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REVIEW

Chromoblastomycosis

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SUMMARY Chromoblastomycosis (CBM), also known as chromomycosis, is one of the most prevalent implantation fungal infections, being the most common of the gamut of mycoses caused by melanized or brown-pigmented fungi. CBM is mainly a tropical or subtropical disease that may affect individuals with certain risk factors around the world. The following characteristics are associated with this disease: (i) traumatic inoculation by implantation from an environmental source, leading to an initial cutaneous lesion at the inoculation site; (ii) chronic and progressive cutaneous and subcutaneous tissular involvement associated with fibrotic and granulomatous reactions associated with microabscesses and often with tissue proliferation; (iii) a nonprotective T helper type 2 (Th2) immune response with ineffective humoral involvement; and (iv) the presence of muriform (sclerotic) cells embedded in the affected tissue. CBM lesions are clinically polymorphic and are commonly misdiagnosed as various other infectious and noninfectious diseases. In its more severe clinical forms, CBM may cause an incapacity for labor due to fibrotic sequelae and also due to a series of clinical complications, and if not recognized at an early stage, this disease can be refractory to antifungal therapy.

KEYWORDS black fungi, chromoblastomycosis, chromomycosis, melanized fungi, muriform (sclerotic) cells, neglected disease

INTRODUCTION

Neglected tropical diseases (NTDs) include a diverse series of endemic tropical and subtropical diseases that prevail in tropical or subtropical zones worldwide. They usually affect individuals living in low-income regions of Asia, Africa, and Latin America. NTDs normally affect populations who do not travel aboard, with little political voice and low visibility. According to the World Health Organization (WHO), the prevalence of NTDs is linked to poverty and disadvantage. Those who suffer most from NTDs are mainly the poorest populations, often living in remote rural areas, urban slums, and conflict zones. With little health care attention and political support, NTDs are not under the radar of public health systems, and they are not a part of their priority lists (1). Several endemic diseases, including helminthic, protozoal, bacterial, and viral infections but not fungal diseases other than mycetoma (implantation mycosis), are defined as "neglected diseases" by the WHO (1, 2).

Implantation mycoses are also classified as "subcutaneous mycoses" and refer to a diverse group of heterogeneous fungal diseases in which the mode of infection comprises several types of transcutaneous trauma (3, 4). The list of implantation mycoses includes global infections such as implantation phaeohyphomycosis (PHM) and entomophthoromycosis as well as endemic mycoses such as sporotrichosis, eumycetoma, lacaziosis (lobomycosis), and chromoblastomycosis (CBM) (3–9). Also known as chromomycosis, CBM is one of the more prevalent implantation fungal infections, being the most common of the gamut of diseases due to melanized or brown-pigmented fungi. CBM is observed mostly in persons living in tropical and subtropical zones around the planet. This disease is characterized by (i) traumatic inoculation by implantation from an environmental source, leading to an initial cutaneous lesion at

the inoculation site; (ii) progressive and chronic involvement of cutaneous and subcutaneous tissular structures and a fibrous granulomatous response with embedded microabscesses and often with tissue proliferation; (iii) a nonprotective T helper type 2 (Th2) immune response with ineffective humoral involvement; and (iv) the presence of muriform (sclerotic) cells in the affected tissue. Morphologically, muriform cells constitute an aggregation of 2 to 4 fungal cells with transverse and longitudinal septation (9–13). CBM lesions are clinically polymorphic and are commonly misdiagnosed as various other infectious and noninfectious diseases. In advanced cases, this disease may lead to an incapacity for labor due to fibrotic sequelae and a series of clinical complications, and if not recognized at an early stage, this disease may become refractory to therapy (13–15).

Chromoblastomycosis is an orphan neglected disease. Its global burden is comparable to or greater than that of mycetoma, and like mycetoma, it is primarily an occupational fungal disease. Due to its global distribution, its impact on the impoverished, and its refractoriness, it should be considered a true neglected disease as defined by the WHO (2, 14–16).

A BRIEF HISTORY OF CHROMOBLASTOMYCOSIS

The priority of the description of the first case of CBM was a point of controversy for many decades. The disease was reported in 1914 in Brazil by Maximilliano Willibaldo Rudolph, who wrote Über die Brasilianische Figueira ("*About the Brazilian fig tree*") in a German journal (17, 18). Rudolph, who worked as a clinician in the State of Minas Gerais in central Brazil, noticed six patients with warty lesions on the lower limbs, popularly known as "fig tree." Rudolph reported the isolation of two black and velvety cultures from four of these patients; the microscopic features of these organisms were quite similar to those of *Fonsecaea pedrosoi*, one of the most common etiological agents of CBM in this geographical region (3). Before Rudolph's article, there was some clinical and epidemiological evidence that cases of mycetoma described in Madagascar in 1903 and 1909 by Bruas and Fontoynont, respectively, were not "Madura foot" or mycetoma cases but possibly CBM (19, 20). Similarly, Hoffmann (21) noted that in 1904, Guiteras had observed cases of "chapa" (plate) in Cuba, a popular name for a disease resembling CBM infection (21).

The beginning of scientific research on this disease started in 1911 in the city of São Paulo, Brazil, when Pedroso and Gomes (22) observed cases of verrucous dermatitis in four Brazilian patients. After excluding leprosy, those researchers observed the presence of spherical brownish cells in skin biopsy specimens, corresponding to current muriform cells, the hallmark of CBM diagnosis. The disease was initially considered to be closely associated with blastomycosis, and consequently, those authors named the disease black blastomycosis. The cultivation of the patients' skin lesions yielded dark fungal colonies, which were later classified as *Phialophora verrucosa* (23). Emile Brumpt sent the isolates to Paris, France, for accurate mycological identification. Because of issues related to Word War I conflagration, the cases described by Pedroso and Gomes were published only in 1920 (22). In 1915, Lane and Medlar, in separate publications, reported the first North American case of CBM, which was observed in an Italian patient living in Boston, MA (24, 25). The patient presented with a warty violet plaque lesion on the right buttock simulating verrucous tuberculosis, but muriform cells were depicted upon histopathological examination. Lane described the disease as "a new blastomycosis," while Medlar classified the isolate as *P. verrucosa* (24, 25). After studying the isolates from the Brazilian cases reported by Pedroso and Gomes, Brumpt concluded that they were not compatible with *P. verrucosa* but belonged to a new species, *Hormodendrum pedrosoi* (26). In 1936 in Argentina, Pablo Negroni, after detailed mycological studies of CBM agents, created the genus *Fonsecaea* and validated the species *F. pedrosoi* (27).

The name "chromoblastomycosis" was employed for the first time in 1922 by Terra et al. to differentiate a cutaneous fungal disease observed in Brazil from the confusing clinical syndrome known as "verrucous dermatitis" (28). Because the new name "chro-

TABLE 1 Popular and medical names of chromoblastomycosis around the world

Name(s)	Country(ies)	Reference(s)
Popular		
Chapa (plate)	Cuba	21
Figueira (fig tree)	Brazil	17, 18
Formigueiro (tingling)	Brazil	22
Sundo	South Africa	318
Sustra	South Africa	318
Foratra, Gajo-miala, Didra	Madagascar	19, 20
Medical		
Black blastomycosis	Brazil	22
Yellow blastomycosis	China	134
Chromomycotic dermatitis	Brazil	22, 28
Verrucous dermatitis	United States, Brazil	22, 24, 25
Guitera's disease		
Pedroso's and Carrión's disease	Brazil	22, 250
Lane and Medlar disease	United States	24, 25
Chromomycosis	Brazil, United States	29, 104
Chromoblastomycosis		22, 30

moblastomycosis" suggests that the etiological agents of the disease show yeast budding forms in tissue, Moore and Almeida proposed a new denomination, "chromomycosis," as a replacement of "chromoblastomycosis" (29). With time, the name chromomycosis was used as an umbrella to encompass a heterogenic and diverse group of mycotic diseases caused by a wide spectrum of melanized (dark-pigmented) fungi. This problem was finally corrected in 1974 by Ajello et al., who created a new term, "phaeohyphomycosis" (PHM), to define all infections clinically and pathologically distinct from chromoblastomycosis (30). A variety of popular and scientific names used to refer to CBM in different countries is depicted in Table 1.

According to International Society for Human and Animal Mycology (ISHAM) mycosis rule denominations, the term chromoblastomycosis was the proper one. Currently, CBM is classified by the International Classification of Diseases (ICD) as follows: ICD-9 no. 117.2 and ICD 10-B43 (31).

TAXONOMY

Etiology

When CBM is defined as an implantation mycosis leading to the hyperproliferation of host tissue, combined with the presence of a fungal pathogenic phase in the form of muriform cells, most of the agents of this disease are members of a single order in the fungal kingdom, the *Chaetothyriales*. Within this order is a single family, the *Herpotrichiellaceae*. The restricted distribution of CBM, with only the single exception of *Chaetomium* (32), indicates that this host-fungus interaction is highly specific because CBM is nearly exclusively found in patients with fully functional immunity. The less specific counterpart disease caused by black fungi, PHM, usually involves a course with tissue necrosis rather than proliferation, has a much wider spectrum of causative agents throughout the fungal kingdom, and is associated mostly with immune disorders.

The *Chaetothyriales* are particularly known by the genus *Exophiala*, comprising so-called "black yeasts," which are able to reproduce by budding. The majority of their relatives, including all the CBM agents, are strictly filamentous. Melanin is consistently present in reproductive and vegetative cells, and therefore, colonies of *Chaetothyriales* are typically olivaceous, dark gray, or black shades due to the presence of dihydroxynaphthalene (DHN)-derived melanin, a hydrophobic, negatively charged compound with a high molecular weight produced by phenolic and/or indolic oxidative polymerization (33). Growth of the *Chaetothyriales* is invariably slow. Generic distinction is made by the morphology of their clonal mode of reproduction. In *Rhinocladiella*, conidia are produced sympodially on elongate cellular extensions; in *Fonsecaea*, they are clustered on denticles and arranged in short chains; in the *Cladophialophora* genus,

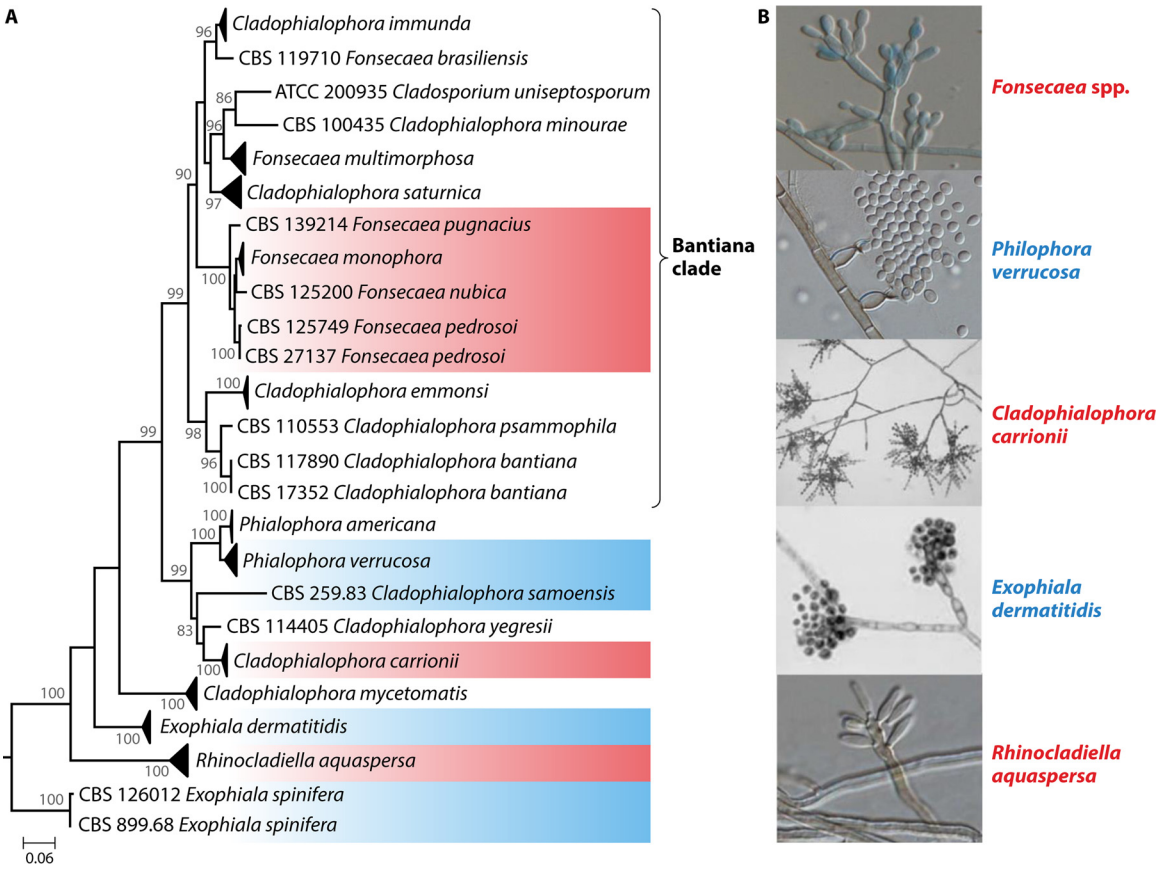

FIG 1 Phylogeny of a representative selection of species of the *Chaetothyriales*, based on the confidently aligned LSU rDNA D1-D2 domains of LSU sequences constructed by the maximum likelihood method implemented in MEGA 5.10. Bootstrap values of >80% from 500 resampled data sets are shown with branches. Morphologies of the species concerned are shown at the right. *Exophiala spinifera* was used as the outgroup.

they are arranged in long, dry chains; in *Phialophora* and in *Cyphellophora*, they are produced in slimy heads through collarettes, with *Cyphellophora* being differentiated by curved, mostly septate conidia; in *Veronaea*, long conidiophores produce sympodial, two-celled conidia; and in *Exophiala*, the cells producing conidia are annellidic, while the delivered cells show intensive budding. Different morphotypes may occur next to each other in a single strain, and the genera outlined above lack phylogenetic significance. Species of the same genus are often morphologically indistinguishable from each other; for reliable distinction of species, sequencing of diagnostic genes is necessary (34). On the other hand, although the number species related to the etiology of CBM has increased after molecular taxonomy, no clinical or therapeutic association has been attributed to the new genotyping of species.

Molecular Phylogeny

Today, the guiding principle to display taxonomic relationships among fungi is molecular phylogeny, since phenotypic characteristics are poorly informative. Partial ribosomal DNA (rDNA) large-subunit (LSU) sequences are sufficiently conserved to show relationships at the ordinal or family level. Pathogenic species are polyphyletic within the *Chaetothyriales*, being dispersed all over the tree. The main agents of CBM are limited to three clusters. A *Fonsecaea* cluster, nested in the "bantiana clade" (34, 35), contains prevalent agents of CBM, *Fonsecaea pedrosoi* and *F. monophora* (36, 37) (Fig. 1). Uncommon, recently described agents in this clade are *F. nubica* and *F. pugnacius* (38, 39). Other fungi in the bantiana clade are *Cladophialophora* species related to *C. bantiana*, the main agent of primary brain infection, and several species isolated from

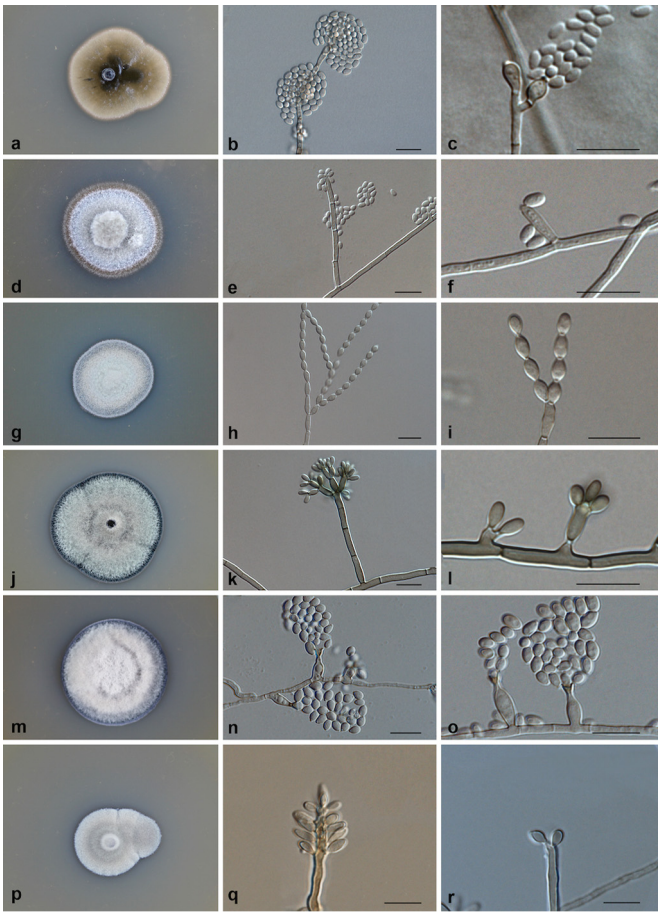

FIG 2 (a to c) *Exophiala dermatitidis* CBS 748.88. (a) Colony on malt extract agar (MEA) after 3 and 4 weeks of incubation at 30°C; (b) conidial head; (c) conidiophore and conidia. (d to f) *Exophiala spinifera* CBS 899.68. (d) Colony on MEA after 3 weeks of incubation; (e) conidiophore and conidia clustered at the apex of the conidiophore; (f) conidiophore and liberated conidia. (g to i) *Cladophialophora carrionii* CBS 166.54. (g) Colony on MEA after 3 weeks of incubation; (h) branching conidial system and conidial chains; (i) conidial chains. (j to l) *Fonsecaea pedrosoi* CBS 273.66. (j) Colony on MEA after 3 weeks of incubation; (k) conidiophores and conidia; (l) phialides and conidia. (m to o) *Phialophora verrucosa* BMU 07506. (m) Colony on MEA; (n) phialides and conidia; (o) flask-shaped phialides and conidia. (p to r) *Rhinocladiella aquaspersa* CBS 122635. (p) Colony on MEA after 3 weeks of incubation; (q) conidiophore and conidia; (r) young conidia and conidiophore. All cultures were incubated at 30°C.

disseminated infections but also some saprobes that are not known to be involved in human disease (40).

