

Chromoblastomycosis due to *Fonsecaea monophora* misdiagnosed as sporotrichosis and cutaneous tuberculosis in a pulmonary tuberculosis patient



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ABSTRACT

Chromoblastomycosis is caused by dematiaceous fungi. It develops after inoculation of the organism into the skin. We report a case of chromoblastomycosis in a pulmonary tuberculosis patient without known history of trauma. The lesions were initially diagnosed as sporotrichosis and skin tuberculosis. Histopathology of scales and skin biopsy specimen revealed sclerotic bodies, the hallmark of *chromoblastomycosis*. The causative organism was identified as *Fonsecaea monophora* by rDNA ITS sequencing. The lesions recovered markedly after two month treatment with oral terbinafine 250 mg daily according to drug sensitive test *in vitro* in combination with local thermotherapy

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1. Introduction

Chromoblastomycosis is a chronic subcutaneous fungal infection in immunocompetent individuals, clinically characterized by verrucous skin eruptions, most commonly on lower extremities [1]. Among factors affecting clinical outcome, etiological agents have an important position. To date, several species have been proven as a causative agent of the disease, of which *Fonsecaea pedrosoi*, *F. monophora*, *Fonsecaea nubica*, *Cladophialophora carrionii*, *Phialophora verrucosa* and *Rhinocladiella aquaspersa* are common agents. Several *Exophiala* species (*Exophiala jeanselmei*, *Exophiala spinifera* and *Exophiala dermatitidis*) have been confirmed as occasional agents of the disease [2]. A history of mechanical trauma or injury to the site of infection marks the prevalent portal of entry.

In southern China, *Fonsecaea pedrosoi* and *F. monophora* are main causative agents causing chromoblastomycosis, and patients generally are immunocompetent [3]. Here, we present a case caused by *F. monophora* in a pulmonary tuberculosis patient without known history of trauma. The agent was isolated from skin scrapings and identified by phenotypic and molecular markers. The patient was managed successfully with oral terbinafine

in combination with topical thermotherapy.

2. Case

A 63-year-old male farmer from Jiangxi Province, southern China presented with asymptomatic verrucous plaques covered with scales on the back side of right hand and a small erythematous was on right forearm on 3 January 2016 (**at day 0**). The lesion had started as an asymptomatic, red papule on the back of his hand, and slowly enlarged over a period of 10 years. Concomitantly, a similar maculopapule appeared on his left forearm. A history of trauma or inoculation was not recalled. Patient was diagnosed with pulmonary tuberculosis at the beginning of the skin infection in a local hospital **at day-10 years**. He was given with intravenous injection levofloxacin at 0.4 g per day plus oral rifampicin at 0.6 g per day for ten months. The pulmonary tuberculosis healed completely. However, there was no improvement in skin lesion, which recovered slightly in summer and enlarged in winter.

One year ago (**at day-1 years**), the patient's lesion was diagnosed as sporotrichosis in a local hospital; however, no response was achieved to a 2-month routine course of potassium iodide treatment. Then, because of a suspicion of cutaneous tuberculosis, treatment with intravenous injection of levofloxacin at 0.4 g per

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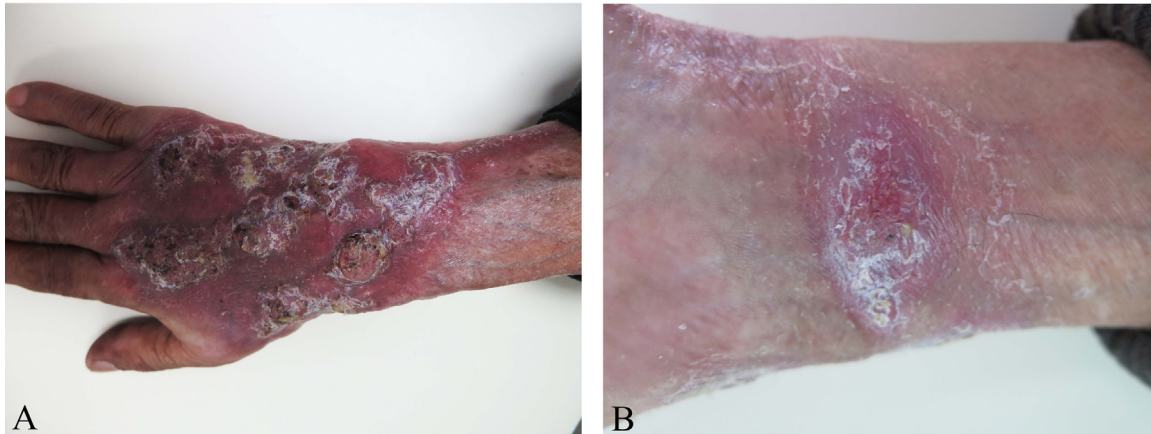


