrist)          | HBP / DM      | L wrist |

(Continued)
Mycological results

For all patients, *Sporothrix* spp. was isolated from cutaneous specimens (lesion exudate or skin biopsy). Additionally, in 43.9% (n = 18) of the cases, the fungus was also isolated from other specimens: nasal swab (17.1%, n = 7), synovial fluid (12.2%, n = 5), oral swab (9.8%, n = 4), blood (7.3%, n = 3), bronchoalveolar lavage, lymph node, cerebrospinal fluid (4.9%, n = 2, each), and nasal biopsy, larynx, and urine (2.4%, n = 1, each). Bone biopsy was performed in 4 (9.8%) patients. One was the first case of our cohort (in 2002 – case 31), with a granulomatous inflammatory infiltrate and rare yeast-like structures in histopathology (Fig 1); the second (case 26) presented a chronic disease, with knee bone destruction and a biopsy was performed, with the isolation of the fungus in culture, despite an unspecific inflammatory process [22]; the third was already referred from another institution with the bone diagnosis (case 34), and the fourth (case 8) was submitted to a bone debridement and, due to the extensive destruction, the surgeon decided to amputate the fourth left finger with the visualization of the fungus in the histopathology.

Molecular identification of the clinical isolates

The nine (22%) analyzed isolates were identified as *S. brasiliensis* by the T3B PCR fingerprinting. Four of these were previously reported (case 4: as case 1 in [18]; case 5: in [12,19] and as case 7 in [20]; case 6: as case 2 in [18]; case 7: in [21]; case 18: in [10]; case 26: as case 6 in [20] and in [22]; case 28: as case 1 in [23] and as case 12 in [24] and case 35: as case 2 in [23] and as case 14 in [24].
case 7 in [20]; case 6: as case 2 in [18] and case 26: as case 6 in [20] and in [22]). The other five patients, with the causative species still unpublished, are cases 8, 17, 28, 31 and 37.

**Bone involvement**

In this series, the initial imaging diagnosis was by radiography (73.2%, n = 30), computed tomography (14.6%, n = 6), magnetic resonance (7.3%, n = 3) and bone scintigraphy (2.4%, n = 1) (Fig 2).

The most common radiological findings were the well circumscribed medullary lytic lesions with preservation of the cortical bone (80.5%, n = 33).

Multifocal bone involvement (lesions in more than one anatomical segment) was diagnosed in 61% (n = 25) of the patients (Fig 3), while 39% (n = 16) presented unifocal involvement (lesions in only one anatomical segment, Fig 4).
The bones of the hands were the most affected ones (58.5%, n = 24), followed by the bones of the feet (43.9%, n = 18) and tibia (26.8%, n = 11, Table 1). The feet, in the multifocal involvement, were affected in 64% of the cases whereas in unifocal involvement, this occurred in 12.5%. So, the calculated risk ratio for a patient with the multifocal involvement to present lesion in the bones of the feet is 5.12 (95% CI: 1.36–19.35; p = 0.001), when compared to the group with unifocal involvement.

The comparative analysis by association tests of the multifocal and unifocal groups (Table 2) showed that the multifocal group was characterized by a higher proportion of males (p = 0.0045) with immunosuppressive conditions (p = 0.0014), notably HIV infection (p = 0.0109). When HIV-infected patients were compared to other patients, they were younger (median age: 38 years) and predominantly men (76.2%, n = 16). All these patients had disseminated cutaneous lesions, with multifocal bone involvement in 81% (n = 17, Fig 5). The diagnosis of HIV was concomitant with the diagnosis of sporotrichosis (or during the investigation of cutaneous sporotrichosis lesions) in 38.1% (n = 8) of the cases and 85.7% of the coinfected patients (n = 18) did not use antiretroviral therapy (they were either in treatment abandonment or had not started it yet). The CD4+ T cell count at the time of the diagnosis of sporotrichosis ranged from 1 to 348 cells/mm^3, with 81% (17/21) below 200 cells/mm^3, median of 46 cells/mm^3 and viral load between undetectable and log 5.74.