Cladophialophora carrionii (41, 42) is located in a separate cluster ("carrionii clade") along with the recently described species *C. samoensis*, causing chromoblastomycosis in Samoa (33). This clade also includes *Phialophora verrucosa*, which has already been isolated from CBM lesions (40, 43, 44).

Rhinocladiella aquaspersa, another consistent agent of CBM (45–48), is located at a significant distance from both clades and is currently not assigned to any phylogenetic group within the *Herpotrichiellaceae*. Occasional infections by *Exophiala* species have been reported, which mostly cause other types of infections, i.e., *Exophiala jeanselmei*, *E. dermatitidis*, and *E. spinifera* (8, 49–54), each located in separate clades (Fig. 1). All agents are flanked by species that cause other types of disease and by environmental species (55, 56).

Molecular identification of individual species is done with the rDNA internal transcribed spacer (ITS) region (35). For distinction of closely related *Fonsecaea* or *Phialophora* species, an additional gene such as translation elongation factor 1α (*TEF1*) or a partial β-tubulin gene (*BT2*) may be recommended (35, 57) (Fig. 2). The cytochrome

P450 cluster involved in melanin synthesis and hydrocarbon degradation might play an important role in the virulence of the *Chaetothyriales*, which probably differs from the other virulence factors of fungi reported previously, such as *Lac* and *HmgA* (58), and other virulence factors (59–65). Likewise, the *ACT1*, *BT2*, and *Cdc42* genes are effectively involved in cell cycle stages and the formation of the actin cytoskeleton (35), which has been related to morphogenetic switching to muriform cells, which are considered the invasive phase of agents of CBM (66) and which are also used for species distinction (35, 57).

Biodiversity

The genus *Fonsecaea* comprises four species that cause CBM: *F. pedrosoi*, *F. monophora*, *F. nubica*, and *F. pugnacius*. *Fonsecaea monophora* and *F. pugnacius* show significant neurotropism, eventually leading to dissemination to the brain and other organs (38, 39, 41) or causing primary brain infection without skin lesions, which are clinical forms of PHM, because no muriform cells are seen in tissues (40, 67). All species of *Fonsecaea* have felt-like, gray-olivaceous colonies. Hyphae are regular, melanized, and branched in the apical part. Terminal cells show 1 to 4 denticles, each bearing a single-celled, broadly clavate conidium, which in turn produces 1 to 2 smaller conidia on denticles (34). Additionally, particularly in media poor in nutrients, phialides with slimy heads of conidia emerging from large collarettes may be produced (Fig. 2). The taxonomy of *Fonsecaea* was revised previously by de Hoog et al. (41) and Najafzadeh et al. (57). New species such as *F. nubica* and *F. pugnacius* were identified with sequences of the ITS and *cdc42*, *BT2*, and *ACT1* genes, eventually supplemented with amplified fragment length polymorphism (AFLP) profiles (38, 39). Pathogenic species of *Fonsecaea* present optimum development at 33°C, with a thermotolerance of growth at 37°C. These cardinal temperatures are slightly higher than those of strictly environmental species (56).

The two *Cladophialophora* species causing CBM, i.e., *C. carrionii* and *C. samoensis*, form grayish-green, dry colonies that profusely sporulate, with conidia being arranged in long, densely branched chains composing a shrub-like conidial system (Fig. 2). Also, in these species, phialophora-like conidia may be produced on nutritionally poor media. Species are phenotypically identical; distinction is made by ITS sequencing (33). *Phialophora verrucosa* is monomorphic for flask-shaped phialides with large, dark, funnel-shaped collarettes at the top, from which slimy heads of ellipsoidal, one-celled conidia are produced. Colonies are olivaceous-black and grow moderately rapidly (24, 25, 34, 68). Disseminated forms of the disease have also been reported but without unambiguous muriform cells in tissue (44), and thus, they may be considered PHM. Some similar cases in China concerned patients with CARD9 mutations (69).

Rhinocladiella aquaspersa forms erect, dark brown, well-differentiated conidiophores, which produce abundant conidia alongside the terminal parts of conidiophores (45, 70). Conidia are subhyaline, ellipsoidal to clavate, and produced from darkened scars. As such, the species is recognizable by microscopic morphology. Colonies are restricted, velvety, and olivaceous-black. Most cases have been reported from South America (46–48).

Exophiala is the only genus of the *Chaetothyriales* that produces a yeast-like phase. Liberated cells inflate and reproduce by budding or become subspherical and gradually change over to hyphae via a string of more or less inflated cells known as the "torulose mycelium," which is highly diagnostic for *Exophiala*. Colonies are black, slow growing, and initially mucous and often become dry and hyphal with age, giving the impression that the colony has become contaminated. *Exophiala spinifera* has long, erect conidiophores with long annellated zones, and *E. dermatitidis* has several very short annellated zones next to each other on a single cell (34). Almost all other *Exophiala* species have to be distinguished by sequencing (55, 71, 72).

The currently recognized increased diversity of species related to CBM has limited clinical or epidemiological consequences, since risk factors and treatment regimens are largely the same for all species.

TABLE 2 Different types of trauma associated with implantation of chromoblastomycosis agents

Type of trauma	Sources of trauma	References
Plants	Wood, straw, grass, thorns, palm trees, bamboo, coconut shells, cactaceae	3, 88, 89, 75, 77–80, 82–84, 90, 92, 96
Animals	Insect stings, cow stomp, buck rear, cock spine, caterpillar contact, leech bite	155, 240, 250
Agricultural tools	Hoes, axes, knives, mills	3, 16, 240
Other	Bricks, shoes, fall, car crashes, natural disasters (hurricanes and flooding, etc.)	3, 9–11, 157, 158

EPIDEMIOLOGY

Potential Environmental Sources of Infection

Melanized fungi and their relatives are also denominated "dematiaceous," "phaeoid," or simply "black" fungi (30, 67, 73). This denomination refers to fungi containing melanin in their cell walls microscopically visible by a gross brown, olivaceous, or black pigmentation period. Although many authors claimed to have obtained melanized fungi from natural sources, in most of those reports, no molecular identification was performed. Agents of CBM in the *Chaetothyriales* constitute only a small fraction of this group. Members of this order are enriched where toxic monoaromates are prevalent. Particularly, human-made niches are occupied, such as wood treated by phenolic preservatives, toxic mine waste, or oil-polluted soils. In nature, monoaromates are found in trace amounts in plant debris, thorns, and wood cortex, which provide microhabitats for these fungi (74). There is a strong correlation between the traumatic implantation of potential natural sources of infection and CBM lesions (74–80). This route of infection may be supported by clinical reports of patients exhibiting the presence of fragments of plant material at the site where they experienced a previous trauma. On rare occasions, wood fragments containing muriform cell-like structures were histopathologically observed in patients with CBM (77, 81–84).

CBM is strongly associated with agricultural activities, which further underscores the occupational nature of this disease (16). During labor activities, individuals living in areas of endemicity are probably infected through diverse traumas related to environmental materials (80, 84–88) (Table 2). Remarkably, however, etiological agents of CBM are difficult to recover from their environment due to their saprobiotic lifestyle: direct isolation by the use of selective methods usually yields saprobic counterparts of the pathogens, while the pathogens are almost exclusively restricted to warm-blooded hosts. Ruben et al. and Fernández-Zeppenfeldt et al. reported the identification of *Cladophialophora* in cactus plants (83, 89), but de Hoog et al. showed that this was a molecular sibling, *C. yegresii* (33). Vicente et al. reported the identification of mainly saprobic *Fonsecaea* species such as *F. minima* and *F. erecta* in plant thorns close to the habitat of patients with CBM due to *F. pedrosoi*, while pathogenic strains were recovered only exceptionally (56, 74, 76). In 1937, Conant thought that *Cadophora americana* recovered from wood pulp was identical to *Phialophora verrucosa*, which potentially caused CBM (90), but recently, Feng et al. proved that the *P. verrucosa* complex contains clinical species next to environmental species (91). Phenotypically identified isolates of *F. pedrosoi* and *C. carrionii* have been isolated from plant debris, grass, tree cortex, and also abandoned wasp nests (75–78, 92–97). After the isolation of a strain identified as *F. pedrosoi* from a spiny plant (*Mimosa pudica*), Salgado et al. suggested that it might be a probable natural source of CBM infection in the north of Brazil (74), but molecular proof was not provided. Several species of *Palmacea* (palm trees) have also been recognized as natural habitats of melanized fungi (72–75). A probable infection source was described in an area of endemicity in the State of Maranhão, located on the border of the Brazilian Amazon rainforest, where several agricultural communities were working on harvesting babassu (*Orbignya phalerata*), a wild palmacea specimen (78–80).

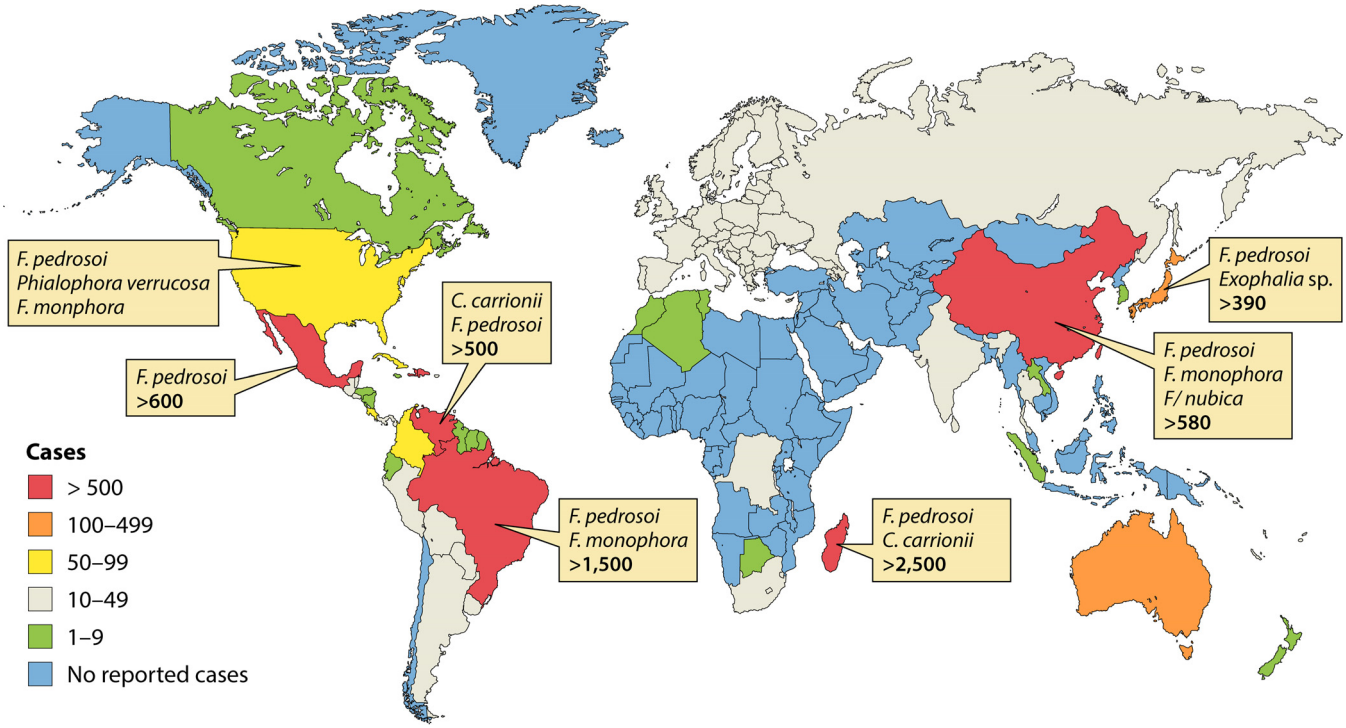

FIG 3 Global distribution of chromoblastomycosis based on reported case series.

Local dwellers collect the babassu nuts, which are rich in oil and are a well-known component used by industrial companies for the manufacture of international beauty products. Members of the *Chaetothyriales* are indeed enriched on babassu shell fragments, which are hypothetically considered a risk factor for the development of cutaneous lesions after labor trauma (78, 80, 84). The invasive potential of agents differs significantly between species and is as yet only fragmentarily understood. CBM is a human disease, and the scarce reports of this infection in other animals, such as amphibians and mammals, are considered PHM, because typical muriform cells were lacking (98–101).

Given the above-described ambiguous results, we hypothesize that saprobic and opportunistic counterparts may occur in a single environmental sample but occupy different microhabitats. Due to this nutritional deviation, saprobic species can be isolated by standard methods (56, 74), while opportunists and pathogens are selected when samples are enriched via a mammalian vector (75). We also hypothesize that both invasive and noninvasive fungi may be present in the same environmental sample, where they each have a different microhabitat. Methods are available to isolate saprobic fungi from the environment, but selective methods for pathogens are needed. The use of mammalian vectors, as done previously by Gezuele, Mok, and others, should be repeated with the support of state-of-the-art identification methods (74–78).

Geographic Distribution

The species most frequently associated with CBM belong to the genera *Fonsecaea* and *Cladophialophora*. Infections due to *Rhinocladiella* are less frequent, while a few cases were associated with members of the *Phialophora* and/or *Exophiala* genus. *F. pedrosoi* and *C. carrionii* infections are normally observed in tropical and subtropical areas of endemicity around the world. There are several reports addressing the reservoir of the most common CBM agents: *C. carrionii* occurs in semiarid areas, whereas *Fonsecaea pedrosoi* is associated with humid climates (3, 9, 11–13, 85, 86) (Fig. 3).

Similarly to most of the endemic mycoses, CBM is not a notifiable disease. As a consequence, there is no precise assessment of either the incidence or prevalence of

this mycosis. Instead, data gathered from surveys and case series suggest that the incidence of CBM ranges from 1:6,800 (Madagascar) to 1:8,625,000 (United States) (3, 16). The disease is prevalent in tropical and subtropical regions of the planet, mainly between latitudes of 30°N and 30°S. The majority of cases are reported from Latin America, the Caribbean, Africa, and Asia. Brazil, Mexico, Venezuela, India, Australia, and southern China report the majority of the series of patients.

Chromoblastomycosis in the Americas

Although Lane and Medlar described one of the first cases of CBM in the United States in 1915 (24, 25), the United States is not an area where this mycosis is endemic. The second case of CBM in the United States was not described until 1933 in Texas; this patient presented with ulcerated and nodular lesions on the right foot (96). Over subsequent decades, scattered cases have been described, particularly from Texas and Louisiana (84, 100–107).

In comparison, implantation mycoses in Mexico are a frequent health problem. Sporotrichosis and CBM (108) are the most common forms of implantation mycosis in this country. A review of the cases (109) reported until 2013 identified 603 mycologically and/or histologically proven cases, supporting the premise that Mexico should be considered a region where the disease is highly endemic (110, 111). *Fonsecaea pedrosoi* is the most common etiological agent of CBM (95.8%), although disease caused by *C. carrionii*, *P. verrucosa*, *R. aquaspersa*, and *E. spinifera* has also been reported (12, 111). Most strains were identified by morphology; data from only a few of these reports have been confirmed by molecular methods.