Fig. 1. Lesions caused by *Fonsecaea monophora* on the patient's right wrist and the back side of right hand, even after treatment with 10% potassium iodide solution for 2 months.

day plus oral rifampicin at 0.6 g per day was resumed for one month (**during day-10 months to day-8 months**). However, there was no improvement of the lesion.

Physical examination revealed purple, verrucous plaques covered with scales on the back of his right hand. A small (2×2 cm) erythematous was seen on right forearm (**at day 0**) (Fig. 1A and B). Results of routine hematological examination and urine analysis were within normal limits. Serological tests for HIV and anti-nuclear antibodies (e.g., anti-dsDNA antibody and anti-ssDNA antibody) were negative and chest radiography was unremarkable. Histopathology with haematoxylin and eosin staining of the epidermis showed irregular acanthosis, and a granulomatous response with histiocytes, plasma cells, polymorphonuclear cells and giant cells including muriform cells in the dermis (Fig. 2). These results led to a clinical diagnosis of chromoblastomycosis. Direct examination of 10% potassium hydroxide wet mounts from the lesion debris revealed brown muriform cells (Fig. 3A) confirming the clinical diagnosis.

Mycological culture was performed (**at day 0**). Portions of both skin biopsies and skin debris were inoculated onto culture media attempting to recover the etiologic agent. Primary isolation of the fungus was performed on agar slants of Sabouraud's glucose agar (SGA) containing chloramphenicol (CMP, 0.125 g/l) and incubated at 25 °C and 37 °C for two weeks. Colonies (OA) were restricted, slightly heaped, powdery to velvety or hairy, olivaceous black; colony reverse olivaceous black. The strain was tolerant to cycloheximide (CHX) and grew well at 25 °C and 37 °C on CHX-

containing SGA. Microscopically, conidiophores were suberect, olivaceous brown, apically densely branched. Conidiogenous cells were in dense clusters, with broad, flat, pale pigmented scars. Conidia were barrel-shaped, clustered, often remaining attached, smooth-walled, brown, or falling off as short branchlets. Phialides were occasionally produced (Fig. 3C). The fungus was identified as a *Fonsecaea* species based on these morphological characteristics.

The isolated agent was reconfirmed by sequencing of ITS 1 and ITS 4 region of rDNA and compared with sequences deposited in GenBank by Blast program and to an edited research database on black fungi maintained at CBS. Based on these analyses, the fungus was identified as *Fonsecaea monophora*. The isolated agent was deposited in the Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College in Nanjing, Jiangsu, under accession number CMCCf 2160004.

At day +10, the *in vitro* susceptibility of the strain to eight antifungal agents was determined using the microdilution method in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) M38A [4]. The minimum inhibitory concentrations (MICs) were defined as the lowest concentration at which no growth occurred which led to the following results: itraconazole, 0.06 µg/ml; ketoconazole, 0.5 µg/ml; fluconazole, 16 µg/ml; miconazole, 2 µg/ml; voriconazole, 0.06 µg/ml; anidulafungin, > 4 µg/ml; amphotericin B, 16 µg/ml; terbinafine, 0.06 µg/ml.