Treatment and evolution

Table 3 depicts antifungal drugs and dosages used for each patient. Itraconazole was used by all patients at doses ranging from 200 to 600 mg/day. Combination of drugs was required for 78% (n = 32) of the patients. The most widely used therapeutic regimen was intravenous AMB, complemented with oral itraconazole. The median treatment time was 16.7 months (1.5 to 99.2 months). In 73.2% of the cases (n = 30) there was at least one hospitalization, 33.3% (n = 10) motivated by bone sporotrichosis. By the end of the study, 53.7% (n = 22) of patients were considered cured. Among immunocompetent patients, 84.6% (11/13) achieved this outcome, while only 39.3% (11/28) of immunocompromised patients cured. Thus, the calculated risk ratio for an immunosuppressed patient to cure from bone sporotrichosis was 0.35 (i.e., 1/2.84) (95% CI: 0.15–0.82, p = 0.02), compared to an immunocompetent patient (S1 Table). Eight patients (19.5%) were still under treatment, two (4.9%) were lost to follow-up and nine (22.0%) died (mainly related to AIDS).

Among the 22 cured patients, we had access to the initial ESR and CRP measures of 15 and 13 patients, respectively, and both markers in 12 patients (S2 Table). In general, these markers were elevated at the onset of the condition and tended to decrease (median: -50% [interquartile range (IQR): -73.7%;2.6%] for the ESR and -77.6% [IQR: -89.5%;-49.7%] for the CRP) over the course of treatment.
Sequelaes occurred in five (12.2%) patients. Amputations occurred in case 8, already described, case 15, also due to an intense destruction of the fourth right finger, despite of 12 months of itraconazole use (400 mg/day) and case 1, the patient ripped out his own necrotic fourth right toe. In case 26, due to osteoarticular sporotrichosis, the patient had bilateral ankylosis of knees, becoming wheelchair bound, while case 10 lost soft tissues of the second right finger, with impairment of movements, also with ankylosis.

In the multivariate analysis, a higher ratio of cure was associated to unifocal bone involvement, $4.84$ (95% CI: 1.79–13.08); $p < 0.01$, compared to multifocal involvement, and white color, $2.49$ (95% CI: 0.99–6.28); $p = 0.05$, compared to non-white patients.

**Discussion**

Sporotrichosis is an expanding zoonotic hyperendemia in the state of Rio de Janeiro, Brazil, mainly affecting women in their forties, who keep contact with sick cats and perform peri domiciliary activities [2,3,25]. *Sporothrix brasiliensis* is associated with atypical and potentially severe cases, probably due to its greater virulence compared to other species of the genus [26–
The exclusive molecular identification of *S. brasiliensis* corroborates the predominance of this species within this region, and its role in severe clinical cases [26]. This study, including 41 patients with bone sporotrichosis over 18 years, is the largest worldwide institutional series on the subject.