The disease has also been described throughout Central America, from Guatemala to Panama (111, 112). Large numbers of cases have been described in Costa Rica (113, 114), Panama (115), Honduras (111, 116), El Salvador (112), Nicaragua, and Guatemala (12), in decreasing order. Most cases arise in the tropical rainforest. The epidemiological data are similar to those for Mexico, with the prevailing fungus isolated being *F. pedrosoi*, although other species have also been reported. Among these reports was the exceptional report of CBM caused by *Chaetomium funicola* in Chiriquí, western Panama (117).

In the Caribbean, unlike Central America, disease is the main cause of implantation mycosis; around 600 cases have been reported (113). Of these cases, 450 patients from the Dominican Republic were described by Isa-Isa (114). Most of these patients were from the humid southern forest region, which is considered to be a focus of endemicity, similar to the Brazilian Amazonian region or Madagascar (118, 119). Approximately 100 cases have been reported in Cuba (37, 120), and fewer cases have been reported in Puerto Rico (121), Jamaica, and Haiti (114, 122). While *F. pedrosoi* is the most common etiological agent of CBM in the Caribbean, its molecular sibling *F. monophora* may also occur, as was uncovered in Cuba (37) only after sequencing. *Cladophialophora carrionii* was encountered in Puerto Rico (121).

In South America, most of the cases of CBM have been described in Brazil. With the exception of Chile, CBM has been reported in all South American countries. After Brazil, Venezuela and Colombia account for most cases of CBM. Within Brazil, CBM is endemic in many geographical areas, especially in the northern regions, where 872 cases were retrospectively reported during the last decades (3, 79, 123, 124). Although 332 cases were reported from other provinces, a significant decrease in the number of new CBM cases has been observed, especially in the southern regions of this country (125, 126). The mean annual incidences of cases of CBM reported in Brazil were 6.4/year (71 cases/11 years) for the state of Paraná (southern region), 5.9/year (325 cases/55 years) for Pará 45 (northern region), 4.3/year (13 cases/3 years) for Maranhão (northeastern region), and 2.6/year (73 cases/28 years) for Rio Grande do Sul (southern region) (8, 13, 123–126). The principal etiological agent of CBM in Brazil is *F. pedrosoi* (which may include its molecular siblings), followed by sporadic reports of *P. verrucosa* and *E. spinifera*.

Following a pattern similar to that observed for paracoccidioidomycosis, the number of new cases of CBM is decreasing in some Brazilian regions. This is thought to be a consequence of several modifications of agricultural methods, including the massive use of agricultural azole fungicides and progressive agriculture mechanization, resulting in a diminution of risk factors due to occupational exposure (127–129).

Chromoblastomycosis in Asia and Oceania

Japan has the highest incidence of CBM among populations in Asia (1/416,000). The first Japanese case was described in 1930 by Kano, who reported a female patient with an unusual CBM-like infection in the facial region, caused by *Hormiscium dermatitidis* (currently *Exophiala dermatitidis*) (130). Since the first description, several Japanese authors reported ~700 cases of CBM infections (130–133). Several hundred cases have been reported from Mainland China since the first description by Yew in 1951 (134, 135). The cases were distributed all over the country, covering more than 21 provinces. The highest prevalence rates were found for Guangdong Province (84/196) and Shandong Province (38/196) based on literature published from 1990 to 2015 (136). There may be areas of hyperendemicity; for example, in a study of CBM conducted by Dai et al. in Zhangqiu City, Shandong Province, up to 300 cases of CBM were found in 1998 (135).

India has long been known for autochthonous CBM infections, with more than 100 cases being reported since the first description in 1950 (137, 138). In other counties, such as Sri Lanka, Pakistan, Thailand, and Malaysia, CBM cases have regularly been reported, demonstrating its endemic character in South and East Asia (139–144).

In Oceania, CBM was first described in Australia, and to date, approximately 200 cases have been reported. CBM in Australia is caused mainly by *C. carrionii*, due to the prevailing arid climatic conditions. There have been a few reported cases from other countries in Oceania, such as New Zealand and Solomon Islands (145–148).

Chromoblastomycosis in Africa

Madagascar represents the most important focus of CBM described to date in the world. Retrospective data collected by Brygoo and Segretain and Esterre et al. at the Institut Pasteur of Madagascar during a 40-year period revealed one of the world's largest case repositories of CBM, consisting of 1,323 confirmed cases observed between 1955 and 1995 (19, 20, 85, 86). Those authors described two distinct ecosystems in the island, one in the north, with a tropical rainforest climate, where *F. pedrosoi* predominates, and other in the south, with an arid and dry geographic region, where *C. carrionii* causes 41% of chromoblastomycotic infections. The isolation of the latter species from the Malagasy spiny desertic region suggests that continuous deforestation, in order to produce charcoal and for house construction, constitutes an environmental risk factor associated with this disease (85, 86). Unfortunately, updated epidemiological studies from the African continent are scarce, and the actual burden of CBM in Africa may be underestimated.

Chromoblastomycosis in Europe

CBM is a disease observed mainly in tropical and subtropical regions (Latin America, Asia, and Africa), but there are many imported cases in Europe. A recent review of CBM in Europe revealed a total of 31 probable cases (149). The authors of that study suggested that the disease was considered to be autochthonous in some cases. One of these cases was an infection acquired in a mine from locally harvested mine wood and caused by *F. monophora* (149). As CBM is uncommon in Europe, cases may have been misdiagnosed as cutaneous tuberculosis, squamous cell carcinoma, psoriasis, PHM, and sporotrichosis or other infectious and noninfectious conditions that may mimic CBM.

Demographics and Risk Factors

Judging from most of the reported case series, CBM involves mainly adult males. The sex distribution in a case series reported in the southern region of Brazil showed that

disease was prevalent in males (4:1) (126). In two other studies involving 390 cases in the same region, the sex ratio distribution was much higher for males (17:1) (123, 124). The difference related to sex distribution in patients with CBM may be related to hormonal protection, as observed for paracoccidioidomycosis patients (150). In these systemic mycoses, females are protected from clinical manifestations by β-estradiol, whereas in CBM, hormonal protection may be related to progesterone (150, 151). Cytosol receptors have been identified in *P. verrucosa*, and its *in vitro* growth was found to be influenced by progesterone and testosterone hormones but not by estradiol (152). Previous explanations of skewed male/female ratios, such as different occupational risks of agricultural labor, are largely erroneous.

In the Amazon, the age distribution ranged from 25 to 85 years, with the most affected group being between 41 and 70 years old (86%) (123, 124). In Mexico, in a series of 603 cases, the disease predominated in adult males (66%) >38 years of age. Although the age range extended from 9 years to 90 years, children were rarely affected (1.2%) (109). In China, the majority of CBM patients are males (6.7:1), with a mean age of 54.75 years (range, 10 to 81 years) (136). Although CBM in children is infrequent, from 1992 to 2004, 22 cases (aged 2 to 19 years) of *C. carrionii* infections were reported in the semiarid state of Falcón, Venezuela, (88).

Genetic susceptibility may also participate in adaptations of the etiological agents to the host tissue environment. In a Brazilian study, 32 nonconsanguineous white CBM patients and 77 healthy controls who were matched according to gender, age, ethnic background, profession, and geographical region were studied for the distribution of HLA-A, -B, -C, -DR, and -DQ. The frequency of only HLA-A29 was significantly increased. This antigen was present in 28% of patients, as opposed to 4% of the controls ($P = 0.03$) (153). These findings suggest a possible genetic susceptibility to CBM. The relative risk to develop disease for patients carrying HLA-A29 was estimated to be 10. In another study conducted in Venezuela, Yegres-Rodriguez also indicated a genetic susceptibility to CBM in the population of the endemic area of Falcon State (154).

Chromoblastomycosis is considered an occupational disease around the world, affecting farm laborers, gardeners, lumberjacks, vendors of farm products, and other workers exposed to contaminated soil and plant materials (3, 12, 13, 16). Similarly to eumycotic mycetoma infections, the lack of protective shoes, gloves, or garments in association with poor hygienic habits and deficient nutrition may favor the development of clinical forms of CBM after infection by implantation (13, 16, 125). As reported in Madagascar, other groups of laborers besides charcoal producers deal with environmental occupational hazards (78–80, 85, 86). For instance, there is some evidence in India that CBM may be acquired during manual work in black tea cultivation at the Gardens of Assam, northeast region, and also in rubber plantations in the central districts of Kerala and nearby Western Ghats (155). Less frequently, CBM has been observed in immunosuppressed hosts, usually in solid-organ transplant recipients and in association with neoplastic diseases (156). Similarly to implantation mucormycosis after natural disasters, several cases of CBM were reported in the United States after Hurricane Ike (157, 158).

PATHOGENESIS AND HOST DEFENSE

Recent studies have shown that impaired fungal clearance in CBM infections is due mainly to the enhanced virulence and pathogenicity of the etiological agents (159–161). Several potential virulence factors are probably involved in this disease, including modifications of the cell surface, hydrophobicity, remodeling of the fungal cell wall, secretion of proteolytic and hydrolytic enzymes, adhesion molecules, incorporation of aromatic hydrocarbons, assembly of siderophores, and especially the presence of melanin. Most of the virulence and pathogenic factors observed in chromoblastomycotic infections are largely similar to those for infections by other pathogenic fungi (160–163). Factors that are significant for the pathogenicity of CBM are melanin, muriform cells, cell adhesion, and hydrophobicity.

The host immune mechanisms against CBM, including cellular and humoral re-

sponses, are poorly understood. Some work has shown the significance of the cellular response in the host-fungus interaction, suggesting that fungal persistence *in situ* is the main factor responsible for the evolution of CBM.

Cell Morphology and Architecture

Melanized fungi are polymorphic organisms. Due to their plasticity and adaptability to several organic and inorganic environments, melanized fungi may show a great diversity in their morphology. When causing PHM, in clinical specimens, the etiological agents may present a series of morphological shapes, isolated or in combination: septated (toruloid) hyphae, pseudohyphae, and yeast-like and vesicular components. When causing black grain mycetoma, melanized fungi usually present as dark black grains composed of short distorted hyphae associated with vesicular elements (3, 67, 73). It is believed that hyphae and conidia are found abundantly in nature and are easily replicated in simple media such as Sabouraud agar (56, 77). In contrast, resistance forms are usually found only under extreme environmental stress conditions, such as very high or very low temperatures, extreme pHs, and nutrient-deficient soils. These resistance forms may also survive in rocks and in plants (164, 165).

After transcutaneous implantation, propagules of CBM agents present a unique cellular and morphological plasticity. During infection, cell differentiation becomes meristematic, with isodiametric swelling and cross-septation. The resulting muriform cells (166) have also been denominated "Medlar bodies" or, alternatively, "copper pennies," "chromo or fumagoid bodies," and "sclerotic or meristematic cells" (118). "Meristematic" is a botanical term for the expansion of cells, and for black fungi, it is used mostly for the description of clumpy thalli of rock-inhabiting extremophiles (167). The word meristem comes from the Greek word merizein, meaning to divide. The term "sclerotic" is related to "sclerotia," which are made of compacted masses of latent hyphae (168). The term "muriform cell" is restricted to cells of the *Chaetothyriales* with meristematic growth that serve as invasive forms in living tissue, either human or plant (169). Muriform cells may be single or clustered. They have a round-to-polyhedral form in a darkly pigmented thick wall with transverse and longitudinal cross-walls. The muriform cell is considered to be a mechanism for evolutionary adaptation to enable survival inside the microenvironment of the host (118). It is directly associated with an intense granulomatous response as well as with the evasion of immune mechanisms signaling the onset of disease chronicity. The time of conversion from conidia to muriform cells in *in vitro* studies was estimated to be 6 days (58, 170).

The muriform cell arrangement in tissue depicts an optimal surface/volume ratio favoring significant melanin deposition. *Fonsecaea pedrosoi* requires a low concentration (0.1 mM; pH 2.5) of Ca^{2+} to differentiate from mycelia to muriform cells, indicating that the ion concentration may be important in the process of transitioning during CBM (58, 171, 172). Muriform cells collected from lesional tissues can easily differentiate into hyphae and conidia *in vitro* or can be maintained as muriform cells under harsh conditions of low pH and nonoptimal nutritional sources in liquid medium (170). It has been shown that the survival of resistance forms and consequently the emergence of clinical disease caused by *F. pedrosoi* may be strongly associated with the presence of muriform cells and an invasive form inside host tissue (173). Among other factors, melanin is also strongly associated with the process of transition. Furthermore, muriform cells remain a significant differential diagnostic tool to distinguish between CBM and the semantically closely related PHM, in which typically muriform elements are not detected (174). Muriform cells are highly resistant to immune system attack, and therefore, better knowledge of this differentiation process may permit the proposal of different and more efficient therapeutic approaches against CBM (175, 176).

Microbial adherence and hydrophobicity are two of the most important determinants of fungal pathogenesis (176, 177). For CBM, infectious forms may stick to epithelial tissue inside the host, leading to the differentiation of muriform cells that resist killing by the host and permit the evolution of chronic granulomatous inflammation. The presence of extracellular hydrophilic molecules of polysaccharides may

enhance this phenomenon because of hydrophobicity. The production of adhesive conidia by phialides of *F. pedrosoi*, *Exophiala dermatitidis*, *E. spinifera*, and *Cladophialophora carrionii* suggests binding to arthropod or other invertebrate vectors. Thus, a detailed review of the fungal cell wall structure and membrane organization is important to improve knowledge about pathogen-host interactions. Cell wall glycoproteins and glycolipids appear to function as epitopes, indicating their use in immunodiagnosis and potential therapy focused on the stimulation of the humoral response.

Ceramide monohexosides (CMHs) have been identified in membranes and cellular walls of pathogenic and nonpathogenic fungi, presenting a unique ceramide moiety compared to those of mammalian CMHs (177, 178). CMHs seem to have a pivotal function in the host immune response, and they may be associated with fungal differentiation. Cerebroside expression in CBM cells has been strongly associated with muriform cell transition and melanin deposition. In particular, structural but not immunogenic CMH diversity has been observed among different forms of *F. pedrosoi*. Identical CMH structures have been demonstrated for the conidial and mycelial forms, while the CMH moiety in muriform cells possesses an additional −OH group bound to the backbone of the lipid structure. Furthermore, muriform cells maintain their resistance to CMH antibodies, while their recognition occurs only at melanin-depleted cell wall regions. Three possibilities have been raised based on the above-described results: first, CMHs are absent from muriform cells; second, CMH may have a different structure on muriform cells that would interfere with the recognition of monoclonal antibodies (MAbs); and third, the melanin structure on muriform cells impairs the linkage of anti-CMH antibodies to the membrane target (178). Indeed, chemical removal of melanin by alkali augmented the reaction of anti-CMH antibodies with muriform cells.

The glycoprotein structure of conidial cells of *F. pedrosoi* consists of two linear polymers, i.e., residual α-(1→6)-bound mannose and β-(1→6)-bound galactofuranose, with replacements of both polymers by an α-(1→2) linkage with residues of glucose. The structure of the glycoprotein additionally contains large amounts of O-linked oligosaccharides, particularly a hexanose, in a comb-like structure (179). O-mannosylation defines the form of the cell wall and cellular specialization and participates in the virulence of the fungus (180, 181). Therefore, the specific above-mentioned structure might play a crucial role in clinics, in strategies for the development of new drugs, and for the detection of *F. pedrosoi* in the future (179).

Protein phosphorylation and dephosphorylation are important for immunomodulation, influencing the host reaction to invading fungal pathogens (176, 182). The contact and invasion of *F. pedrosoi* in epithelial cells and macrophages may be associated with the activity of fungal protein kinases (183). In particular, inhibitors of protein kinase, such as genistein and stauporine, when used for pretreating either macrophages or *F. pedrosoi* conidia before infection, may inhibit cellular invasion. Notably, pretreatment of conidia had an effect simply on interactions with epithelial cells, with no influence on macrophages (183). Specific peptidase inhibitors (PIs) against human immunodeficiency virus significantly affect peptidase secretion and growth of *F. pedrosoi*, interfering with fungus-host cellular contacts (184). Peptidase activity was impaired in a dose-dependent way, with nelfinavir producing the greatest inhibitory result. The growth of *Fonsecaea pedrosoi* was importantly affected after exposure to PIs, while the conidial structure presented significant morphological changes comprising cytoplasm invagination, cell wall detachment, widening of fungal vacuoles, and anomalous cell division (184).

Virulence Factors

Thermotolerance is one of the important virulence factors among members of the *Chaetothyriales*. Pathogenic *Fonsecaea* species have an optimum growth temperature of 33°C and a maximum growth temperature of 37°C (185). These cardinal temperatures are slightly higher than those of strictly environmental species. In comparison, *Cladophialophora bantiana*, which infects the central nervous system and the respiratory system of humans, can grow at 40°C (186, 187). Similar differences in optimum and

maximum growth temperatures seem to guide the predilection for either cold- or warm-blooded hosts in *Exophiala* species (188).