The patient received empirical treatment with oral itraconazole 200 mg and terbinafine 250 mg daily for 2 weeks (**during day**

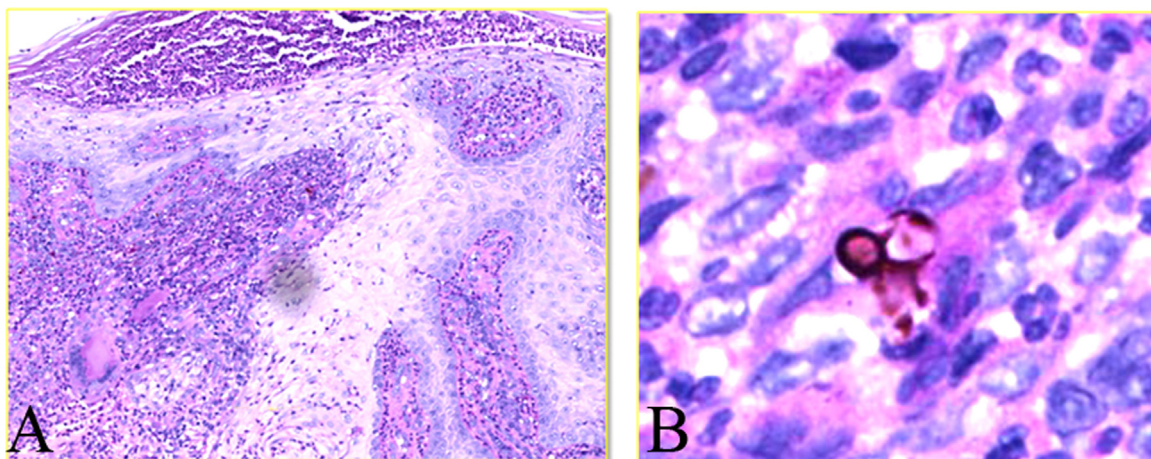


Fig. 2. Chronic granulomatous inflammation: epidermis with irregular acanthosis; dermis consisting of lymphocytes, plasma cells, neutrophils, eosinophils, macrophages, and multinucleated giant cells. Some giant cells containing muriform bodies (periodic acid-Schiff stain; original magnification, $\times 400$).

