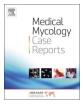
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Differential distribution patterns of Fonsecaea agents of chromoblastomycosis, exemplified by the first case due to F. monophora from Argentina

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ABSTRACT

Chromoblastomycosis is a mutilating infection of the skin and subcutaneous tissues caused by melanized fungi belonging to the order Chaetothyriales. Proven cases of the main agent, *Fonsecaea pedrosoi* are mainly limited to (sub)tropical, humid climates of Latin and Central America and the Caribbean. *Fonsecaea monophora* has a global distribution along the equator. Cases outside the (sub)tropics have thus far mostly been considered to have been imported, but here we report the first endemic case by *F. monophora* from Argentina. Patient was a 82-year-old rural female worker from Corrientes, a province with a dry continental climate.

1. Introduction

Originally described by Pedroso and Gomes in 1914, in the City of São Paulo, Brazil, chromoblastomycosis is an infection of the skin and subcutaneous tissue which is hypothesized to appear after traumatic implantation of plant material contaminated with fungal elements. The injury occurs mainly in the lower extremities and commonly affects male individuals. The correct diagnosis of chromoblastomycosis is based on clinical, mycological and pathological data. The hallmark of the disease is the presence of sclerotic bodies in infected skin. The disease has a worldwide distribution in tropical and subtropical regions of Africa, Latin and Central America, Australia and East Asia, but in each climate zone it is caused by different prevalent members of Chaetothyriales, i.e. genera Fonsecaea, Cladophialophora and Rhinocladiella. The most commonly isolated agent in humid tropical climates is F. pedrosoi, whereas C. carrionii is prevalent in semiarid climates [1]. Although species distinction is not possible with mere phenotypic characters, only a part of the cases in the literature were based on sequence data. Fonsecaea has recently been revised and now contains three species: F. pedrosoi, F. monophora, and F. nubica. These siblings are slightly different in their clinical spectra: F. pedrosoi and F. nubica seem to be strictly associated with chromoblastomycosis, whereas F. monophora also has been isolated from brain abscesses,

cervical lymph nodes, and bile [2]. Also their climatic conditions seem to differ. The aim of this report is to present the first case of chromoblastomycosis in Argentina caused by *F. monophora* outside the main endemic area of the disease. In Argentina, published cases thus far have invariably been attributed to *F. pedrosoi*, but this identification may be questionable.

2. Case

A 82-year-old rural woman worker from Corrientes, a northeastern province in Argentina was admitted to the hospital with a tumor in the form of thick scaly erythematous plaque on the left hand, with a slow expansion of about 2 years duration. There was no recall of previous injury. Physical examination evidenced a dry tumoral lesion of 4×3 cm covered by thick scales, without necrosis or secretion. Initial clinical diagnosis was that of tumor, but histopathological data soon suggested that a fungal infection was concerned. From direct examination of a skin biopsy, muriform cells, characteristic of chromoblastomycosis, were observed (Day 0) (Day 0). The sample was cultured on Sabouraud glucose agar (SGA, Laboratorios Britania, Buenos Aires, Argentina) at 28 °C for four weeks and a black, hairy colony was grown (Day 28). Microscopic examination of the culture showed elliptical, brownish conidia arising in short chains from differentiated

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Fig. 1. Muriform cells from the skin biopsy.

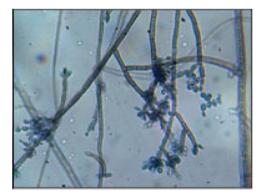


Fig. 2. Microscopic examination from culture identified as Fonsecaea genera.

conidiophores. The fungus was identified as belonging to the genus *Fonsecaea* (Figs. 1 and 2).

Since *Fonsecaea* species are morphologically indistinguishable, the rDNA internal transcribed spacer (ITS) was sequenced with primers V9G and LS266, in addition to partial coding genes CDC42, LAC, and HmgA, which were amplified with *cdc42-SF1s* and *cdc42-SR1s*, *LacIS* and *LacIAS*, and *HmgA-F2s*, *HmgA-R12s* and *HmgA-R22s*, respectively [3]. All sequence results matched 100% with the type strain of *F. monophora*, CBS 269.37, for which ITS and CDC42 (GenBank AY366914 and GU197491, respectively, can be considered as diagnostic. The strain was deposited in the reference collection of CBS (housed at Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands)).

All sequence results matched 100% with F. monophora. Initially the lesion was treated with topical Imiquimod, (5% cream once daily) for 124 days, and significant clinical improvement was achieved. However, after 186 days another lesion appeared in the distal edge of the previous lesion that was rough, 2 \times 1 cm in size. Besides, residual macula evidenced hypochromic areas, and erythematous, atrophic lesions secondary to the above process appeared. Thus, another skin biopsy was taken. In the direct examination, muriform cells were again observed. Treatment with itraconazoIe (ITZ) was started with a dose of 400 mg/ day at day 207. To allow desirable serum drug concentration, ITZ measurement was performed after nine days from the first dose by bioassay technique, since pharmacokinetics and metabolism of this azole depends on numerous factors that might result in inappropriate therapeutic response [4]. The first serum value was low $(0.35 \,\mu\text{g/mL})$ because the patient had not good adherence to follow the treatment. Then the dose was adjusted and values were high (21,7 μ g/mL), reason for which the dose was adjusted again to achieve a value within the expected range of 5-15 µg/mL. Measurements were performed regularly. Skin change was clinically observed during ITZ therapy with significant improvement of the lesion within 248 days of ITZ treatment. Fig. 3 shows the lesions observed in our patient.

Minimum inhibitory concentrations (MIC) were determined according to CLSI guidelines document M38-A2 [5]. MIC values in μ g/mL were as follows: 2, 64, 0.015, 0.015 for amphotercin B (AMB), fluconazole (FCZ), ITZ, and voriconazole (VCZ), respectively.