The rigid cellular wall of the fungus contrasts with the dynamic flux of structural molecules necessary for differentiation, growth, and adaptation to the host. Different membrane and cell wall components may contribute to CBM fungal virulence, including melanin, chitin synthases (CHSs), a set of hydrolytic enzymes (phosphatases, phospholipases, lipases, esterases, ecto-ATPases, peptidases, DNases, ureases, and gelatinases), lipids, galactomannans, and cerebrosides. The differentiation of hyphae and conidia into muriform cells may contribute to virulence, considering the increase of the thickness of the wall and the formation of muriform cells that resist host responses.

Lipids or lipid-free cell wall fractions extracted from different CBM agents induce significant granulomatous reactions in mice (189, 190). Indeed, live muriform cells have a high capacity for the induction of Langerhans giant-cell formation *in vitro* (185). Acid-labile galactofuranosyl residues are responsible for the immunogenic property of galactomannans isolated from three *Fonsecaea* species (191). Cerebrosides, the major neutral glycosphingolipids of fungal cells, composed of a monosaccharide linked to an *N*-acyl sphingoid ceramide, elicit antibody production that inhibits *F. pedrosoi* growth and enhances the mouse macrophage-killing function (192).

Chitin is formed by a beta-1,3-linked glucan and polymers of *N*-acetylglucosamine occurring in different conformations, suggesting a role in fungal structure and differentiation (193). Chitin synthesis depends on the enzymatic activity of chitin synthases I, II, and III, which participate in cytokinesis, septum synthesis, and bud scar formation (194), respectively, with each one having its catalytic activity encoded by different genes, CHS1, CHS2, and CHS3, respectively (195), that have conserved sequences in *F. pedrosoi* (196). The black yeast *Exophiala dermatitidis*, occasionally involved in CBM (166), possesses a structural gene for a class V CHS, WdCHS5 (194). WdCHS5 is essential for fungal growth at 37°C and the expansion of muriform cells, indicating a critical importance of the class V CHS for virulence (197). Muriform cells of *F. pedrosoi* were demonstrated to have a chitin-like component that mediates Th17 development by inhibiting dectin-1, impairing the immune response, and contributing to CBM chronicity (32) (Fig. 4).

Melanin

Melanin is a complex, hydrophobic, negatively charged macromolecule that includes indolic or phenolic polymers (32, 73). In CBM agents, this polymer can be derived from either L-3,4-dihydroxyphenylalanine (L-DOPA) or DHN (32, 60). Melanin is broadly found in nature. It is considered to be an immunologically active compound functioning as an important virulence factor in different pathogenic fungi (32, 198). Three possible mechanisms have been proposed to be associated with its contribution to the enhanced resistance of fungi against immune host cells: (i) protection against proteolytic enzymes, (ii) protection against oxygen or nitrogen derivatives, and (iii) reduction of phagocytosis. Melanin derived from DHN is synthesized by the polyketide path, a route starting with acetyl coenzyme A (acetyl-CoA) (199). Interestingly, the inhibition of this specific biochemical process with tricyclazole may be used to induce morphological changes in the cellular wall of *F. pedrosoi* leading to diminished resistance of the fungus to mechanical lysis and macrophage killing (200, 201). Melanin is mainly stored, as revealed by transmission electron microscopy, in concentric layers in intracellular vesicles, known as melanosomes, similarly to mammalian cells (201). Further studies have demonstrated that *F. pedrosoi* produces not only cell wall-associated but also extracellular melanin, pyomelanin, which may accumulate, e.g., in mutants of the tyrosine degradation pathway, as observed for spontaneous mutants of *F. monophora* (202). In particular, melanin may be detected inside phagocytic vacuoles together with engulfed fungi.

During infection, melanin deposition from *F. pedrosoi* interferes with nitric oxide (NO) production and inhibits phagocytosis (202), which is not reverted with gamma interferon (IFN-γ) and lipopolysaccharide (LPS). This phagocytosis inhibition may also

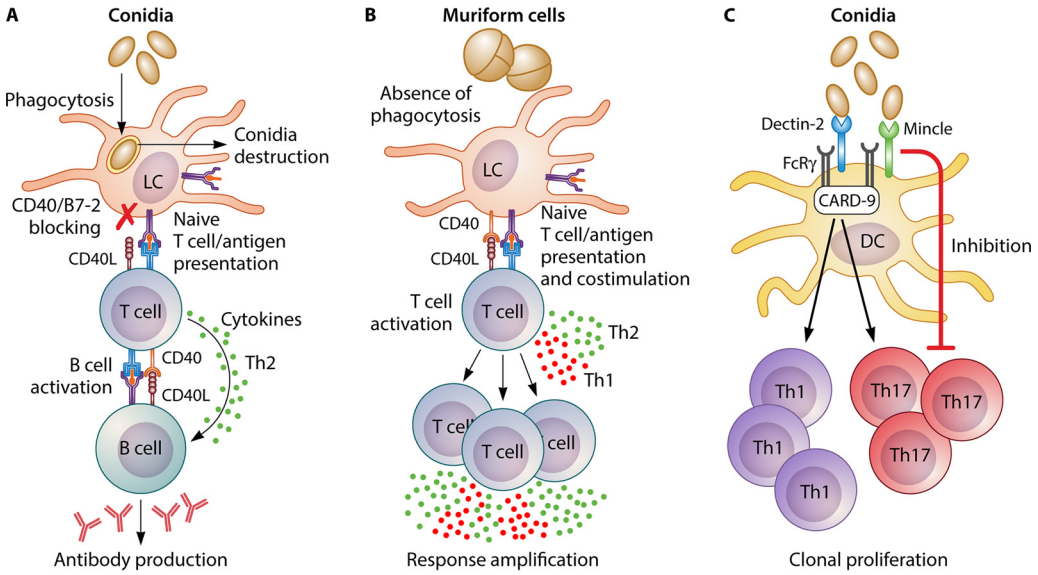

FIG 4 CBM immunology. Epidermal Langerhans cells (LCs) are at the front line of defense and may respond differently to conidia or meristematic cells. (A) After recognition of conidia, engulfment and destruction occur, followed by inhibition of costimulatory molecules. Conidial antigens may be presented to T cells, which activates B cells to produce antibodies against CBM pathogens. (B) If muriform cells are present, although there is no phagocytosis by LCs, naive T cells may be activated and proliferate, amplifying the immune response. (C) Recognition of conidia by dendritic cells (DCs) can be made by the C-type lectin dectin-2 or Mincle. However, the dectin-2 pathway leads to T-cell activation with proliferation of Th1 and Th17 cells, while Mincle signaling inhibits the same process, in a clear demonstration of PRR antagonism of the immune response following recognition of CBM fungi by DCs.

be observed after *in vitro* incubation with whole melanized cell walls. The immunomodulating role of melanin has also been shown for CMH recognition by antibodies on specific regions of the fungal cell wall where melanin was poorly expressed, implying that inhibition of melanin biosynthesis by drugs might render muriform cells more vulnerable to antibodies against CMH (190). As demonstrated by flow cytometry and immunofluorescence analyses, antibodies to melanin were reactive with mycelia, conidia, and muriform cells along with ghost particles (203). Muriform cells derived from clinical specimens were immunogenic, leading to interactions with antibodies. Opsonization of *Fonsecaea pedrosoi* conidia gradually led to increased polymorphonuclear leukocyte (PMN) attack and phagocytosis (178).

Extracellular Enzymes and Metabolites

Proteolytic enzymes, such as peptidases, have multiple tasks in consecutive stages of the pathogen-host interaction enabling the bypass of the host defense, leading to either digestion or host surface destruction. Proteolytic enzymes can be detrimental to various components of the host defense mechanisms, leading to immunological escape or antimicrobial resistance (204, 205). Kneipp et al. demonstrated that muriform cells, in comparison to conidia and mycelia, have high phosphatase activity associated with pathogenicity (206, 207). Phosphorylated substrates were hydrolyzed by surface ecto-phosphatases. The activity of the enzymes was increased on low-acidic-pH compounds and was blocked by specific compounds such as sodium fluoride and sodium molybdate (207).

A series of hydrolytic enzymes is produced and secreted by CBM agents. The walls of *F. pedrosoi* muriform cells present higher phosphatase activity than do conidia or hyphae (207), and phosphatase activity enhances host cell adhesion in both *F. pedrosoi* (206) and *Rhinocladiella aquaspersa* (208). The cytolytic effect of ATP suggests that ecto-ATPases found at the surface of *F. pedrosoi* may favor fungus survival in hostile environments such as the human body (209, 210). Peptidases secreted by *F. pedrosoi* are able to cleave human plasma proteins, such as immunoglobulins and albumin, and components of the matrix, such as fibronectin, while metalloproteinase inhibitors

impair conidial growth and differentiation (211) These findings were recently confirmed in *Phialophora verrucosa* (212). HIV aspartyl peptidase inhibitors strongly abrogate aspartyl proteolytic activity (204), greatly affect *F. pedrosoi* ultrastructure, diminish adhesion to epithelial cells, and increase susceptibility to killing by macrophage cells, indicating possible therapeutic use in CBM patients (184).

Innate Immune Response

Among the cells of innate immunity, macrophages seem to have an important function in regulating fungal growth. CBM fungal cells can be detected in intracytoplasmic vacuoles of skin macrophages (213). Sotto et al. investigated the cellular immune response, especially the distribution of antigens and antigen-presenting cells (APCs), in lesional biopsy specimens of patients with CBM (214). Notably, most antigens were observed as homogeneous or granulated material in the cytoplasm of macrophages, indicating that phagocytes are involved in innate immunity against CBM agents (214). Similar antigens accumulated in hypertrophic FXIIIa$^+$ dendritic cells (DCs) (214). However, chronic granulomatous infectious diseases are usually characterized by numerous macrophages on lesional tissue. On the other hand, activated macrophages seem to have a fungistatic rather than fungicidal role in CBM, enabling the survival and proliferation of *F. pedrosoi* inside macrophages. In particular, Rozental et al. demonstrated a fungistatic role of activated macrophages in delaying germ tube and hypha formation (215). Macrophage fungicidal activity is dependent on the etiological agent of CBM (216). Higher digestive activity was observed in cases of *F. pedrosoi*, *C. carrionii*, and *R. aquaspersa* infections than in infections by other microorganisms (216). Phagocytosis mediated by complement was more significant in *R. aquaspersa* and *P. verrucosa* than in *F. pedrosoi* infection and was suppressed by mannan; killing was significant only in *R. aquaspersa*. These findings indicate fungicidal activity of resident macrophages against *R. aquaspersa* but little or no activity against *C. carrionii*, *P. verrucosa*, and *F. pedrosoi*.

Bocca et al. showed impaired macrophage function upon *F. pedrosoi* infection (217), with inhibition of NO production, even after culture with IFN-γ and LPS. In addition, decreases in the levels of CD80 (B7-1) and major histocompatibility complex class II (MHC-II) have been shown. da Silva et al. reported that phagocytosis of CBM fungi was cell type dependent (171). Langerhans cells (LCs) isolated from the skin of BALB/c mice were phagocytic only against conidia and not against muriform cells of *F. pedrosoi*. In addition, maturation of LCs, evaluated by the amounts of CD40 and CD86, was blocked only by conidia, demonstrating a significant function of the innate immunity of LCs in infection by *F. pedrosoi*. Judging from these results, muriform cells induce disease exacerbation with a Th1 response, and conidia divert this to a Th2 antibody response (Fig. 4).

The cytokine profile depends on the severity of CBM. Mazo Fávero Gimenes et al. demonstrated that patients with more severe clinical forms produced predominantly interleukin-10 (IL-10) with inhibition of IFN-γ, resulting in low-level induction of T-cell proliferation (218), while patients with mild CBM presented increased IFN-γ and decreased IL-10 production with effective T-cell proliferation (199). Mild forms of CBM favor a Th1 profile due to a good immunological response that may inhibit disease development, while, in contrast, moderate forms of CBM trigger an intermediate response between Th1 and Th2. Furthermore, CBM patients secrete large amounts of tumor necrosis factor alpha (TNF-α). Furthermore, *F. pedrosoi* and *R. aquaspersa* induce macrophage IL-1 secretion, whereas *C. carrionii* triggers IL-6 production, suggesting that IL production is fungal species specific (218).

Sousa et al. showed that in severe CBM, patients presented more IL-10 and less HLA-DR and costimulatory molecules than did patients with mild CBM (219). Immune therapy with anti-IL-10 or with recombinant IL-12 upregulated HLA-DR and costimulatory molecules, thus reestablishing an antigen-specific Th1 cellular response. These results also demonstrate different profiles of monocytes from CBM patients with distinct clinical forms (219). The impact of chemotherapy for CBM on the cellular

immune response was analyzed by Gimenes et al., who evaluated the production of IL-10, TNF-α, and IFN-γ as well as the proliferation of peripheral blood mononuclear cells (PBMCs) from patients with CBM at different time points of therapy (220). In this study, after treatment for 6 months, cells from CBM patients proliferated after contact with fungal antigens, producing high IFN-γ levels. After 1 year of treatment, T-cell proliferation and IFN-γ secretion were diminished, followed by IL-10 augmentation. da Silva et al. showed that CBM patients presented large serum amounts of transforming growth factor β (TGF-β), which were decreased after therapy with itraconazole (ITZ) for 3 months, correlating these findings with fast clinical enhancement (221). On the other hand, after therapy for 6 to 12 months, the amounts of TGF-β increased to the levels found before therapy, which was clinically correlated with slow enhancement in clinics and the persistence of fungal cells and fibrotic lesions (221).

The absence of fungal identification mediated by Toll-like receptors (TLRs) led to flawed cytokine production against *F. pedrosoi*, which was restored after the addition of TLR ligands *in vitro*, followed by *in vivo* protection from infection in an animal model (222). Chronicity developed after failure of costimulation of pattern recognition receptors (PRRs) (206). In cases of *Fonsecaea pedrosoi*, initial fungal recognition is mediated only by C-type lectin receptors (CLRs), leading to the flawed stimulation of proinflammatory compounds. TLR costimulation restored inflammation in response to *F. pedrosoi*, also requiring signaling by CLRs through the Syk/CARD9 pathway. Administration of exogenous TLR ligands facilitated the clearance of fungal infection *in vivo* (222).

PMNs are the main regulatory cells of the characteristic CBM granuloma (223). Abscesses rich in PMNs show abrogated engulfment of muriform fungi, whose *in situ* existence is thought to be a crucial factor in the chronicity of the inflammatory reaction (118). PMNs appear to competently eliminate *F. pedrosoi* extracellularly by using antibody-independent mechanisms, including reactive oxygen species production and the liberation of substrates (224, 225). Host receptors, such as β-glucans, may also participate in the fungicidal role of PMNs by ligation to dectin-1 (226) (Fig. 4).

DCs are significant immune regulators in several fungal infections. Mature DCs present a unique ability to prime and polarize naive lymphocytes toward a Th1 response, while immature DCs enhance a Th2 response, inducing immune tolerance. DCs are responsible for surveillance and primary protection upon invasion of melanized fungi on the skin (227). Patients with severe CBM may have T-helper-cell activation and an upregulation of HLA-DR and costimulatory molecules enhanced by DCs. The authors of that study concluded that an altered T-cell response in *F. pedrosoi* infection induces fungal disease exacerbation (227) (Table 3).

Adaptive Immune Response

Cell-mediated immunity (CMI) shows increased antigenic specificity and memory but develops more slowly than innate immunity (58, 176). Stimulation of $CD4^+$ cells by macrophages responsible for the engulfment of *F. pedrosoi* is essential to prevent CBM development (228). Impairment of cell-mediated immunity in CBM patients led to an inefficient reaction to fungally derived antigens, resulting in the maintenance of CBM-related fungi in lesional skin (229). A delayed-type hypersensitivity (DTH) reaction in *F. pedrosoi* CBM indicates that inflammation may be mediated by T cells (227). The immunophenotypic profile associated with CMI in the chronic granulomatous process leading to CBM cutaneous lesions showed predominantly macrophages but also T-helper and cytotoxic T cells, besides B cells (85, 230).

