The implementation of the comprehensive diagnostic and monitoring programme for workers during the COVID-19 pandemic in our centre allowed us to detect this case. We believe that this is important for the good control of hospital-acquired infection.

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https://doi.org/10.1016/j.eimce.2022.11.004

Chromoblastomycosis due to *Cladophialophora immunda*: An emerging pathogen in immunocompromised patients?

Cromoblastomicosis por Cladophialophora immunda*: ¿un patógeno emergente en pacientes inmunocomprometidos?*

Chromoblastomycosis is a chronic granulomatous infection of the skin and subcutaneous tissue caused by traumatic inoculation of dematiaceous (pigmented) fungi present in soil, plants and decaying wood.[1] It is more common in tropical and subtropical climates, although autochthonous cases have been described in regions with a temperate climate.[2] The highest incidence is recorded in men between 20 and 60 years of age, probably due to greater occupational exposure, and it is responsible for 90% of cases.[1] Its diagnosis is fundamentally based on the identification in the tissue of thick-walled pigmented cellular structures known as Medlar bodies, sclerotic bodies or muriform cells, which represent the invasive form of the fungus and are pathognomonic of this entity.[3] In addition, culture is recommended and, for the identification of the species, the sequencing of internal transcribed spacer regions [ITS] of fungal ribosomal DNA (rDNA) is the most appropriate.[3] We present a case of chromoblastomycosis due to *Cladophialophora immunda*, a species recently identified in soils contaminated with hydrocarbons,[4,5] in a kidney transplant patient.

The case is a 56-year-old man originally from Guinea–Bissau, resident in Spain for 30 years, with a history of end-stage kidney disease secondary to interstitial uric acid nephropathy and who had undergone a kidney transplant from a cadaveric donor on immunosuppressive treatment with tacrolimus 4.75 mg/day, mycophenolic acid 180 mg/8 h and prednisone 5 mg/day. Ten months after the transplant, he attended a dermatology consultation due to a fast-growing multinodular exophytic tumor lesion in the external metatarsal area of his right foot (Fig. 1A). The patient denied previous trauma and had not travelled to his country of origin in the previous year. He did not report fever or other accompanying symptoms and nor did he present locoregional adenopathies. The serologies were negative and the blood count and blood biochemistry showed no significant findings. A skin biopsy of the main lesion was performed for histology and microbiology studies. The histology revealed pseudoepitheliomatous epidermal hyperplasia associated with an intense dermal inflammatory process comprised of suppurative granulomas and multinucleated giant cells. Both in the cytoplasm of these cells and among the neutrophils, numerous rounded brownish fungal structures were observed, sometimes clustered in chains, and haematoxylin and eosin (H&E) and Grocott staining revealed the presence of septa (muriform cells) (Fig. 2A–C). Direct examination with calcofluor white was negative for yeasts, hyphae or pseudohyphae. After three days of incubation, fungal growth was observed at 30°C in Sabouraud chloramphenicol gentamicin agar and potato dextrose agar with chloramphenicol, but not in media with cycloheximide. Macroscopically, the colonies were velvety, dark olive-grey in colour and black on the reverse side (Fig. 2D), with a maximum growth temperature of 37°C. Septate, branched, pale brown hyphae were observed microscopically, with poorly differentiated conidiophores producing ellipsoidal conidia forming long coherent chains, without dark scars (Fig. 2E and F). These characteristics permitted the identification of the genus *Cladophialophora.* Species identification was performed by sequencing ITS regions of the rDNA. The aligned sequence was a 99% match for *C. immunda*, CBS 126867, GenBank accession number MH864254.1. On further questioning, the patient said he had been working in a paint factory. With the diagnosis of chromoblastomycosis due to *Cladophialophora immunda*, treatment was initiated with terbinafine 250 mg/24 h for 4 weeks, with subsequent curettage and electrocoagulation of the residual lesion and a new histological and microbiological study that confirmed

DOI of original article: https://doi.org/10.1016/j.eimc.2022.02.010.