The cases evaluated and reported came mainly from areas with low socioeconomic conditions in the metropolitan region of Rio de Janeiro, following the distribution of the so called sporotrichosis "belt" [25]. Since 2013, sporotrichosis became a compulsory reportable disease in the state of Rio de Janeiro, and the clinical support for the patients with sporotrichosis was decentralized, with only the most serious cases being referred to the INI-Fiocruz. This reflected in the increase of cases with bone involvement in this institution, probably with an important selection bias, represented by the 9% of cases with bone involvement seen in 2016 (Graph 1). It draws our attention, the immunosuppression present in our cohort, not only due to HIV infection, but also to alcoholism and corroborates that immunosuppression is a factor associated with invasive forms and bone involvement in sporotrichosis [2,16]. It is worth noting that sporotrichosis was a key to HIV diagnosis in many patients, and that most of the patients were not on antiretroviral therapy, highlighting the opportunistic behavior of sporotrichosis and the importance of investigating disseminated disease. We believe that a maintained hyperendemia of sporotrichosis leads to the overlap with the HIV pandemic that also reflects in a predominance of men and a lower median age in this group. Other different remarkable aspects are the large percentage of non-white HIV patients with low schooling that can be understood as indicators of vulnerability of the population exposed to both diseases, culminating in more severe cases of sporotrichosis [28,29]. Alcoholism is another recognized risk factor for disseminated disease and bone involvement [16]. Previous reviews also highlighted the presence of comorbidities: Gladstone and Littman [6] found 27.2% of comorbidities among the 22 cases reviewed, with alcoholism present in 4.5% of them. Lederer et al. [13] reported a case of bone sporotrichosis, reviewed other 20 cases from 1980 to 2015 and found 52% of comorbidities, with alcoholism present in 23.8% and HIV infection in 14.2%. In our study, besides HIV infection, it was difficult to assess the impact of alcoholism alone, as the casuistic size is small, and more than half of these alcoholics also had HIV coinfection. Gregory et al. [30] demonstrated the potential deleterious clinical effects of the overlap of these two immunosuppressive
| Case | Bone involvement | Treatment for sporotrichosis | Time<sup>a</sup> (months) | Outcome |
|------|------------------|-----------------------------|---------------------------|---------|
| 1    | Multifocal       | ITZ 200mg 12mo, ITZ 400mg 24mo / AMB (d): ~ 1g | 37.7 | Cure (amputation)<sup>b</sup> |
| 2    | Multifocal       | ITZ 200mg 4mo / AMB (d): 9.2, AMB (l): 6.8g / TRB 250mg 1mo / PSZ 8mo | 37.6 | Death<sup>b</sup> |
| 3    | Multifocal       | ITZ 400mg 12mo, ITZ 200mg 17mo / AMB (d): 6.4g / TRB 250mg 6.5mo | 25.9 | Cure |
| 4    | Multifocal       | ITZ 200/400mg since August 2011 / AMB (d): 1.5g / TRB 500mg 2mo | 68.8 | Treating<sup>c</sup> |
| 5    | Multifocal       | ITZ 200mg 1mo / AMB (d): 2.5g, ANF (l): 12.8g / TRB 17mo / PSZ 15mo | 19.4 | Death |
| 6    | Multifocal       | ITZ 200mg 1mo, ITZ 400mg 14.5mo / AMB (l): 14.2g / TRB 13mo / PSZ 3mo | 44.4 | Death<sup>c</sup> |
| 7    | Multifocal       | ITZ 400mg 60mo / ITZ 200mg since September 2017 / AMB (d) 1.5g / TRB 500mg 22mo | 48.2 | Treating<sup>c</sup> |
| 8    | Unifocal         | ITZ 100mg 3mo, ITZ 200mg 1mo, ITZ 400mg 1.5mo | 1.5 | Cure (amputation) |
| 9    | Unifocal         | ITZ 400mg 12mo | 19.3 | Cure |
| 10   | Unifocal         | ITZ 200mg 4mo, ITZ 100mg 3.5mo | 3.5 | Cure<sup>c</sup> |
| 11   | Unifocal         | ITZ 100mg 1mo, ITZ 200mg 9mo | 14.4 | Lost to follow-up |
| 12   | Unifocal         | ITZ 200mg 2mo, ITZ 300 mg 14mo / AMB (l): 3.6g | 16.7 | Cure |
| 13   | Unifocal         | ITZ 400mg 43mo | 43 | Cure |
| 14   | Unifocal         | ITZ 100mg 2mo, ITZ 400mg 6mo | 6 | Cure |
| 15   | Unifocal         | ITZ 400mg 19mo / AMB (d): 295mg, AMB (l): 1.2g / TRB 250mg 9mo | 5.6 | Cure (amputation) |
| 16   | Multifocal       | ITZ 400mg 13mo / AMB (d): 500mg, AMB (l): 4.4g / TRB 250mg 4mo | 18.2 | Cure |
| 17   | Unifocal         | ITZ 200mg 2mo, ITZ 400mg ~7mo | 7.1 | Cure |
| 18   | Multifocal       | ITZ 100mg ~12mo, ITZ 200/400mg ~5mo / AMB (d): 315mg, AMB (l): 900mg | 21.8 | Death |
| 19   | Multifocal       | ITZ 400mg 16mo / AMB (d): 500mg | 14.5 | Cure |
| 20   | Multifocal       | ITZ 200mg 1mo, ITZ 400mg 1mo (abandonment), ITZ 200mg since August 2017 / AMB (d): 3.8g | 28.9 | Treating<sup>c</sup> |
| 21   | Unifocal         | ITZ 400mg irregular use / AMB (d): 1.85g, AMB (l): ~9.6g / TRB 250mg 1.5mo, TRB 500mg ~3mo / PSZ ~3weeks | 8.5 | Death<sup>c</sup> |
| 22   | Multifocal       | ITZ 200mg 14mo / AMB (d): 1g | 10.5 | Cure |
| 23   | Unifocal         | ITZ 400mg 8mo / AMB (d): 2.1g / PSZ since September 2017 | 15.2 | Treating |