A study in nude mice inoculated with *F. pedrosoi* revealed more diffuse granulomas during infection, with a haphazard fungal distribution, corroborating the crucial function of T cells in CBM control (231). $CD4^+$ lymphocytes have a role in controlling CBM by secreting IFN-γ in order to increase cellular immunity responses against *F. pedrosoi* (218, 220, 232). Upon mouse inoculation with live conidia, increased T-helper-cell entrance into lymph nodes was observed (232). After inoculation of *F. pedrosoi* into the peritoneum of mice depleted of T helper or cytotoxic T cells, a high fungal burden was observed in the spleen and liver of $CD4^+$-depleted animals, in contrast to $CD8^+$-

TABLE 3 Innate immune responses to agents of chromoblastomycosis[a]

Component of innate host defense	Specimen type or method	Aim of the study	Result(s)	Reference
Antigen-presenting cells	Skin specimens from patients with CBM	Role of MPs and DCs in CBM	Accumulation of fungal antigens in cytoplasm of skin MPs and dermal FXIIIa$^+$ DCs	214
	In vitro interaction of *F. pedrosoi* with *in vivo*-activated MPs	Role of MPs in CBM	Fungistatic (not fungicidal) role of MPs in *F. pedrosoi* delaying formation of germ tube and hyphae	215
	Phagocytic index, cytokine and NO production by MPs	MP fungicidal activity is fungal species dependent	Higher phagocytic index for *F. pedrosoi*, *C. carrionii*, and *R. aquaspersa*; complement-mediated phagocytosis is more important for *P. verrucosa* and *R. aquaspersa*	216
	In vitro assays and *in vivo* model of CBM	Impaired MP function during *F. pedrosoi* infection	Impaired NO production of MPs and downregulation of MHC-II and CD80 expression	217
	Conidia of *F. pedrosoi* and mouse peritoneal MPs	Role of MPs in CBM	Ingestion of conidia by a typical phagocytic process, with formation of phagosomes	224
	Specimens from patients with severe and mild forms of CBM	Role of DCs in severe forms of CBM	DCs induced CD4$^+$ T-cell activation *in vitro*, expression of HLA-DR and costimulatory molecules CD86, TNF-α, IL-10, and IL-12; inappropriate T-cell response in *F. pedrosoi* infection	319
	F. pedrosoi conidia or muriform cells with LCs from BALB/c mice	Cell type-dependent phagocytosis of CBM fungi	LC phagocytosis in conidia but not in muriform cells; inhibition of LC maturation by conidia but not by muriform cells	171
Cytokine production	Specimens from patients with severe and mild forms of CBM	Cytokine profile was dependent on fungal species and infection severity	IL-1 production in *F. pedrosoi* and *R. aquaspersa* infections, while IL-6 production in *C. carrionii* infection; severe form of CBM showed increased IL-10 levels, decreased IFN-γ levels, and inefficient T-cell proliferation; mild form of CBM showed decreased IL-10 levels, increased IFN-γ levels, and efficient T-cell proliferation	219
	Specimens from patients with severe and mild forms of CBM	Cytokine profile was dependent on infection severity	Severe form of CBM showed increased IL-10 and decreased HLA-DR and costimulatory molecule expression	219
	Specimens from patients with CBM	Impact of therapy at different time points on cytokine profile and PBMC proliferation	After 6 mo of treatment, increased IFN-γ levels, while after 1 yr, decreased proliferation of T cells and IFN-γ levels and increased IL-10 levels	220
	Specimens from patients with CBM	Impact of itraconazole on cytokine profile	After 3 mo of itraconazole, decreased plasma levels of TGF-β (clinical improvement); after 6–12 mo, reincreased TGF-β levels (fibrotic scars or slow clinical improvement)	221
	In vitro and *in vivo* CBM experimental model	Defective production of proinflammatory cytokines due to a lack of specific PRR costimulation in *F. pedrosoi infection*	Due to a lack of fungus recognition by TLRs, recognition was done primarily by CLRs; exogenous administration of TLR ligands helped clear *F. pedrosoi* infection *in vivo*	319
PMNs	*In vitro* interaction of *F. pedrosoi* with PMNs	Role of PMNs in CBM	PMNs associated with killed extracellular fungi with induction of oxidative burst	178

[a]MPs, macrophages; DCs, dendritic cells; CBM, chromoblastomycosis; TLRs, Toll-like receptors; PMNs: polymorphonuclear leukocytes; CLRs, C-type lectin receptors.

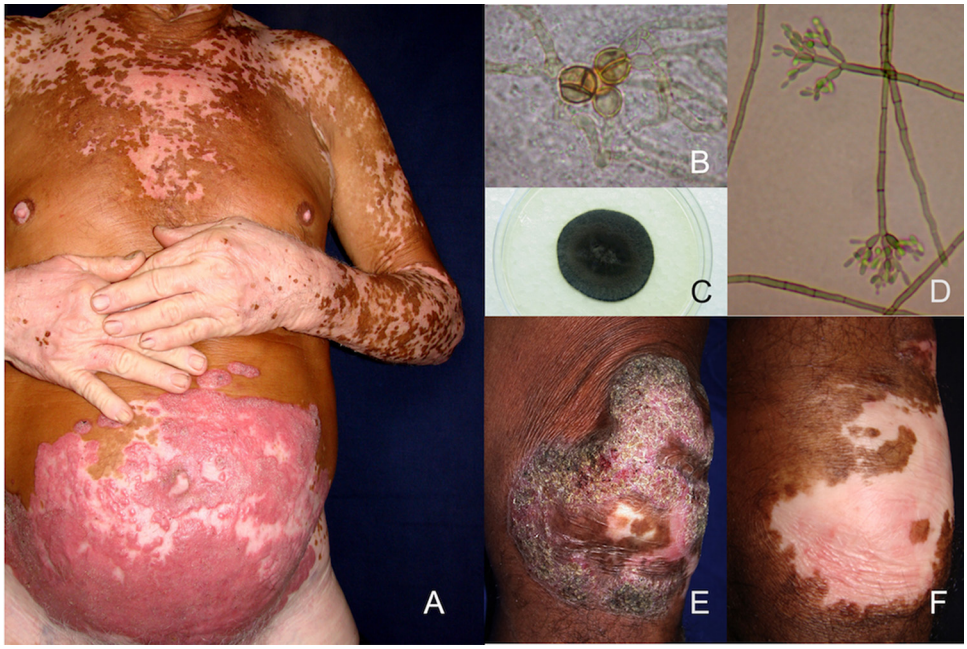

FIG 5 Vitiligo and chromoblastomycosis. (A) A 62-year-old Brazilian man presented with a 10-year history of a slow-growing plaque lesion, which started as a small nodule near his navel and spread centrifugally until it reached 30 cm in diameter. Concomitantly, he noted the presence of interwoven achromic patches, which extended to other regions of his skin after 5 years of having the disease restricted to the abdominal area. (B) A skin scraping from the plaque lesion revealed the presence of muriform cells. (C and D) After 3 weeks of culture in Mycosel, a grayish colony grew (C), and micromorphology depicted *Fonsecaea pedrosoi* structures (D). (E and F) An association of CBM and vitiligo has not been reported, but the presence of antimelanin antibodies in CBM patients is known. Most of the cases that were treated with itraconazole were healed, leaving an achromic patch (F) in place of the previous verrucous/plaque lesions (E). This patient developed vitiligo after CBM, which could be related to the presence of antimelanin antibodies.

depleted mice, which were not affected. Furthermore, $CD4^+$-depleted mice had decreased DTH and produced smaller amounts of IFN-γ than wild-type animals (232).

The Th17 function in CBM is not well defined. Interleukin-17 and IL-22 seem to play a critical role in mucocutaneous host defense (233). *CARD9* mutations in four patients with PHM presented significantly decreased cytokine production and fewer Th17 cells, with a diminished immune response to *P. verrucosa* (69, 234) (Fig. 4).

The humoral immune response is less frequently investigated for CBM infection, since previous studies have shown that this arm of the host defense may not be effective against melanized fungus infections (58). Esterre et al. demonstrated a decrease in antibody titers after antifungal therapy in CBM patients (235). For the severe form of CBM, increased production of IgG has been observed. A specific humoral immune response may develop in individuals who live in regions of endemicity and who were previously exposed to the fungus, but this does not clearly correlate with the severity of CBM (86). This corresponds to data from a previous study in which levels of antibodies against neutrophils correlated with CBM presenting chronic and widespread lesions (236). More than 1 year after finishing CBM treatment, patients may present positive serology.

Some antibodies may be protective against CBM. Antibodies against *F. pedrosoi* melanin enhance the efficacy of phagocytes in inhibiting fungal growth (237). Some patients locally become achromic during the evolution of the disease or upon treatment, indicating that antimelanin antibodies have cross-reacted with human melanocytes, a phenomenon known as vitiligo (Fig. 5).

Coupling of an antiglucosylceramide (anti-GlcCer) MAb on *F. pedrosoi* conidia diminished the fungal burden, enhancing the engulfment and destruction of *F. pedrosoi* by murine cells. Among other factors, immunoglobulin levels may interfere with the host response against melanized fungus (58) (Table 4).

TABLE 4 Adaptive immune response to chromoblastomycosis agents

Method or specimen type(s)	Aim of the study	Result(s)	Reference(s)
Murine infection with *F. pedrosoi*	Role of cell-mediated immunity in *F. pedrosoi* infection	Activation of T helper cells by MPs involved in fungal phagocytosis	228
Specimens from 8 patients with CBM	Role of cell-mediated immunity in chronic forms of CBM	Impaired cell-mediated immunity; inefficient response to fungal antigens, leading to fungal persistence (chronicity)	229
Delayed-type skin tests in guinea pigs infected with *F. pedrosoi*	Role of cell-mediated immunity in CBM infection	Delayed-type hypersensitivity reaction indicating a T-cell-mediated response	227
Skin biopsy specimens from CBM patients	Role of cell-mediated immunity in CBM infection	Inflammatory response to CBM showed distinct subtypes ($CD4^+$ and $CD8^+$) of T cells as well as B cells; skin verrucous plaques showed Th2 responses, while erythematous atrophic plaques showed Th1 responses	230
Athymic mice infected with *F. pedrosoi*	Role of T-cell-mediated immunity in CBM infection	Lack of T cells in CBM leads to transformation of granulomas in a more diffuse and confluent context with random fungal distribution	231
Immunization of $CD4^+$- or $CD8^+$-deficient mice with *F. pedrosoi* conidia	Role of T-cell-mediated immunity in CBM infection	Mice lacking $CD4^+$ presented diminished delayed-type hypersensitivity and produced lower IFN-γ levels (more severe disease); mice lacking $CD8^+$ cells presented unaltered levels of IFN-γ	232
Specimens from 4 patients with *P. verrucosa* infection	Role of CARD9 mutations in *P. verrucosa infection*	Decreased cytokine and Th17 cell production	69, 234
Specimens from patients with CBM infection	Role of therapy with terbinafine in antibody production during CBM	Decreased antibody titers in patients with CBM during chemotherapy	235
Specimens from patients with CBM infection	Antineutrophilic antibody levels in CBM infection	Antineutrophilic antibody levels correlated proportionally with chronicity and extent of lesions	236
Serum samples from *F. pedrosoi*-infected human patients	Role of melanin in antifungal antibody production	Melanin induces production of antifungal antibodies and induces efficacy of phagocytes, leading to fungal growth inhibition	202, 203
Serum samples from CBM-infected human patients	Role of melanin in antifungal antibody production	Interactions between fungi and antimelanin antibodies inhibited fungal growth	237
Glucosylceramide purified from mycelia of *F. pedrosoi*	Role of a MAb to glucosylceramide in CBM	Treatment of conidia with MAb against glucosylceramide resulted in fungal growth reduction; MAb enhanced phagocytosis and killing of *F. pedrosoi*	192

CLINICAL MANIFESTATIONS

Initial Cutaneous Lesions

After several kinds of micro- or macrotraumatic wounds, infection initiates after infectious propagules of the etiological agents gain entrance through the cutaneous barrier, usually in exposed and nonprotected areas of the body (3, 5, 12, 13). Feet, knees, lower legs, and hands are the most common sites, but infections of other regions, such as the trunk, nose, ears, eyelids, shoulders, and buttocks, have also been reported (8, 11–13, 85, 86, 125, 238). The period between inoculation and the onset of the initial lesion is uncertain and may range from weeks to months; some patients do not recall any inoculation.

The initial lesion may begin as an erythematous macular skin lesion and progresses to a pink and smooth papular lesion. With time, it may manifest as a papulosquamous lesion and evolve with polymorphic aspects, which may be confused with several infectious and noninfectious diseases (Fig. 6A). The initial lesion may spread locally and produce satellite lesions. At this point, pruritus is the main clinical manifestation. At this early stage, the patient mostly does not seek medical help; this stage is rarely seen by the clinician, and if not diagnosed, the initial lesions may progress while assuming several types of clinical forms with different grades of severity (9, 239, 240) (Fig. 6 and 7).

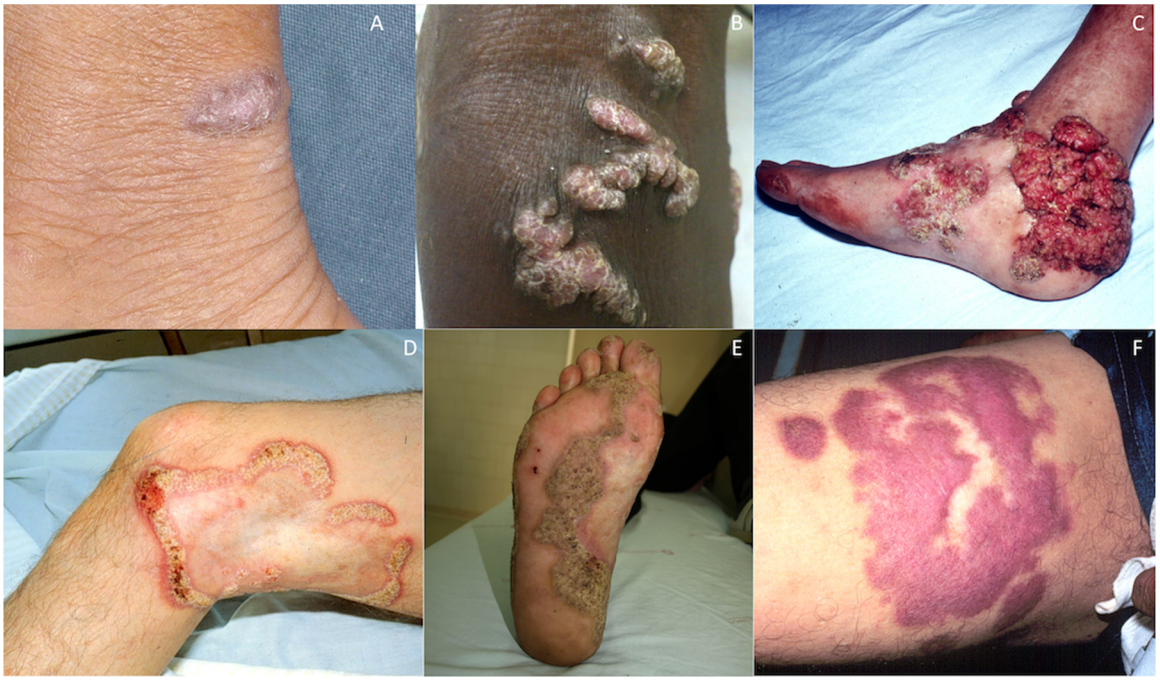

FIG 6 Clinical types of lesions observed in patients with chromoblastomycosis. (A) Initial lesion with a 3-month duration in the lower leg. (B) Confluent nodular lesions on the knee. (C) Tumoral (cauliflower-like) lesion on the posterior part of the foot. (D) Cicatricial lesion with verruca showing serpiginous and verrucous contours. (E) Hyperkeratotic verrucous lesion on the sole of the foot. (F) Soft violaceous plaque lesion in the root of the thigh.

Clinical Classification and Severity

Similarly to leprosy, leishmaniasis, and other parasitic diseases, CBM and PHM represent two poles of a wide spectrum of diseases caused by distinct species of melanized fungi (118). Clinically, the boundaries of the spectrum are imprecise. Both

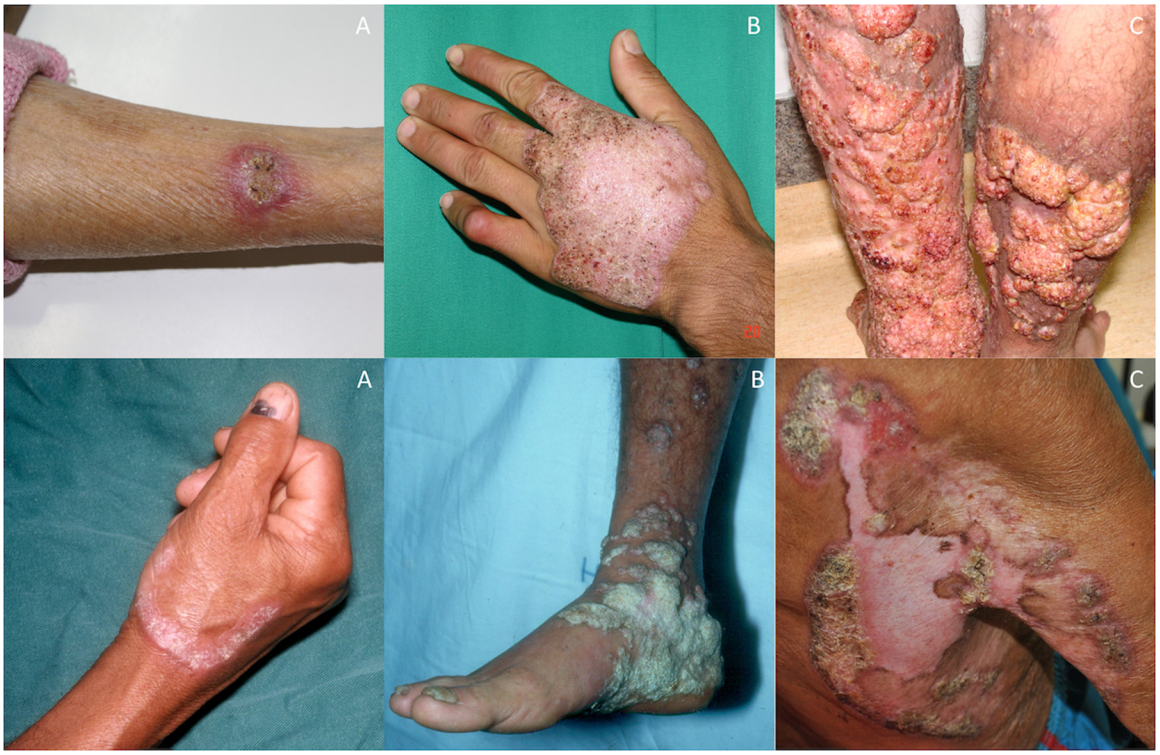

FIG 7 Lesions of chromoblastomycosis with different severity grades. (A) Mild forms; (B) moderate forms; (C) severe forms.