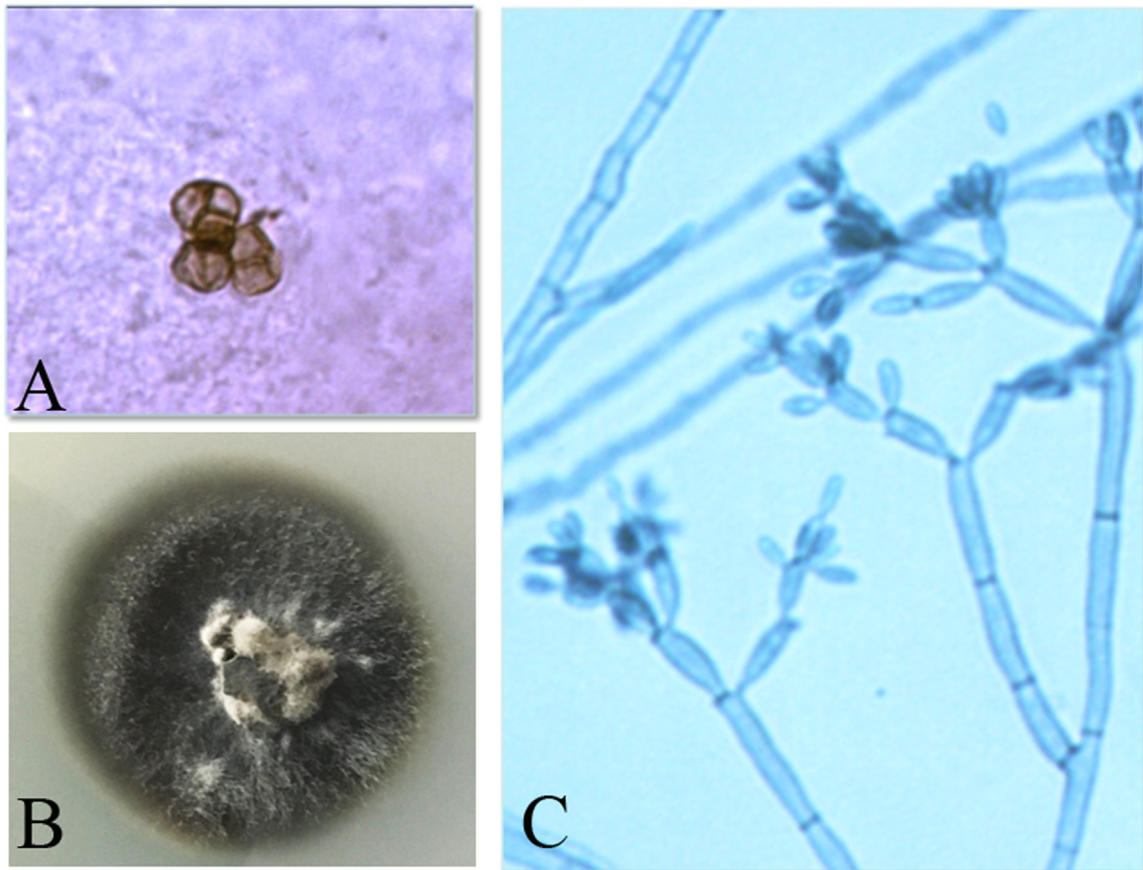


Fig. 3. Direct light microscopy of nail clips displaying multiform cells (original magnification, $\times 400$) (A). Culture in Sabouraud agar after culturing for 3 weeks, at 25 °C, presenting powdery to velvety or hairy, olivaceous black colony (B). Light microscopy of lactophenol cotton blue preparation of colonies grown on Sabouraud's dextrose agar presenting with Conidiophores suberect, olivaceous brown, apically densely branched. Conidiogenous cells in dense clusters, with broad, flat, pale pigmented scars (original magnification, $\times 400$) (C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

+15 to day +30). Based on the antifungal susceptibility testing, itraconazole should be most effective, but since oral itraconazole application induces itching, treatment with oral terbinafine 250 mg daily with terbinafine cream twice per day was continued. Over 2 months of treatment, the skin lesions showed marked reduction (during day +31 to day +61). The drug was well tolerated by the patient without showing any side-effects. Because the agent was not thermotolerant, local thermotherapy was also used, and the patient responded well to the combined therapy.

3. Discussion

Chromoblastomycosis is a chronic subcutaneous mycosis caused by dematiaceous fungi. The disease has been reported worldwide, but is more prevalent in tropical and subtropical regions of Southern Africa, Madagascar, Latin America, East Asia, and Australia [2]. Chromoblastomycosis is caused by several species in the ascomycete order *Chaetothyriales*, comprising the black yeasts and relatives, mainly *Fonsecaea pedrosoi* (syn.: *Fonsecaea compacta*), *Rhinocladiella aquaspersa*, *Cladophialophora carrionii*, and occasionally *Phialophora verrucosa*. Agents in *Exophiala* are highly exceptional and usually cause other types of infection without muriform cells.

Several species are potential etiologic agents of the disease in the endemic areas of southern China, with *Fonsecaea* species traditionally being referred to as *F. pedrosoi* [3]. During a recent taxonomic revision of the genus *Fonsecaea*, three species were recognized to be commonly involved in chromoblastomycosis, viz.