| Case | Bone involvement | Treatment for sporotrichosis | Time<sup>a</sup> (months) | Outcome |
|------|------------------|-----------------------------|---------------------------|---------|
| 24   | Multifocal       | ITZ 400mg 14mo / AMB (d): 700mg, AMB (l): 6.9g | 14.7 | Cure |
| 25   | Multifocal       | ITZ 400/200mg ~12mo (relapse), ITZ 400mg since November 2011 / AMB (d): 2.3g | 99.2 | Treating |
| 26   | Multifocal       | ITZ 200mg 2mo, ITZ 300mg 10mo / TRB 250/500mg 70mo | 93.7 | Cure |
| 27   | Multifocal       | ITZ 200mg (irregular), ITZ 400mg 10mo | 6.5 | Lost to follow-up |
| 28   | Multifocal       | ITZ 600mg 2mo, ITZ 400mg 50mo / AMB (d): ~1g | 52.8 | Cure |
| 29   | Multifocal       | ITZ 400mg since March 2015 / AMB (d): 400mg, AMB (l): 10.6g / TRB 500mg 16mo | 35.8 | Treating |
| 30   | Multifocal       | ITZ 400mg 12mo / AMB (d): 2g, AMB (l): 5.5g / TRB 250mg 2mo | 12.1 | Cure |
| 31   | Multifocal       | ITZ 400mg ~16mo / AMB (d): ~8.3g | 26.1 | Cure |
| 32   | Unifocal         | ITZ 200mg 2mo / ITZ 400mg 12mo / AMB (d): 320mg, AMB (l): 2.2g | 15.3 | Cure |
| 33   | Unifocal         | ITZ 200mg 4mo, ITZ 400mg 5mo | 5 | Cure |
| 34   | Unifocal         | ITZ 400mg 9no / AMB (d): 150mg, AMB (l): 3.6g | 13.9 | Death |
| 35   | Unifocal         | ITZ 400mg 5.5mo / AMB (d): 1g | 14.2 | Cure |
| 36   | Multifocal       | ITZ 200mg 1mo, ITZ 400mg 22mo / AMB (d): 4.1g / TRB 250mg ~20mo | 24.1 | Cure |
| 37   | Multifocal       | ITZ 200mg 3mo / AMB (d): 2g | 3.5 | Death |
| 38   | Multifocal       | ITZ 400mg since November 2016 / AMB (d): 3.4g, AMB (l): 7.2g | 6 | Treating<sup>c</sup> |
| 39   | Unifocal         | ITZ 200mg 1.5mo, ITZ 400mg 1mo (abandonment), ITZ 400mg since July 2017 | 15.4 | Treating<sup>c</sup> |
| 40   | Multifocal       | ITZ 200mg 9no, ITZ 400mg 21mo / AMB (d): 300mg, AMB (l): 12.4g | 28.3 | Death |
| 41   | Multifocal       | ITZ 400mg 21mo / AMB (d): ~1.2g | 21.3 | Cure |

ITZ: itraconazole; AMB: amphotericin B; (d) deoxycholate; (l) lipid formulation; --: approximate dose; TRB: terbinafine; PSZ: posaconazole; g: grams; mg: milligrams; mo: month(s); a: total time of treatment for bone sporotrichosis; b: self-amputation of one phalanx but with multifocal involvement; c: multiple abandonment or irregular treatment.

https://doi.org/10.1371/journal.pntd.0009250.t003
conditions. Besides, alcoholism affects the therapeutic adherence, which may directly impair the clinical evolution.