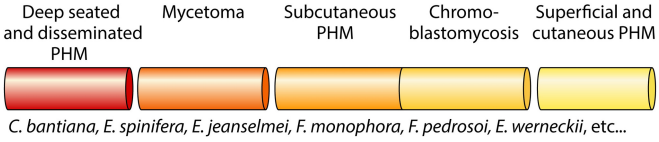

FIG 8 Spectrum of fungal diseases caused by melanized fungi. PHM, phaeohyphomycosis.

diseases may depict plaque and nodular lesions types, and some etiological agents may cause both types of infections. Both infections can be found in either immunocompetent or immunosuppressed hosts, but CBM is prevalent in immunocompetent patients, while PHM is mostly not (Fig. 8). Earlier literature reports referred to an involvement of deep organic sites such as brain and lungs as CBM, but such cases should be considered PHM (241–243). CBM affects mainly the skin and the subcutaneous tissue; dissemination to deeper organs is extremely rare (39, 244).

Several species of the *Herpotrichiellaceae* are related to the etiology of CBM, but no link with a specific type of lesion or mild to severe grades of this disease has been associated with any etiological agent.

Dissemination is hypothesized to occur slowly by continuous spread, leading to new satellite lesions, while noncontiguous or remote-site lesions may result from autoinoculation due to itching. Lymphatic spread to somewhat remote sites has been described in a few cases (245, 246). After months or even years, if not surgically removed, the initial CBM lesion evolves, assuming polymorphic clinical aspects.

To describe the wide clinical spectrum of CBM lesions, several classifications were proposed (247–249). Among these, the classification introduced by Arturo Carrión in 1950 is still valid and very helpful for clinicians facing this mycosis because it is based upon dermatological definitions of cutaneous lesions (249). Over time, this classification has been used by several authors for different types of lesions: nodular, tumoral (cauliflower-like), verrucous, scarring, and plaque (3, 239, 249) (Table 5). Recently, Lu et al. (87) referred to pseudovacuolar and eczematous types in patients with a short time of evolution and showing mild to moderate severities of disease and a favorable response to therapy. In more severe and advanced cases, patients may present a combination of lesion types, one of which may predominate (Table 5 and Fig. 6). In view of patient management and a favorable disease prognosis, CBM lesions must be classified according to the predominant clinical type and severity grade (3) (Table 5 and Fig. 7). The different grades of severity are related mainly to the time of evolution, the site involved, the patient's hygienic habits, the patient's compliance with antifungal therapy, and impaired innate host defense mechanisms, including primary immunodeficiencies such as CARD9 mutations (69, 234).

Complications and Sequelae

Unlike other implantation mycoses such as sporotrichosis and mycetoma, CBM is limited to subcutaneous tissues, and it does not affect fascia, tendons, muscles, and osteoarticular sites (3, 108). However, CBM progresses slowly and by contiguity produces fibrotic changes and lymphatic stasis, leading to lymphedema, which in some cases resembles elephantiasis. Secondary recurrent bacterial infection is another frequently observed complication of CBM. This process exacerbates the commitment of lymphatic vessels (12–15, 246).

Initial lesions and mild forms are oligosymptomatic, and usually, they do not lead to medical consultation and do not interfere with the patient's daily activities. With time, pruritus is the predominant complaint, which may be intense and accompanied by local pain in patients depicting moderate clinical forms. If not treated, with time, the severity increases, and CBM lesions are associated with complications such as edema and secondary bacterial infections, leading to a limitation of or an inability to work (13). During therapy, CBM lesions may present an intense fibrotic reaction resulting in scarring. Facial lesions may produce eyelid retraction, leading to several grades of ectropion, xerophthalmia, and keratitis. Associations of CBM with several infectious

TABLE 5 Clinical classification, severity gradation, and criteria for interruption of therapy in patients with chromoblastomycosis[a]

Type of lesion	Description of lesion	Severity of disease
Nodular	Moderately elevated, fairly soft, dull to pink violaceous growth; surface is smooth, verrucous, or scaly; over time, lesions may gradually become tumorous[b]	
Verrucous	Hyperkeratosis is the outstanding feature; warty dry lesions; frequently encountered along the border of the foot[c]	Mild, with a solitary plaque or nodule <5 cm in diam[d]
Tumorous	Tumor-like masses, prominent, papillomatous, sometimes lobulated; "cauliflower like"; surface is partly or entirely covered with epidermal debris and crusts; more exuberant on lower extremities[e]	Moderate, with solitary or multiple lesions as nodular, verrucous, or plaque types existing alone or in combination, covering 1 or 2 adjacent cutaneous regions and measuring <15 cm in diam[f]
Cicatricial	Nonelevated lesions that enlarge by peripheral extension with atrophic scarring, while healing takes place at the center; may expand centrifugally, usually with an annular, arciform, or serpiginous outline; tend to cover extensive areas of the body[g]	Severe, with any type of lesion alone or in combination covering extensive cutaneous regions whether adjacent or nonadjacent[h]
Plaque	Least common type; slightly elevated with areas of infiltration of various sizes and shapes; reddish to violaceous, presenting a scaly surface, sometimes showing marked lines of cleavage; generally found on the higher portions of the limbs, shoulders, and buttocks[i]	
Mixed form[j]	Association of the seven basic types of lesions; usually observed in patients showing severe and advanced stages of the disease[d]	

[a]Data from references 9, 13, 67, 121, 239, 248, and 265.
[b]See Fig. 6B.
[c]See Fig. 6E.
[d]See Fig. 7C.
[e]See Fig. 6C.
[f]See Fig. 7B.
[g]See Fig. 6D.
[h]See Fig. 7D.
[i]See Fig. 6F.
[j]See Fig. 1E.

diseases have been reported, including osteomyelitis, paracoccidioidomycosis, leishmaniasis, and leprosy (126, 250–254). These coinfections may increase the progression of both diseases, resulting in prolonged antifungal therapy and increased toxicity related to the respective therapies.

In advanced cases, chronic lymphedema, ankylosis, and malignant transformation are observed (13, 15, 246, 255–259). The latter is the most aggressive and disabling CBM-associated complication, leading mostly to squamous cell carcinoma (255–259). Recently, Azevedo et al. reported seven cases from Brazil and reviewed another 10 reported cases (259). The mean duration of CBM was 23 years, ranging from 5 to 36 years. Neoplastic transformation occurred independently of the applied antifungal therapy, and most patients underwent curative amputation. According to those authors, it is difficult to establish if the association of CBM, chronic inflammation, and bacterial infection may play a role as a carcinogenic or cocarcinogenic factor (259). Figures 9 and 10 depict the clinical aspects of the most frequent complications and sequelae related to CBM.

Differential Diagnosis

CBM lesions are chronic, indolent, and clinically polymorphic. They can mimic a wide spectrum of diseases with infectious and noninfectious causes. It is important to differentiate CBM from other endemic diseases occurring in several geographic areas, especially in patients presenting with prolonged cutaneous and or subcutaneous lesions. Diagnosis must be confirmed by histopathology and mycological examination (Table 6 and Fig. 11).

LABORATORY DIAGNOSIS

Mycology

The diagnosis of CBM requires laboratory confirmation by direct mycological examination and/or histopathology. The visualization of muriform cells in clinical specimens

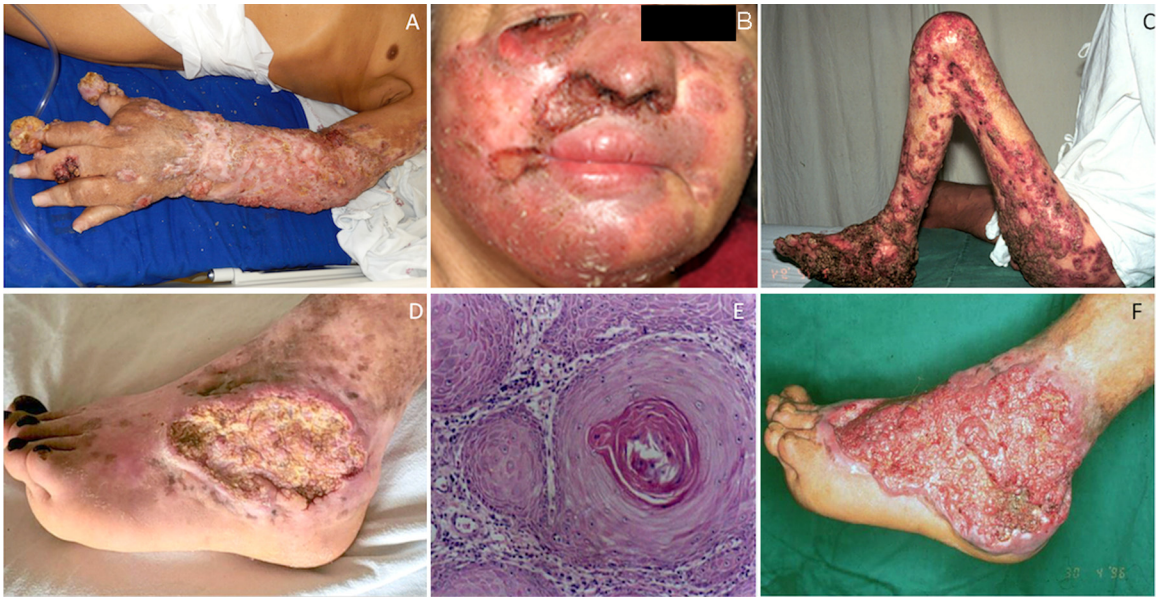

FIG 9 Complications and sequelae related to severe forms of chromoblastomycosis. (A) Chronic lymphedema hyperkeratotic lesions in the upper limb. (B) Ectropium, secondary bacterial infection, and facial lymphedema. (C) Ankylosis of the knee. (D) Neoplastic transformation of a foot lesion. Shown is an ulcerative lesion with chronic bacterial infection. (E) Skin biopsy specimen taken 80 months later showing a well-differentiated epidermoid carcinoma with typical nuclear atypias and "corn pearls" in the middle of neoplastic cell blocks. Shown is an HE-stained section at a ×400 magnification. (F) Vegetant and papillomatous lesions resulting from the association of chromoblastomycosis with neoplastic lesions.

is compulsory for confirmation of the diagnosis of this disease. Pigmented fungal elements may easily be found superficially at the lesion, and they look like small black dots (cayenne pepper appearance). These structures observed by the naked eye represent small hematic crusts, cellular debris, and fungal structures resulting from

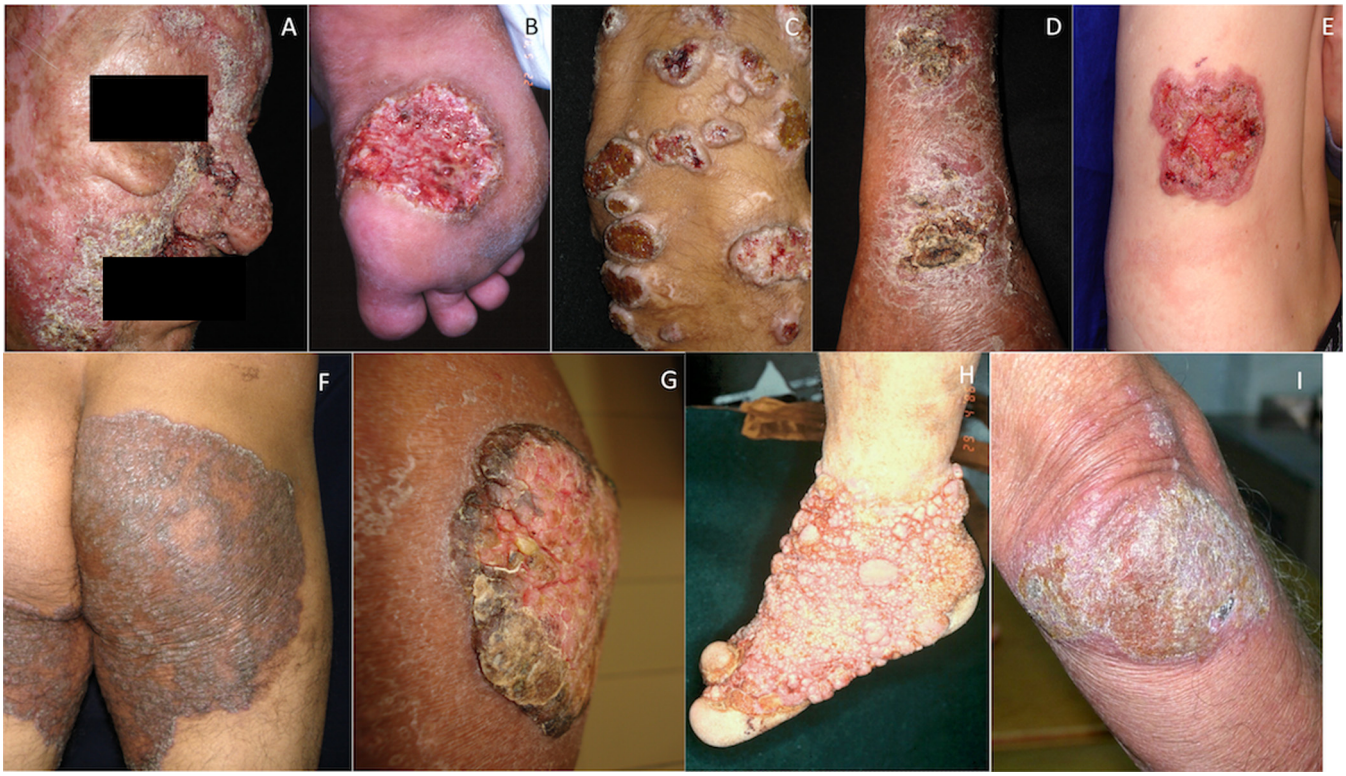

FIG 10 Differential diagnosis of chromoblastomycosis. (A) Coccidioidomycosis; (B) paracoccidioidomycosis; (C) phaeohyphomycosis; (D) sporotrichosis; (E) deep dermatophytosis associated with CARD9 homozygous mutation; (F) verrucous tuberculosis; (G) Bowen disease; (H) mossy foot; (I) mycosis fungoid.

TABLE 6 Main differential diagnoses of chromoblastomycosis

Type of disease, agent	Associated disease(s)
Infectious	
Fungi	Systemic mycoses, including coccidioidomycosis, blastomycosis, and paracoccidioidomycosis; implantation mycoses, including fixed sporotrichosis, eumycetoma, phaeohyphomycosis, and lacaziosis; and cutaneous mycoses, including granulomatous dermatophytosis, majocchi granuloma, and granulomatous candidiasis
Bacteria	Ecthyma, cutaneous tuberculosis, leprosy, actinomycetoma, nocardiosis, botryomycosis, tertiary syphilis, yaws, mycobacteriosis (e.g., *Mycobacterium marinum* and *M. fortuitum*)
Protozoa	Cutaneous leishmaniasis, rhinosporidiosis
Viruses	Verrucae, papillomas
Helminths	Filariosis
Noninfectious	Squamous cell carcinoma, mycosis fungoides, Bowen disease, psoriasis, sarcoidosis, systemic lupus erythematosus, mossy foot, and others

transepithelial elimination (Fig. 11A). Skin scrapings containing crusts, cellular debris, and tissue fragments are clarified by using a 10 to 40% potassium hydroxide (KOH) solution. Single or clustered muriform cells are depicted as round to polyhedral (chestnut-like) cells with a diameter of 5 to 12 μm. They are typically dark pigmented, thick walled, and crossed by both transverse and longitudinal septa resembling a brown brick wall (Fig. 11B). The calcofluor staining method may be helpful for diagnosis if fungal cells are scarce.