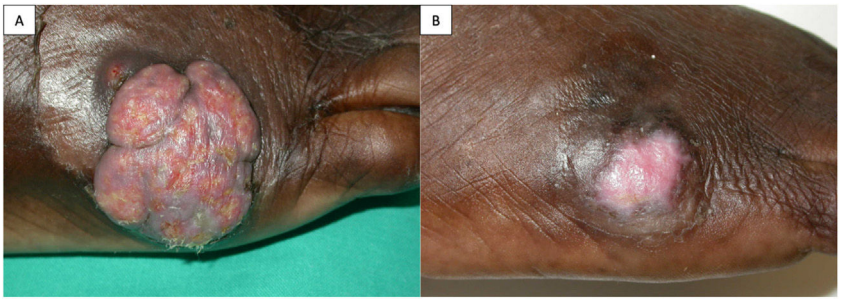

Fig. 1. (A) Multinodular tumour lesion in the external metatarsal area of the right foot. B. Scar plaque after treatment with terbinafine and surgical debridement.

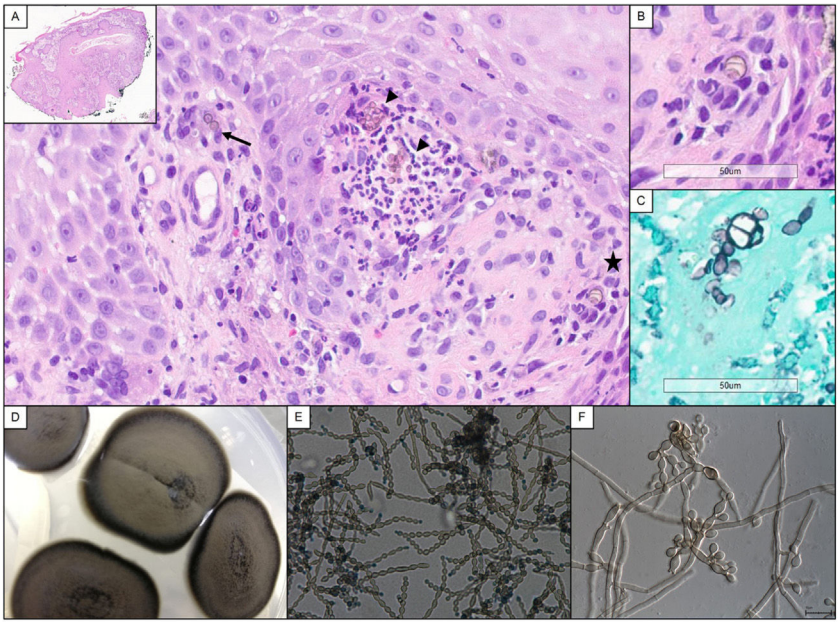

Fig. 2. (A) Histological image in which brown fungal structures can be seen in the ridges of the papillary dermis, both in the cytoplasm of a multinucleated giant cell (arrow) and in a suppurative focus (arrowhead). The presence of a septate fungal structure or muriform body (star) is also noteworthy. In the upper left corner, panoramic view showing pseudoepitheliomatous hyperplasia (H&E, ×400). (B) Detail of the muriform cell in image A with digital magnification (H&E, ×400). (C) Fungal microorganisms highlighted with Grocott's methenamine silver stain also showing thick-walled septate bodies, digitally augmented (H&E, ×400). (D) Macroscopic fungal growth, day 25 to 30°C on Sabouraud dextrose agar. (E) Microscopic observation of a fresh preparation of potato-chloramphenicol agar (×400). (F) Detail of conidiophores, from malt extract agar (×1,000).

microbiological cure (Fig. 1B). It did not prove necessary to reduce the immunosuppressive treatment. After two years of follow-up, no recurrence has been observed.