The initial mycological diagnosis was established by culture of exudate or fragment of cutaneous lesion, non-invasive and easy to perform tests. Bone biopsy in the context of osteomyelitis is indicated mainly to confirm the etiological agent and guide the correct treatment, being essential in cases of isolated osteoarticular involvement without cutaneous lesions [9]. In the four cases that this procedure was performed, the diagnosis of cutaneous sporotrichosis was previously known.

Radiography was the most used exam for screening and diagnosis of osteomyelitis. Although less sensible than scintigraphy (important for screening), computed tomography and magnetic resonance (for diagnosis), it is less expensive and available in our institution.

Two distinct clinical presentations were observed: unifocal bone involvement by contiguity of cutaneous lesions, mainly in women without immunosuppression, like the zoonotic profile of sporotrichosis in Rio de Janeiro; and asymptomatic multifocal bone involvement, in immunosuppressed men. The bones of the hands were the most affected ones, probably because cats usually scratch and bite the hands of people taking care of them. The feet had an important differential percentage in multifocal involvement, in relation to the unifocal form, and the tibia lesions were present only in the multifocal form, hence the importance to search bone involvement in these sites in disseminated sporotrichosis. We recommend a special attention to the feet, when searching for bone disease in patients with disseminated sporotrichosis, based on our findings herein presented and on the significant odds ratio encountered. In previous reviews, the tibia was the most affected bone [6,13], present in unifocal and multifocal disease.

The combination of AMB and itraconazole was the most used therapeutic option. Doses and time of treatment were individualized mainly in multifocal forms. In several cases, the cumulative dose of AMB was higher than that usually recommended in the literature, as well as the time of itraconazole use [16]. Terbinafine was used as a therapeutic option mostly in cases of drug interactions or intolerance to itraconazole, while posaconazole was used in isolated, severe cases, in which the central nervous system was simultaneously affected. Clinical cure was associated with the zoonotic profile of white patients and unifocal presentation. Furthermore, the lower cure rate among the patients with the multifocal presentation probably reflects the high percentage of immunosuppression in this group, mainly patients with HIV who do not adhere to the treatment, presenting severe conditions requiring multiple admissions and long follow-up periods. These data show how important strategies for adherence to treatment in selected groups are. Also, the need for new affordable antifungals, with good bone penetration, tolerable adverse effects, and less drug interactions. In the literature, several antifungal regimens for the treatment of osteomyelitis have been reported, with cure or improvement in most of them.

The ESR and CRP trend to decrease suggests a relationship between these markers and bone disease activity, something established for bacterial osteomyelitis [31]. Nevertheless, because this is a retrospective study in which we did not obtain data from the entire sample and many patients presented other infectious comorbidities, such as HIV, this analysis may be compromised. It seems reasonable to recommend the measurement of both markers in the follow-up of patients with bone sporotrichosis, but in cases of divergence between them and images, the later should prevail. A prospective study with many cases, strict follow-up, and measurements may help to answer this question, but this would probably demand a multicenter research.

Those with relapse were associated with involvement of several bones and immunosuppression, as reported in a review [13]. Aesthetic, functional and disabling sequelae occurred in five patients. In the literature, there are few data focusing on sequelae from bone sporotrichosis.
Gladstone et al. [6] reported cases with the need for surgical debridement and highlighted a permanent articular dysfunction following osteoarticular involvement.

This study presents limitations inherent to a retrospective study using secondary data, of patients treated over 18 years in a reference hospital. However, the observation of this cohort allowed us to ratify the bone sporotrichosis as a challenging chronic condition, with prolonged course of treatment, often with poor results. So, it is particularly important to early diagnose patients with both presentations, unifocal and multifocal osteomyelitis, and prompt an appropriate treatment, to obtain cure, without sequelae.

Supporting information
S1 Table. Univariate and multivariate analyses of possible predictors to cure, of the patients with bone sporotrichosis treated at the INI-Fiocruz between 1999 and 2016. (DOCX)
S2 Table. Erythrocyte sedimentation rate (mm/h) and high sensitivity C-reactive protein (mg/dl) values at the onset of the disease and at the end of treatment, in cured patients with bone sporotrichosis, followed up at INI-Fiocruz, from 1999 to 2016. (DOCX)
S1 STROBE Checklist. Strobe checklist. (DOC)

Acknowledgments
To Marcellly Macena, from the Laboratory of Mycology, for the recovery of the isolates.