Muriform cells near the surface may germinate with filaments (Fig. 11C). The sensitivity of direct examination ranges from 90 to 100%. This method is fast, easy, and inexpensive. Therapy may be started upon the demonstration of muriform cells;

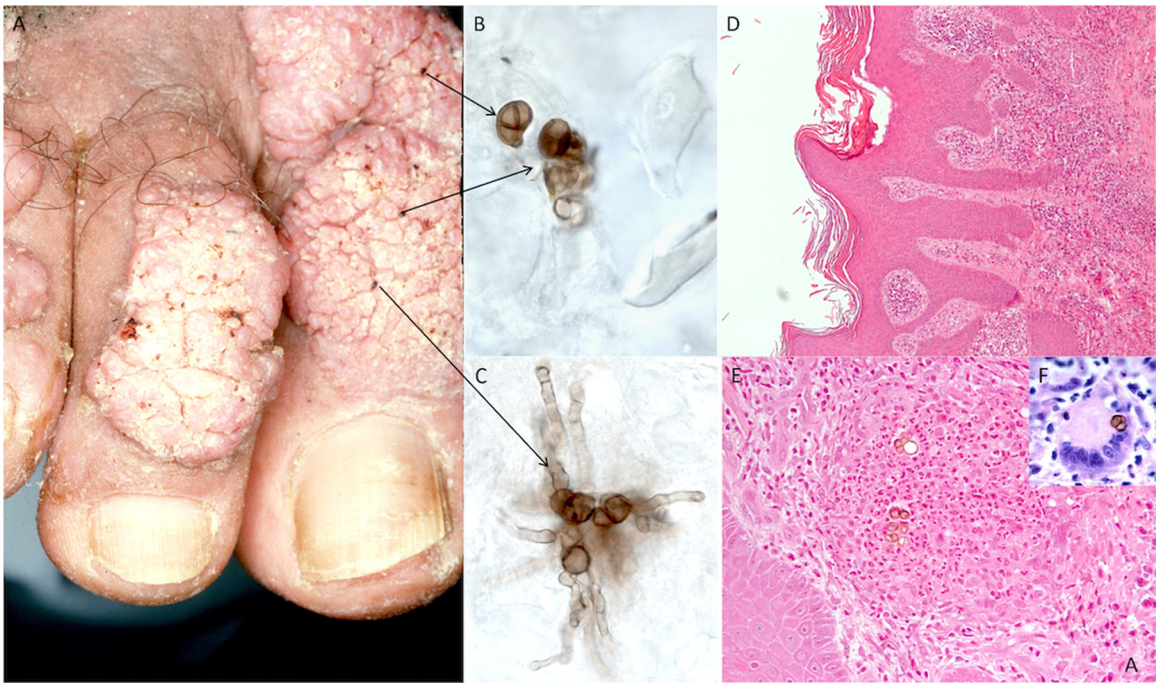

FIG 11 Laboratory diagnosis of chromoblastomycosis. (A) Skin scrapes and biopsy specimens should be taken from the "black dot" area (arrows). (B and C) Direct examination shows muriform cells (B), which may germinate and form filaments near the cutaneous surface (C). (D to F) In HE-stained sections, tissue reaction with hyperkeratosis, pseudoepitheliomatous hyperplasia (D), may be observed, associated with neutrophilic microabscesses containing muriform cells (E), which may also be found in Langerhans cells (F).

however, culture identification is important because *Fonsecaea* species may be less sensitive to antifungals than *C. carrionii* (260, 261). In addition, identification may contribute to data on the epidemiology and biodiversity of the etiological agents worldwide (262).

When grown in routine culture media, most of the causative agents of CBM tend to form slow-growing, dark-pigmented colonies. Exceptions are sporadic cases caused by *Exophiala* spp., which may show an initial black-yeast aspect. CBM agents are not inhibited by cycloheximide or chloramphenicol, enabling the use of selective media to avoid rapidly growing contaminants. The incubation time is up to 6 weeks. Initial colonies are deep green, becoming velvety and darkening with time. In contrast to black yeasts, CBM agents lack an initial yeast phase. Microscopic examination allows identification to the genus level (Fig. 2). Further identification to the species level requires molecular sequencing, for which the rDNA ITS barcoding gene is recommended. In addition, taxonomic studies may apply specific genes, such as those encoding β-tubulin, translation elongation factor 1α (35, 37, 54), and others.

The tissular response is not specific in CBM specimens, and it may be similar to the tissue reactions observed for most implantation mycoses. Histopathological examination of tissue shows muriform cells that may be inside giant cells or multinucleate giant cells of the Langerhans giant-cell type, identified by routine hematoxylin-eosin (HE) staining. Gomori-Grocott and Fontana-Masson stains are sensitive for the detection of fungal cells where fungal elements are scarce. Hyperkeratosis, pseudoepitheliomatous hyperplasia of the epidermis, pyogranulomatous reactions, and irregular acanthosis alternating with areas of atrophy are the most important histological characteristics of CBM (143, 223, 263). The dermis typically contains a dense granulomatous inflammatory infiltrate, with different grades of fibrosis, associated with mononuclear cells (histiocytes, lymphocytes, and plasma cells), epithelioid cells, giant cells, and polymorphonuclear cells (Fig. 11D to F). Two different kinds of inflammatory responses were suggested by d'Avila et al.: amorphous suppurative granulomas and true tuberculoid granulomas (230). Suppurative granulomas demonstrated pseudoepitheliomatous hyperplasia, microabscesses with large numbers of fungi, higher numbers of dermal capillary vessels, and fibrosis. True tuberculoid granulomas demonstrated atrophy of the epidermis or light acanthosis, well-formed granulomas with Langerhans giant cells, epithelioid cell lymphocytes, abscesses, and microabscesses. Fragmented fungal elements may be observed in the cytoplasm of giant multinucleated cells.

Immunodiagnosis

Similarly to other implantation mycoses, serological and intradermal tests have not been standardized for CBM and are not used in the routine laboratory. However, according to data from in-house serological studies, such tests may be helpful for seroepidemiological and diagnostic purposes. Esterre et al. developed an enzyme-linked immunosorbent assay (ELISA) technique (235). Those authors obtained positive reactions in 6.2% of samples in western Madagascar, showing the existence of asymptomatic individuals. Vidal et al. studied 60 serum samples from Brazilian patients with CBM by immunodiffusion and ELISA for IgG anti-*Fonsecaea* antibodies (237). They observed variable positivity with both techniques but a specificity of 90%. Intradermal reactions prepared with culture filtrates (chromomycin) were also employed for epidemiological surveys, suggesting the presence of delayed hypersensitivity to CBM infection in healthy individuals living in areas of endemicity (264, 265).

TREATMENT AND OUTCOME

During the past century, since the first CBM case was reported by Max Rudolph, several diverse therapeutic regimens were reported in the literature. These regimens include physical therapeutic methods as well as topical and systemic therapy with antifungal agents. With the exception of small initial lesions, which can be excised surgically, CBM lesions are refractory, and healing is almost impossible to achieve, especially in its moderate to severe clinical presentations (14, 15). Similarly to most of

the NTDs, randomized and comparative clinical trails are lacking, and the only evidence for the selection of optimal therapy is based on open clinical studies and also expert opinions. In general, patients showing severe and advanced clinical forms of disease require a long duration of continuous systemic antifungal treatment (240, 266, 267).

Treatment with Physical Methods

In most cases, the various modalities of physical methods that are available are used as adjuvant therapy in combination with antifungal agents and include surgery, thermotherapy, laser therapy, and photodynamic therapy (PDT).

Conventional Surgery

Without a doubt, surgery is the best physical method for the treatment of CBM. Excisional surgery is strongly recommended for all initial small and well-delimitated cutaneous lesions. Surgery may also be used in conjunction with ITZ or terbinafine (TBF) treatment. Scattered reports of surgical removal of larger lesions in association with skin grafting are available, but there is a risk that this might lead to dissemination of the infection. Other physical methods, such as Moh's surgery (268) and iontophoresis, are no longer used (12, 13, 240).

Cryotherapy

Cryotherapy or cryosurgery uses liquid nitrogen, the coldest cryogenic agent (−196°C) with the greatest freezing capability, to stimulate inflammatory reactions and necrosis of the affected tissue. Cryotherapy is recommended mostly for small lesions. With larger lesions (>15 cm^2), cryotherapy should be performed in sections and at different time intervals. Larger skin folds should be avoided in order to prevent secondary fibrosis and reduce the risk of sequelae such as retractile scars. This method can be applied by using a cotton-tipped applicator or spray devices (269, 270). The freezing time for CBM cryotherapy with liquid nitrogen ranges from 30 s to 4 min according to the extent of the lesion. This therapy is also recommended for patients who have previously been treated with antifungal agents that led to a reduction of the size of the lesions. The most frequent adverse events related to cryotherapy include local pain, edema, blisters, postinflammatory hypopigmentation, hypertrophic scars, and secondary bacterial infection. In order to avoid dissemination of lesions to adjacent areas after cryotherapy, antifungal drugs, e.g., ITZ or TBF, should be administered in combination with physical methods (271, 272). In general, cryotherapy is convenient, cost-effective, and efficient but requires perseverance from both the patient and the physician.

Heat Therapy

The maximum growth temperature of causative pathogens of CBM is 42 to 46°C, and therefore, the application of heat therapy, either in combination therapy or as monotherapy, yields favorable results. Heat therapy has particularly been applied in Japan (273, 274). A 1-month application of a disposable chemical pocket warmer occluded with a bandage over the lesions 24 h per day resulted in an improvement of lesions and negative microscopic examination and culture results (275, 276). Flattening of lesions can be observed within 4 days, while complete resolution is achieved within ~2 months (275). A follow-up biopsy after 3 months showed cicatrized lesions (277). However, the use of other heat sources, such as electric bed warmers, as monotherapy is insufficiently effective and should be used in combination with other physical therapeutic modalities (278). Local heat therapy for 2 h per day combined with the administration of posaconazole (PCZ) at 400 mg twice per day for an 8-month period led to a significant reduction of lesions, while the combination of heat therapy for 12 h/day and terbinafine (125 to 250 mg daily) led to negative direct examination and culture results within 2 weeks (278, 279). Additional surgical therapy was necessary in recalcitrant cases. Heat therapy is a potential therapeutic option that deserves further clinical investigation.

Laser Therapy

A CO_2 laser emits a 10,600-nm wavelength, promoting photocoagulation. Currently, it is the only laser with very high continuous-wave power (280, 281). With its high precision, minimal tissue damage, and hemostatic capacities, the carbon dioxide laser is an ideal and very useful nonselective ablative laser. Lasers have been applied both as monotherapy and in combination with other treatment modalities (280–282).

Photodynamic Therapy

PDT is a recent therapeutic modality with an effect on CBM similar to that on actinic keratosis or other types of skin cancer. PDT combines visible-light photons of an appropriate wavelength to stimulate intracellular molecules of a photosensitizer (283, 284). The activation of the photosensitizer produces several reactive molecules, including oxygen species, which leads to target cell damage. Antifungal PDT has been successfully used to treat fungal infections such as those caused by dermatophytes, *Candida* species, and *Aspergillus niger*. Lyon et al. reported PDT with a red light-emitting diode (LED) light with a low-cost methylene blue photosensitizer against CBM (283). After 6 PDT sessions, this well-tolerated procedure resulted in substantial improvement and complete remission of the lesions (285). Also, combination therapy with antifungals and PDT has been reported. Hu et al. combined oral terbinafine treatment with weekly 5-aminolevulinic acid (ALA)-PDT in a case of CBM; apparent clinical improvement was achieved within less than a year, and no recurrence was observed (286). PDT is minimally invasive, with few side effects, and may shorten the time of treatment. Adverse effects are mild, such as burning sensations, stinging, or pain. For patients experiencing intense pain and discomfort, the application of a fan, cooling sprays, or analgesics or adjustment of the irradiation time may be considered. PDT is a promising treatment option for a better quality of life for patients. The effectiveness of PDT against the most common species causing CBM has been demonstrated in an *in vitro* study (284). However, the antimicrobial effects of PDT appeared to serve no useful purpose when the light was turned off, so sequential systemic antifungal agents are necessary (284).

In Vitro Antifungal Susceptibility

The clinical outcome for infected patients is determined by the intrinsic antifungal activity of the drug used but also its pharmacological profile and the clinical presentation and severity of the infection. In this scenario, there is a clear need for standardization of *in vitro* susceptibility testing to assist the clinician in achieving optimal treatment (73). There are concerns regarding the reproducibility and clinical correlation of MIC results generated by different methods used on a worldwide scale (287–289). In general, the determination of *in vitro* susceptibility to antifungal drugs is useful to evaluate intrinsic microbiological resistance, but usually, it is not useful for the prediction of the patient's clinical response. A further problem is the use of hyphae and conidia rather than muriform cells as the inoculum. Muriform cells are believed to be highly resistant and may respond differently to exposure to antifungal drugs. Antifungal sensibility test results should always be interpreted with care.

Despite the above-described limitations, numerous studies suggest that *Fonsecaea* species are highly susceptible *in vitro* to several triazole compounds, including ITZ, voriconazole (VCZ), PCZ, and isavuconazole (ISA), but not to fluconazole (FCZ), 5-flucytosine (5-FC), and amphotericin B (AMB) (290–292). There are a few studies addressing putative differences in antifungal susceptibility between species of the same genus. Najafzadeh et al. tested *Fonsecaea pedrosoi*, *F. monophora*, and *F. nubica*, and they all exhibited low MIC values for ITZ, VCZ, PCZ, and ISA. Higher MIC values were documented for all species against D-AMB, anidulafungin (ANI), caspofungin (CAS), and FCZ, while MICs values for VCZ and ISA showed 1- to 2-fold-higher dilutions for *F. pedrosoi* than in *F. nubica* and *F. monophora*. PCZ exhibited significant *in vitro* activity against all species tested, indicating that this expanded-spectrum azole has a potential role in the treatment of CBM (289).

TBF also shows *in vitro* activity against *Fonsecaea* and other etiological agents of CBM (287). This allylamine derivative was employed to treat CBM patients in Japan and Madagascar, with good clinical response (293), but more data are needed, including comparative trials, to more precisely determine the role of this antifungal in the therapy of CBM.

Cladophialophora carrionii and prevalently environmental *Cladophialophora* species (*C. yegresii*, *C. saturnica*, and *C. immunda*) are susceptible *in vitro* to triazoles. Deng et al. revealed high susceptibility values of PCZ, ISA, VCZ, ITZ, and TBF, with slightly higher triazole MIC values for isolates from Latin America than for those from other continents (292). Antifungal combination therapy is sometimes used for severe and invasive infections. Some authors have documented a good response of the combination of ITZ plus 5-FC for refractory CBM cases. No synergism or antagonism against melanized fungi was observed when D-AMB was combined with ITZ or TBF (291). A synergistic interaction was noted for only a single *C. carrionii* isolate with the combination of TBF and ITZ.

In summary, ITZ, PCZ, VCZ, and ISA currently exhibit the best *in vitro* activity against agents of CBM, whereas D-AMB, FCZ, and the echinocandins usually have limited activity. Secondary antifungal resistance is apparently uncommon but should be suspected when patients are not responding to or are relapsing under correct regimens of antifungal therapy. Ideally, serum triazole levels should be monitored during the course of therapy.

First-Line Therapy

According to several open and noncomparative clinical trials, ITZ is the standard therapy for CBM, and it is also the most commonly used antifungal drug. ITZ cure rates range from 15 to 80% (12, 13, 15, 266, 294). The compound is an antimold triazole with a favorable safety profile (295, 296). As for other triazoles, ITZ inhibits the biosynthesis of cell membrane ergosterol via 14-α-sterol demethylase, a cytochrome P450 oxidase coenzyme (295). The loss of ergosterol generates defective cell membranes that lose fluidity and permeability. In contrast to ketoconazole (KTZ), ITZ does not affect human steroidogenesis, including the adrenal response to testosterone and corticotrophin during prolonged periods of continuous therapy (296). The capsule formulation of ITZ shows clinically significant activity against most CBM agents, although it is more effective against *C. carrionii* than against *F. pedrosoi* (289, 290). Doses for adults and adolescents of 200 to 400 mg/day are usually recommended. The duration of treatment varies; however, most cases show improvement within 8 to 10 months (266). Although complete clinical and mycological cure is achieved, this drug is administered mostly until criteria of cure have been observed (13, 15) or lesion reduction has occurred, and cryosurgery can subsequently be used to remove the remaining active cutaneous lesions. Clinical results with ITZ have been variable, limited mainly by gut absorption deficiencies and, consequently, low plasma levels and tissue concentrations, for which reason serum therapeutic drug monitoring is recommended. Success with ITZ was also reported for 6 to 12 months of pulse therapy consisting of sequential periods of 1 week per month of daily administration of 400 mg per day (297, 298). Unfortunately, comparative clinical trials to support this approach are lacking. Drug-drug interactions due to competitive inhibition of the cytochrome P450 3A4 enzyme system by ITZ also need to be addressed in patients receiving other medicines that are metabolized through this pathway.