3. Discussion

Chromoblastomycosis mainly occurs in humans, although cases of infected animals have also been reported [2]. Classical studies in Latin America suggest that mainly rural workers are at risk, with a preponderance of elderly males [2,6]. In children and adolescents the disease prevails in males over females [7]. Similar findings were published in Africa. An increased incidence was observed in elderly women, which might be related to a decrease of steroid hormones supposedly playing a protective role against the disease [8]. In East Asia, however, male/female ratios were more or less equal [9].

In the literature, lesions were predominantly observed at the lower limbs. In our patient, despite she did not recall injury, the route of infection was assumed to be traumatic due the work she performed, which was rural perform. Our case is comparable to studies of e.g. Kim et al. and Kondo et al. where infections occurred at upper extremities [9]. Lower extremity infection occurs more often in males, while females show a preponderance of upper extremity infection. The presentation was of the plaque type, which is one of the more common types of clinical appearance [2,10].

Chromoblastomycosis is generally reported from tropical and subtropical regions with humid climates. This holds true for F. pedrosoi in Latin America, the prevalent agent in the tropical rain forest of the Amazon and adjacent climate zones. Very few strains from Africa have thus far been sequenced, and hence species distributions on this continent remain unclear [8,11]. East Asian cases nearly always concern F. monophora. Until recently, cases outside these endemic areas were considered to have been imported [1]. One of the very few exceptions is that of Pindycka et al., proving endemic occurrence of F. monophora in Northern Europe in coal-miner. Our case is from Corrientes in Northern Argentina, a province with a continental climate of hot and dry summers and relatively cold winters [12]. Patient had never left this area, and thus most probably the infection was acquired locally. It seems that the environmental occurrence of F. monophora covers a much wider ecological diversity than that of F. pedrosoi. Possibly this is linked to the wider pathology of F. monophora, while F. pedrosoi in contrast is strictly associated with chromoblastomycosis.

Corrientes being unknown as an endemic area of chromoblastomycosis may be due to a lack of molecular diagnoses accompanying case reports. Since *Fonsecaea* species are morphologically indistinguishable, diagnosis must be accompanied by sequencing of the rDNA ITS region. Appropriate diagnostics remains a serious problem because physicians usually do not expect a fungal cause for subcutaneous diseases. This was also the case with the patient reported here, where carcinoma was first suspected.

Different antifungal susceptibility profiles may be observed among the different agents. F. monophora was reported to have a better response to treatment compared to F. pedrosoi, although recently resistance was verified in a case of chromoblastomycosis caused by F. monophora [13,14]. Therapy may depend on the etiological agent, size of the lesions, and on the patient's health status and socio-economic parameters. In general, chromoblastomycosis is very difficult to treat and prone to recurrence [15]. Three types of treatment are available, i.e. physical (cryotherapy or surgical excision), chemotherapeutic, and combination therapy. Cryosurgery with liquid nitrogen is an option for small, localized lesions [10]. Best results with antifungal therapy are achieved with ITZ and terbinafine (TBF) in high doses for at least 6-12 months [10,16,17]. Table 1 summarizes MICs of current antifungals given in the recent literature. From these data, ITZ and VCZ seem to be appropriate for therapy. Zhang et al. studied 18 clinical isolates of F. monophora combining ITZ and TBF orally in doses of 200 or 400 mg/



Fig. 3. Lesions observed in the patient in different stages. A. First lesion from which *F. monophora* was isolated. B. Clinical improvement with imiquimol in the area of the previous lesion. However, a new lesion has appeared in the extreme area and the same fungus was isolated. C. Relapse of the lesion. D. Improvement of the lesion after itraconazole treatment.

Table 1 MICs of the F. monophora of different antifungal drugs showed on the literature.

Strains of F monophora	AMB (µg/mL)	FCZ (µg/mL)	ITZ (µg/mL)	VCZ (µg∕ mL)	Reference
N = 1	2	64	0.015	0.015	This report
N = 1	2	ND	0.125	1	[13]
N = 1	ND	ND	1	0.06	[19]
N = 1	2	64	0.125	1	[20]
N = 25	0.5–2	8–64	0.031–0.25	0.12–1	[14]
N = 18	ND	ND	0.008–0.125	ND	[18]

day and 250 or 500 mg/day for ITZ and TBF, respectively [18]. In vitro synergy has been observed between the two drugs in 12 of 18 strains investigated and successful combination therapy was achieved in three patients.

Combination therapy may be an option for refractory or severe clinical forms of the disease [10].

In the present case it was decided to apply local imiquimod treatment given the characteristics of the lesion and the economic capabilities of the patient. Slight improvement was initially achieved. However, a new lesion developed, and then treatment with ITZ (capsule) was indicated at a dose of 400 mg/day. Itraconazole can interact with other drugs leading to plasma values above or below the expected range resulting in an inefficient action. Higher values may confer toxicity, whereas lower values may lead to treatment failure. For this reason the patient was monitored with regular measurements of plasma levels to achieve the desirable plasma concentration.

In conclusion, our case report showed the presence of *F. monophora* in Argentina. The apparent wide geographic distribution of *F. monophora* sheds doubt on earlier cases of chromoblastomycosis from Argentina which had invariably been ascribed to *F. pedrosoi* on the basis of clinical and phenotypic features. Despite topical imiquimod therapy, improvement was observed only when ITZ was indicated and plasma levels achieved the expected range. The patient is still under regular observation and her treatment is continued. More surveillance and

awareness of this disease is necessary for early detection in order to avoid the development of chronic forms that might disable the patient in his daily functioning.

Conflict of interest

There are none.

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