Author Contributions
Conceptualization: Antonio C. F. do Valle, Maria Clara Gutierrez-Galhardo, Dayvison F. S. Freitas.
Data curation: Vanessa Ramos, Dayvison F. S. Freitas.
Formal analysis: Vanessa Ramos, Guis S-M. Astacio, Marcel S. B. Quintana, Maria Clara Gutierrez-Galhardo, Dayvison F. S. Freitas.
Funding acquisition: Manoel M. E. Oliveira, Rosely M. Zancopé-Oliveira.
Investigation: Vanessa Ramos, Guis S-M. Astacio, Priscila M. de Macedo, Marcelo R. Lyra, Rodrigo Almeida-Paes, Manoel M. E. Oliveira, Rosely M. Zancopé-Oliveira, Luciana G. P. Brandão, Maria Clara Gutierrez-Galhardo, Dayvison F. S. Freitas.
Methodology: Vanessa Ramos, Guis S-M. Astacio, Antonio C. F. do Valle, Manoel M. E. Oliveira, Luciana G. P. Brandão, Marcel S. B. Quintana, Maria Clara Gutierrez-Galhardo, Dayvison F. S. Freitas.
Project administration: Antonio C. F. do Valle, Maria Clara Gutierrez-Galhardo, Dayvison F. S. Freitas.
Resources: Antonio C. F. do Valle, Rosely M. Zancopé-Oliveira, Maria Clara Gutierrez-Galhardo.
Software: Marcel S. B. Quintana.
Supervision: Antonio C. F. do Valle, Rosely M. Zancopé-Oliveira, Maria Clara Gutierrez-Galhardo, Dayvison F. S. Freitas.
Validation: Gui S-M. Astacio, Maria Clara Gutierrez-Galhardo.

Writing – original draft: Vanessa Ramos, Maria Clara Gutierrez-Galhardo, Dayvison F. S. Freitas.

Writing – review & editing: Antonio C. F. do Valle, Priscila M. de Macedo, Marcelo R. Lyra, Rodrigo Almeida-Paes, Manoel M. E. Oliveira, Rosely M. Zancopé-Oliveira, Luciana G. P. Brandão, Marcel S. B. Quintana, Maria Clara Gutierrez-Galhardo, Dayvison F. S. Freitas.

References

1. Chakrabarti A, Bonifaz A, Gutierrez-Galhardo MC, Mochizuki T, Li S. Global epidemiology of sporotrichosis. Med Mycol. 2015; 53:3–14. https://doi.org/10.1093/mmy/myu062 PMID: 25526781

2. Barros MB, Almeida-Paes R, Schubach AO. Sporothrix schenckii and sporotrichosis. Clin Microbiol. 2011; 24:633–54. https://doi.org/10.1128/CMR.00007-11 PMID: 21976602

3. Freitas DF, do Valle AC, Almeida-Paes R, Bastos FI, Gutierrez-Galhardo MC. Sporotrichosis in Rio de Janeiro, Brazil: a protracted epidemic yet to be curbed. Clin Infect Dis. 2010; 50:453.

4. Gutierrez-Galhardo MC, Freitas DF, do Valle AC, Almeida-Paes R, Oliveira MM, Zancopé-Oliveira RM. Epidemiologic aspects of sporotrichosis epidemic in Brazil. Curr Fungal Infect Rep. 2015; 9:238–45.