The host-pathogen relationship in this disease is very specific, since the species involved are almost entirely restricted to a single order of the fungal kingdom, the *Chaetothyriales*, and more specifically the *Herpotrichiellaceae* family. The species responsible for the vast majority of cases of chromoblastomycosis belong to the *Fonsecaea and Cladophialophora* genera, predominantly the *Fonsecaea pedrosoi* and *Cladophialophora carrionii* species, followed, to a lesser degree, by the *Rhinocladiella, Phialophora* and *Exophiala* by genera.[1,6–8] From isolated strains of *Cladophialophora*, four new species have been identified in this genus, *Cladophialophora saturnica* and *Cladophialophora immunda* which cause skin infections, *Cladophialophora mycetomatis* related to subcutaneous infection and *Cladophialophora samoënsis*, an endemic agent of chromoblastomycosis.[9] *Cladophialophora immunda* belongs to the b̈antianap̈hylogenetic clade (clade II) of the genus, which contains the main neurotropic species. It is more closely related to environmental species such as *C. saturnica*, the cause of skin infections, or *C. devriesii*, which has been described as a cause of disseminated infections, than to *C. carrionii*, the most prominent aetiologic agent of chromoblastomycosis in the genus, belonging to the c̈arrioniiclade (clade I).[4] *C. immunda* has a thick and melanised cell wall and other physiological adaptations that allow it to cope with extreme physical and chemical conditions. More specifically, it has a special association with environments contaminated by hydrocarbons.[5,10] In fact, its use in bioremediation has been postulated, given its ability to degrade hydrocarbons,[5,10] although little is known about its potential pathogenicity. In our case, the patient reported occupational exposure to acrylic paints, the probable ecological niche of the fungus due to its affinity with environments enriched with monoaromatic hydrocarbons, which are present, for example, in solvents.

Chromoblastomycosis is rare in kidney transplant patients, and in general, in immunosuppressed patients phaeohyphomycosis is the main manifestation of the disease caused by pigmented fungi.[11,12] Phaeohyphomycosis is defined by the presence of pigmented yeast cells, hyphae, or pseudohyphae in the host tissue. A wide spectrum of clinical syndromes can occur, including fungemia, and pulmonary and cerebral involvement, although the cutaneous and subcutaneous forms are the main manifestations.[13] Chromoblastomycosis, on the other hand, does not extend beyond the subcutaneous tissue, although some genera involved, such as *Fonsecaea* and *Exophiala*, show marked neurotropism.[9] The

largest series of pigmented fungal infections in kidney transplant patients was described in Brazil, where only four of 58 cases were chromoblastomycosis.[11] Another Brazilian series of six cases observed transitional forms between phaeohyphomycosis and chromoblastomycosis with the simultaneous presence of melanised pseudohyphae and muriform cells in histological preparations.[12] Our case had characteristics similar to those of the patients described in these series, such as male sex (82%), age (40–60 years), late post-transplant presentation (>90 days) and treatment with corticosteroids (100%) and calcineurin inhibitors (95%).[11,12] In contrast, clinical tumour presentation was infrequent (2%), with verrucous and plaque forms being the most common. The most widely used antifungal drug was itraconazole, combined with partial surgical debridement in approximately 50% of cases. In our patient, we chose terbinafine to avoid the serious drug interaction of itraconazole with tacrolimus, its efficacy being equally demonstrated in combination with the surgical removal of the residual lesion.[14]

The isolation of a fungus with the microscopic characteristics of the genus *Cladophialophora* in an immunosuppressed patient requires ruling out the *C. bantiana* species, an agent that produces phaeohyphomycosis with the capacity for dissemination and neurotropism.[15] On the other hand, given that phenotypic differentiation between the species of the *Exophiala, Cladophialophora* and *Fonsecaea* genera is difficult, the analysis of the sequences of the ITS region has proven very useful for identification[1] and is especially recommended for rare or recently described pathogens.[3] In our case, the lack of growth at 40 °C ruled out *C. bantiana*, and the presence of muriform cells in the biopsy as well as the molecular identification enabled us to conclude that it was chromoblastomycosis by *C. immunda,* a species previously described on a single occasion as a cause of phaeohyphomycosis[5]. Finally, regarding treatment, several studies suggest that standard treatment should include itraconazole plus surgery (AII recommendation),[3] although other alternatives such as terbinafine or potassium iodide have also been used successfully (BIII recommendation).[3,14] For refractory cases, posaconazole is a promising alternative based on experimental and *in vitro*studies.[3]

Funding

No funding was received for this study.

Acknowledgements

To Dr. Rosa Baldellou, for her indispensable participation in the clinical management of the patient and her contribution of the clinical photographs; to Dr. Carmen Yus, for her invaluable collaboration in the histopathological diagnosis; and to Dr. Yolanda Gilaberte, for her decisive support in the coordination and review of the article.

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https://doi.org/10.1016/j.eimce.2022.11.006