F. pedrosoi, *F. monophora*, and *F. nubica*. *F. monophora*, segregated from *F. pedrosoi* on molecular grounds [5], was shown to have a more variable clinical spectrum than *F. pedrosoi*: it proved to be a more general opportunistic pathogen, repeatedly causing neurotropic infections, whereas *F. pedrosoi* and *F. nubica* are exclusively associated with chromoblastomycosis [6]. While morphological differentiation is difficult, molecular analysis is necessary to reliably identify the fungus down to species level. Recently, Xi et al. re-evaluated 24 isolates from chromoblastomycosis patients that were originally identified as *F. pedrosoi* [3]. Using rDNA ITS sequencing, 20 of the strains were found to be *F. monophora*, suggesting that this is the prevalent agent of chromoblastomycosis in southern China. Jiangxi Province has a subtropical monsoon climate, which typically promotes the occurrence of *Fonsecaea* species, whereas *Cladophialophora* agents of the disease are restricted to arid climate zones [2].

The clinical appearance of chromoblastomycosis is polymorphic. Etiologic agents are hypothesized to gain entrance through the skin by traumatic implantation of contaminated material. The majority of lesions are observed on extremities of outdoor workers. Queiroz-Telles et al. [7] described five types of lesions: nodular, tumorous, verrucose, cicatricial and plague, while additional types were proposed in further literature. The fungus causing chromoblastomycosis usually is restricted to subcutaneous tissue; cutaneous dissemination may develop as a result of self-inoculation from the original lesion, perhaps also by local hematogenous or lymphatic spread. In later stages of disease, marked extension to other body parts is common. The host immune status is believed to play a role in the clinical evolution of this disorder

leading to either mild or severe symptoms, even though most cases develop in otherwise healthy individuals; infections caused by *F. monophora* in immunocompromised patients are very rare [8].

The origin of infection in our case remains obscure. We presume that the portal of entry of the fungus must have been through minor trauma, even if such a history was denied. We were not able to trace back the patient's immunological status when he was infected by the fungus. Because patient had a double infection with *Fonsecaea monophora* and *Mycobacterium tuberculosis*, we presume that the patient was under temporary immunosuppression, which likely contributed to the spread of the fungus to cause the right forearm lesion.

Because of clinical polymorphic appearance of chromoblastomycosis, it is difficult to diagnose the disease on the basis of clinical appearance only. Histopathology is identical in all types of chromoblastomycosis, being the hallmark of the disease but without species differentiation. Polymorphonuclear cells and giant cells are formed in the dermis. Muriform cells were easily recognized in routine haematoxylin-eosin stain, but were also visible in KOH wet mounts. In the present case, patient was initially diagnosed with sporotrichosis judging from clinical appearance only, while also an erroneous diagnosis of cutaneous tuberculosis was made because he was unresponsive to potassium iodide treatment and had a history of pulmonary tuberculosis.

Chromoblastomycosis is difficult to treat and relapses may occur. Therefore it is important to consider personalized treatment of the patient based on the severity of the disease and clinical and histopathological features. Nowadays, therapy is based on a few open trials and expert opinion. Itraconazole, either as monotherapy or in combination with other drugs or physical methods, is widely used [9,10]. Recently, the combination of antifungals with photodynamic therapy has successfully been employed in patients presenting with chromoblastomycosis [11]. In the present case, the patient was empirically treated with oral itraconazole and terbinafine and he responded well to this association treatment; however he could not endure itching of itraconazole. Antifungal susceptibility testing confirmed that the agent was also sensitive to terbinafine, and indeed patient responded well to this treatment. Once the causative agent is isolated, it is recommended to perform antifungal susceptibility testing *in vitro* to confirm the appropriate treatment regimen. Because the agent was not tolerant to 42 °C, daily local thermotherapy was effectively used along with drug treatment.

In conclusion, we describe a rare case of chromoblastomycosis due to *Fonsecaea monophora*, in a patient with pulmonary tuberculosis who was initially misdiagnosed with sporotrichosis and skin tuberculosis. To correctly diagnose these infections, pathologists need to be aware of chromoblastomycosis and the characteristics of sclerotic body morphology. In addition, molecular diagnostics should be encouraged to identify the *Fonsecaea* species

involved. Finally, the treatment should be adjusted according to antifungal drug sensitivity testing and a prolonged period of treatment may help to prevent recurrence.

Conflict of interest

The authors have no conflict of interests in connection with this study.

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