5. Pappas PG, Teixeira I, Nolasco D, Holgado W, Bastamante B. Sporotrichosis in Peru: description of an area of hyperendemicity. Clin Infect Dis. 2000; 30:65–70. https://doi.org/10.1086/313607 PMID: 10619735

6. Gladstone JL, Littman ML. Bone sporotrichosis failure of treatment with potassium iodide and sulfadimethoxine and success with amphotericin B. Am J Med. 1971; 51: 121–33. https://doi.org/10.1016/0002-9343(71)90329-9 PMID: 4998882

7. Govender S, Rasool MN, Ngcawene M. Bone sporotrichosis. J Infect. 1989; 19:273–6. https://doi.org/10.1016/S0163-4453(89)90829-3 PMID: 2600443

8. Badley AD, Scoy RE. Long-Term Follow-Up of Multifocal Osteoarticular Sporotrichosis Treated with Itraconazole. Clin Infect Dis. 1996; 23:394–5. https://doi.org/10.1093/clinids/23.2.394 PMID: 8842282

9. Appenzeller S, Amaral TN, Almada MC, Bertolo MB, Marques Neto JF, Samara AM, et al. Sporothrix schenckii infection presented as monarthrosis: report of two cases and review of the literature. Clin Rheumatol. 2006; 25:926–8. https://doi.org/10.1007/s10067-005-0095-z PMID: 16333559

10. Eustace KE, Sampaio FM, Lyra MR, Quintella L, do Valle AC. Cutaneous disseminated sporotrichosis complicated by osteomyelitis. Acta Derm Venereol. 2013; 93:192–3. https://doi.org/10.2340/00015555-1403 PMID: 22656250

11. Aquino GC, Trope BM, Fernandes NC, Engel DC, Ramos e Silva M. Sporotrichosis with Bone Involvement: An Alert to an Occupational Disease. Case Rep Dermatol. 2014; 6:114–8. https://doi.org/10.1080/20472615.2014.8906266 PMID: 24847249

12. Paixão AG, Gutierrez-Gualhardo MC, Almeida-Paes R, Nunes EP, Gonçalves MLC, Chequer GL, et al. The difficult management of disseminated Sporothrix brasiliensis in a patient with advanced AIDS. AIDS Res Ther. 2015; 12:16. https://doi.org/10.1186/s12981-015-0051-1 PMID: 25942926

13. Lederer HT, Sullivan E, Cianflone NC. Sporotrichosis as an unusual case of osteomyelitis: A case report and review of the literature. Med Mycol Case Rep. 2016; 11:31–5. https://doi.org/10.1016/j.mmcr.2016.04.001 PMID: 27136584

14. Aronowitz PB, Gilroy M, Christiansen KN. Disseminated Sporotrichosis with Osteolytic Bone Involvement: J Gen Intern Med. 2017; 32:1063. https://doi.org/10.1007/s11606-017-4048-4 PMID: 28349410

15. Rippon JW. Medical Mycology: the pathogenic fungi and the pathogenic actinomycetes. 3rd ed. Philadelphia: WB Saunders; 1988. p. 325–52.

16. Kauffman CA, Bastamante B, Chapman SW, Pappas PG. Clinical practice guidelines for the management of sporotrichosis: update by the Infectious Diseases Society of America. Clin Infect Dis. 2007; 45:1255–65. https://doi.org/10.1086/522765 PMID: 17968818

17. Oliveira MM, Sampaio P, Almeida-Paes R, Pais C, Gutierrez-Gualhardo MC, Zancopé-Oliveira RM, et al. Rapid Identification of Sporothrix Species by T3B Fingerprinting. J Clin Microbiol. 2012; 50:2159–62. https://doi.org/10.1128/JCM.00450-12 PMID: 22403427

18. Biancardi AL, Freitas DS, Valvissere VR, Andrade HB, Oliveira MM, do Valle AC, et al. Multifocal choroiditis in disseminated sporotrichosis in patients with HIV/AIDS. Retin Cases Brief Rep. 2017; 11:67–70. https://doi.org/10.1097/ICB.0000000000000290 PMID: 28967963
19. Freitas DF, Lima MA, Almeida-Paes R, Lamas CC, do Valle AC, Oliveira MM, et al. Sporotrichosis in the central nervous system caused by Sporothrix brasiliensis. Clin Infect Dis. 2015; 61:663–4. https://doi.org/10.1093/cid/civ361 PMID: 25956895