Terbinafine is the second most frequently used antifungal agent for the treatment of CBM. Terbinafine has cure rates that are similar to those with ITZ (278, 293, 299). It is an orally administered allylamine derivative with fungistatic and fungicidal effects through the inhibition of squalene-epoxidase, which interferes with ergosterol biosynthesis and fungal membrane function. Unlike triazole derivatives, which are metabolized through the cytochrome P450 3A4 pathway, TBF is metabolized through the cytochrome P450 2D6 pathway. Thus, drug-drug interactions are minimal for this allylamine compound. In general, TBF shows good *in vitro* activity against most etiological agents of CBM. The recommended doses are 250 to 500 mg/day; the duration of treatment varies, until mycological cure or resolution of skin lesions is

achieved. TBF may be advantageous over ITZ in that TBF shows fewer drug-drug interactions and may exert a relevant *in vitro* antifibrotic action (118, 293, 300).

Combined Systemic Antifungal Treatment

Combination therapy with systemic antifungal drugs has been used in the salvage therapy scenario for patients with invasive refractory mycoses. A combination of antifungal agents and/or physical methods usually is the last therapeutic option for refractory or advanced clinical presentations of CBM. On the other hand, there is no strong evidence to support the superiority of the combination of two systemic antifungal agents for the treatment of CBM.

The combination of ITZ and TBF is often used in patients presenting refractory disease. In some cases of CBM where ITZ and TBF were used for a prolonged time and failed, Gupta et al. used both drugs in an alternate modality with alternate weeks of ITZ and TBF. This can rescue some cases (301). *In vitro* studies have not demonstrated a synergism or antagonism of this combination (291). This combined therapy is utilized for very special cases that have been unresponsive to previous treatments. A few patients depicting moderate to severe clinical forms were treated with the combination of ITZ and 5-FC, with excellent results. The main issue is that in most of the countries where CBM is present, 5-FC is not available. Moreover, patients need to take a large number of pills per day, resulting in more noncompliance (302–304).

Role of Other Triazoles

The development of new antifungal drugs for invasive fungal infections favored their use in the therapy of most endemic mycoses, including CBM. Among the recent expanded-spectrum triazoles, PCZ is the best potential option for treatment of all clinical presentations of CBM, including severe or refractory clinical forms (305, 306). The broad *in vitro* antifungal spectrum of PCZ includes most of the melanized fungi causing CBM and PHM (289, 290). In addition, the PCZ oral solution is characterized by better pharmacodynamic and pharmacokinetic profiles than those of the ITZ capsule formulation. The recommended dose of the oral suspension of PCZ in patients with CBM is 800 mg/day divided into two doses for long periods of therapy (304). It is expected that in the future, recently licensed PCZ formulations, that is, oral tablets and intravenous solutions, may also be evaluated in PHM and CBM patients. Special emphasis should be focused on delayed-release PCZ tablets. In neoplastic patients, this formulation seems to be minimally affected by factors such as food ingestion, increased gastric pH, impaired motility, or mucositis. Recent data revealed that delayed-release PCZ tablets may also achieve higher average plasmatic concentrations than those achieved with the oral solution and are well tolerated in healthy individuals (307, 308). The combination of PCZ and 5-FC or TBF may be a potential therapeutic armamentarium for refractory cases.

Oral VCZ was also tested in a few cases to treat refractory forms of this disease. Although good clinical results have been achieved with this drug, adverse effects such as visual disturbances and photosensitive cutaneous reactions are not uncommon (309). Isavuconazole, a newly licensed broad-spectrum triazole for the treatment of invasive aspergillosis and mucormycosis, was recently tested for efficacy and tolerability in a small number of patients with PHM and CBM who participated in an international multicentric clinical trial. Similarly to other triazoles, ISA is very effective *in vitro* against melanized fungi, and it may be another therapeutic option for patients with CBM in the future (ClinicalTrials.gov registration no. NCT00634049).

Abandoned Antifungal Agents

Several antifungal regimens have been used in the therapy of patients with CBM in the past with varying results. This group of agents has been used when conventional treatment has failed. The most important agent in this group has been cholecalciferol or vitamin D_2 at doses of 600,000 IU per week. Potassium iodide alone has minimal effects, and the use of oral thiabendazole seems to be ineffective. Treatment with

intravenous D-AMB alone or combined with 5-FC has not been used since the introduction of ITZ during the 1980s. In the past, several authors had also recommended D-AMB by intravenous, intra-arterial, or intralesional injection, with limited success; however, due to prolonged treatment, it easily produces adverse events such as arteritis, necrosis, and local pain. In addition, when the drug was discontinued, infection almost always reactivated. Other drugs used with variable results and severe side effects are ketoconazole, topical 5-fluorouracil, and topical ajoene (15).

Adjuvant Therapy

In recent years, there have been several reports of small case series evaluating the combination of antifungal drugs such as ITZ or TBF with immunoadjuvant compounds such as glucan (310) and topical imiquimod (311). Adjuvant therapy was used mostly in more severe and refractory cases, so the results are widely variable, but in general, in some cases, cure can be obtained, and in others, an important reduction of lesions has been achieved. Glucan is an injectable formulation of (1→3)-β-polyglucoside obtained from *Saccharomyces cerevisiae* in order to activate the Th2 immunophenotype, resulting in higher levels of IL-10 and lower levels of IFN-γ (312). This kind of therapy has been used with success in some cases of leishmaniasis and disseminated paracoccidioidomycosis (313). A few patients have been treated in Brazil with triweekly subcutaneous injections of glucan combined with 200 to 400 mg of ITZ, with some degree of clinical response (310).

Imiquimod is a synthetic amine similar to guanosine, an imidazoquinoline, with an immunomodulatory effect with antitumoral action, enhanced innate and acquired immunity, and powerful antiviral activity. This compound is known by its TLR7-activating property (314). Although there is only a single report of its use for the therapy of CBM, 5% imiquimod applied 4 to 5 times weekly appeared to be a potential adjunctive agent when combined with itraconazole. Side effects are rare, including itching and burning sensations. After prolonged use, a lichenoid infiltrate may develop as a reflection of increased local immunity. Siqueira et al. (315) developed an experimental DNA-hsp65 vaccine to stimulate adaptive immunity, with promising results not only for therapy but also for CBM prophylaxis in animal models. This drug has been combined with itraconazole, with an important improvement of experimental cutaneous lesions (315).

CRITERIA OF CURE

Chromoblastomycosis is a chronic mycosis that is resistant to most treatments and prone to recurrence. Patients depicting moderate to severe clinical forms of this disease still remain a true therapeutic challenge for clinicians (15, 316). According to the most recent case reports and expert opinions, ITZ, PCZ, and TBF are the best therapeutic choices for most patients with proven CBM who require the use of continuous long-duration antifungal therapy from several months to years (12, 13, 15, 266, 293, 294, 305). Consequently, caution should be exercised before complete cure is claimed.

Unlike systemic invasive mycoses such as candidiasis, aspergillosis, histoplasmosis, and cryptococcosis, where immunological and molecular biomarkers are available for patient follow-up, CBM treatment must be monitored by clinical, mycological, and histopathological criteria. In order for clinicians to better decide when to stop therapy, outpatients should be monitored at trimonthly visits. The response to treatment is evaluated by clinical, mycological, and histopathological criteria. A complete clinical response is defined as a definitive resolution of all lesions, with scarring and disappearance of pruritus and local pain. A 2-year follow-up period without recurrence is also necessary. Biopsies are performed every three or four months to assess the mycological and histopathological criteria of cure. A mycological response is achieved when there are no fungal elements upon direct microscopic examination and no culture-based recovery of organisms from tissue fragments. Moreover, stained histological sections should not reveal muriform cells and microabscesses; the granulomatous dermal

TABLE 7 Factors playing a role in therapy of patients with chromoblastomycosis

Factor	Description
Host	Wrong or delayed diagnosis
	Severity of disease, where lymphedema, excessive fibrosis, hardened tissue, and low vascularization are barriers to therapeutic response; drug bioavailability at the site of infection is low
	Secondary bacterial infection and malignant transformation
	Long-duration therapy with systemic antifungal may cause noncompliance to therapy
	Individual immune response where patients with mild forms develop a Th1 response and patients with severe forms develop a Th2 response; high levels of IFN-γ and low levels of IL-10 are important for control of CBM infection
	Innate immunodeficiency, CARD9 mutation
Etiological agent	*Fonsecaea* species infections are more difficult to treat than *C. carrionii* infections
	Treatment discontinuations may lead to fungal resistance
	Muriform cells are difficult to eradicate
Antifungal agents	No standardized *in vitro* tests for melanized fungi
	Muriform cells are not tested *in vitro* for antifungal drugs
	There is no animal model for CBM therapy
	Triazoles, mainly itraconazole, may present erratic absorption
	Therapeutic drug monitoring for itraconazole is usually unavailable
	Itraconazole may present several drug-drug interactions and toxicity
	Posaconazole is still an expensive drug and mostly unavailable in areas of endemicity
	There is no high-quality comparative clinical trial for this disease
Other	Chromoblastomycosis is an orphan and neglected disease affecting mainly low-socioeconomic groups who live in rural environments of areas of endemicity around the world
	Clinical researchers relatively neglect chromoblastomycosis
	Diseases linked to poverty likewise offer little incentive to industry to invest in developing new or better products for a market that cannot pay

infiltrate is typically replaced by chronic inflammation and dense fibrosis in the presence of epidermal atrophy (10, 13, 266).

The prognosis for patients with CBM has improved since expanded-spectrum triazoles have been available. Nevertheless, failure of antifungal therapy and relapse remain a substantial issue. When they occur, the clinician is usually tempted to attribute therapeutic failure to specific drug resistance, as observed in the scenario of invasive mycoses (317). However, acquired or natural resistance of melanized fungi to triazoles is uncommon. Thus, there are many other factors behind antifungal failure in patients with CBM (Table 7).

PREVENTION

Similarly to other fungal infections, there are no available vaccines for implantation mycoses, including CBM. As this disease is caused by several types of transcutaneous trauma, the use of protective equipment such as gloves, shoes, and adequate clothes may reduce the risk of infection by ubiquitously melanized fungi. This may be a key point for individuals with an occupational risk.

CONCLUSIONS

CBM is a neglected fungal disease that is endemic in tropical and subtropical geographical regions of low-income developing countries in Asia, Africa, and Latin America. The burden and medical impact of this implantation mycosis are certainly underestimated. Pathogenic species are polyphyletic within the *Chaetothyriales*. Among the organisms that commonly cause CBM are *Fonsecaea pedrosoi*, *F. monophora*, *Cladophialophora carrionii*, *Rhinocladiella aquaspersa*, *Phialophora* species, and *Exophiala* species. The causes of CBM vary as a function of the global geographic distri-

bution and natural reservoirs. CBM mainly involves adult males and is considered an occupational disease around the world, affecting farm laborers, gardeners, lumberjacks, vendors of farm products, and other workers exposed to contaminated soil and plant materials. Recent studies have shown that impaired fungal clearance in CBM infection is due mainly to the enhanced virulence and pathogenicity of its etiological agents. Factors that may confer increased pathogenicity in CBM include thermotolerance, muriform cells with thick cell walls, cell adhesion, hydrophobicity, and melanin. The circulating cytokine profile in patients with CBM depends on the severity of CBM, such that patients showing severe clinical forms of the disease have evidence of Th1/Th2 dysimmunoregulation with prevalent IL-10 production, low IFN-γ levels, and poor T-cell proliferation. CBM usually develops in exposed and nonprotected cutaneous surfaces of the body, particularly the lower legs, feet, and hands. CBM lesions must be classified according to the predominant clinical type and severity grade. There are five classically defined types of lesions: nodular, tumoral (cauliflower-like), verrucous, scarring, and plaque. The diagnosis of CBM requires laboratory confirmation by direct mycological examination and/or histopathology. Visualization of muriform cells in clinical samples is a cornerstone of the diagnosis of this disease. Treatment includes surgical removal of the initial lesions and antifungal therapy for more advanced clinical forms. Itraconazole is the most commonly used antifungal agent in the treatment of CMB, and posaconazole has a potential role in the treatment of this disease. Other physical therapeutic methods may be helpful and consist of thermotherapy, laser therapy, and photodynamic therapy. Prevention of infection should be directed at reducing environmental traumatic transcutaneous inoculation in susceptible patients.

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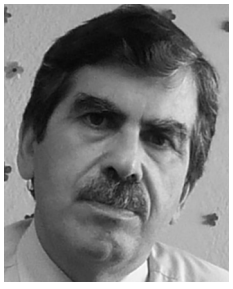

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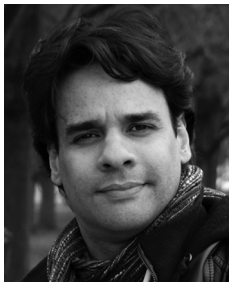

Moises Batista da Silva has an M.S. degree and a Ph.D. in Neuroscience and Cell Biology from the Federal University of Pará (UFPA), Brazil, and completed a postdoctoral fellowship at Colorado State University in Fort Collins, CO. He is currently an Adjunct Professor of microbiology at the Institute of Biological Sciences, where he teaches bacteriology to biology and medical students. His research activities involve epidemiology, immunology, and genetic characterization using standard laboratory or molecular biology techniques for the identification of a number of different tropical infectious diseases. For 15 years, he worked in the Dermato-Immunology Laboratory, a multicenter laboratory that supports the clinical diagnosis of leprosy and other fungal skin diseases in patients at the nearby specialized dermatology reference health center. He assists in the education and motivation of clinicians, nurses, biologists, and biomedical students. His main areas of expertise are in tropical disease with an emphasis on leprosy, chromoblastomycosis, and lobomycosis.

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Zoe Dorothea Pana, M.D., M.Sc., Ph.D., completed her pediatric training at Aristotle University in Thessaloniki, Greece. Her research interests have focused on invasive fungal infections since 2009. Her Ph.D. work focused on innate immunity and susceptibility to infection in immunocompromised hosts. She has completed 2 master's degrees, the first focused on Medical Epidemiology/Statistics and the second in Nanosciences. Her work in nanosciences was based on developing innovative antifungal nanomaterials (nanotubes) against *Candida* biofilms. She has participated in several European and International projects concerning neonatal sepsis, central nervous system infections, and new antifungal treatments and diagnosis options in children. She was recently awarded the Libra Fellowship and is currently a fellow at the Johns Hopkins University Hospital in the Hospital Epidemiology and Infection Control (HEIC) Department. Her current work focuses on health care epidemiology, including antimicrobial resistance and infection prevention.

Arnaldo Lopes Colombo is a Professor of Medicine at the Division of Infectious Diseases of the Federal University of São Paulo (UNIFESP), Brazil, where he was the Vice Chancellor of Research during 2009 to 2012. He is currently the Head of the Special Mycology Laboratory, UNIFESP, which is a reference laboratory in Brazil and Latin America for yeast identification, antifungal susceptibility testing, and characterization of molecular mechanisms of antifungal resistance. Dr. Colombo obtained his M.D. from UNIFESP in 1983, where he also continued his residency training in Internal Medicine and Infectious Diseases. In 1994, he completed 2-year fellowship training in Medical Mycology at the University of Texas Health Science Center in San Antonio, TX. Dr. Colombo has an active research program focused on the investigation of the burden of opportunistic fungal infections in tertiary-care hospitals in Latin America and the emergence of antifungal resistance among *Candida* and *Aspergillus* strains in this region. He is currently senior advisor of the Global Action Fund for Fungal Infection (GAFFI) and Leading International Fungal Education (LIFE).

Thomas J. Walsh, M.D., Ph.D. (hon.), F.A.A.M., F.I.D.S.A., is a Professor of Medicine, Pediatrics, and Microbiology and Immunology at Weill Cornell Medicine of Cornell University and founding Director of the Transplantation-Oncology Infectious Diseases Program and the Infectious Diseases Translational Research Laboratory. He directs a combined clinical and laboratory research program dedicated to improving the lives and care of immunocompromised children and adults. The objective of the program's translational research is to develop new strategies for diagnosis, immunopharmacology, innate host defenses, pharmacokinetics/pharmacodynamics, treatment, and prevention of life-threatening invasive mycoses and other infections in immunocompromised children and adults. The program's current targeted laboratory investigations and clinical trials in medical mycology include invasive candidiasis, pulmonary aspergillosis, mucormycosis, fusariosis, and phaeohyphomycosis. In addition to patient care and translational research, Dr. Walsh has also mentored more than 180 students, fellows, and faculty from more than 30 nations.