20. Almeida-Paes R, Oliveira MM, Freitas DF, do Valle AC, Gutierrez-Galhardo MC, Zancopé-Oliveira RM. Refractory sporotrichosis due to Sporothrix brasiliensis in humans appears to be unrelated to in vivo resistance. Med Mycol. 2017; 55:507–17. https://doi.org/10.1093/mmy/myw103 PMID: 27771622

21. Lyra MR, Nascimento ML, Varon AG, Pimentel MF, Antonio LF, Saheki MN, et al. Immune reconstitution inflammatory syndrome in HIV and sporotrichosis coinfection: report of two cases and review of the literature. Rev Soc Br Med Trop. 2014; 47:806–09. https://doi.org/10.1590/0037-8682-0146-2014 PMID: 25626666

22. Freitas DF, Santos SS, Almeida-Paes R, Oliveira MM, do Valle AC, Gutierrez-Galhardo MC, et al. Increase in virulence of Sporothrix brasiliensis over five years in a patient with chronic disseminated sporotrichosis. Virulence. 2015; 6:112–20. https://doi.org/10.1080/21505594.2015.1014274 PMID: 25668479

23. Gutierrez-Galhardo MC, do Valle AC, Fraga BL, Schubach AO, de Siqueira Hoagland B, Monteiro PC, et al. Disseminated sporotrichosis as a manifestation of immune reconstitution inflammatory syndrome. Mycoses. 2010; 53:78–80. https://doi.org/10.1111/j.1439-0507.2008.01655.x PMID: 19019165

24. Freitas DF, de Siqueira Hoagland B, do Valle AC, Fraga BL, Barros MB, Schubach AO, et al. Sporotrichosis in HIV-infected patients: report of 21 cases of endemic sporotrichosis in Rio de Janeiro, Brazil. Med Mycol. 2012; 50:170–8. https://doi.org/10.3109/13693786.2011.596288 PMID: 21859385

25. Silva MB, Costa MM, Torres CC, Gutierrez-Galhardo MC, do Valle AC, Magalhães MA, et al. Urban Sporotrichosis: A Neglected Epidemic in Rio De Janeiro, Brazil. Cad Saude Publica. 2012; 28:1867–80. [Article in Portuguese] https://doi.org/10.1590/s0102-311x2012001000006 PMID: 23090167

26. Almeida-Paes R, Oliveira MM, Freitas DF, do Valle AC, Zancopé-Oliveira RM, Gutierrez-Galhardo MC. Sporotrichosis in Rio de Janeiro, Brazil: Sporothrix brasiliensis is associated with atypical clinical presentations. PLoS Negl Trop Dis. 2014; 8:e3094. https://doi.org/10.1371/journal.pntd.0003094 PMID: 25233227

27. Della Terra PP, Rodrigues AM, Fernandes GF, Nishikaku AS, Burger E, de Camargo ZP. Exploring virulence and immunogenicity in the emerging pathogen Sporothrix brasiliensis. PLoS Negl Trop Dis. 2017; 11:e0005903. https://doi.org/10.1371/journal.pntd.0005903 PMID: 28854184

28. Schneider MC, Aguiller E, Barbosa da Silva Junior J, Ault SK, Najera P, Martinez J, et al. Elimination of neglected diseases in Latin America and the Caribbean: a mapping of selected diseases. PLoS Negl Trop Dis. 2011; 5:e964 https://doi.org/10.1371/journal.pntd.0000964 PMID: 21359810

29. Travassos C, Laguardia J, Marques PM, Mota JC, Szwarzwald CL. Comparison between two race/skin color classifications in relation to health related outcomes in Brazil. Int J Equity Health. 2011; 10:35. https://doi.org/10.1186/1475-9276-10-35 PMID: 21867222

30. Gregory JB, Amedee AM, Siggins RW, Molina PE, Nelson S, Veazey RS. Alcohol and HIV Effects on the Immune System. Alcohol Res. 2015; 37:287–97. PMID: 26895761

31. Berbari EF, Steckelberg JM, Osmon DR. Osteomyelitis. In: Mandell GL, Bennett JE, Dolin R. Mandell, Douglas and Bennett’s principles and practice of Infectious Diseases. 7th ed. Philadelphia: Churchill Livingstone Elsevier; 2010. p. 1457–65.