Eumycetoma: present status and perspective

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Abstract

The eumycetoma is a severely debilitating chronic progressive fungal cutaneous infection. Classic clinical triad is characterized by painless subcutaneous mass, sinus tracts formation and sero-purulent discharge that contain aggregates of fungal hyphae called grains. Any part of the body can have affected, with extension to muscular or bone, even visceral compromised. The eumycetoma is observed in tropical and subtropical countries; In Latin-America, is reported with less frequency. In endemic areas, antibody presence against etiological agents were higher compared with number of people affected, thus it is supposed that individual genetic susceptibility must exist. Recently, it was reported specific polymorphism in genes CR1, IL-8, NOS2 and chitinase, which were associated with development of eumycetoma. The diagnosis is suggested by clinical presentation; the histopathology and microbiology studies, plus radiologic valuation confirmed diagnosis. *Madurella mycetomatis* is the most informed etiological agent. Using phylogenetic tools new species in genus *Madurella* were reported; moreover, *Trematosphaeria grisea* and *Pseudallescheria boydii* were reclassified. Etiological agent identification is important, because differences in antifungal susceptibility exist. Eumycetoma treatment includes surgery plus antifungal drugs. Identification of etiological agents is primordial, because antifungal resistance could exist. To develop new pharmacological strategies, comprehension of grain formation physiology and drugs effects are necessary.

KEY WORDS: Eumycetoma. Madura foot. *Madurella*. *Trematosphaeria*. *Pseudallescheria*.

Introduction

Mycetoma is a subcutaneous, granulomatous-type, chronic infectious disease that is endemic in tropical and subtropical areas, which can be caused by more than 56 different microorganisms, including bacteria, then being called actinomycetoma, and fungi, then being called eumycetoma¹. In this review we will focus on eumycetoma. The characteristic clinical triad is comprised by not-too-painful volume increase, formation of fistulae and purulent or seropurulent exudate containing "grains". It can extend and compromise deep structures, which results in destruction, deformity and loss of function, sometimes even being fatal. The foot is the most commonly affected topology, accounting for 82% of cases (Fig. 1 and 2). It can occur in other localizations, such as the knees, arms and legs,

head and neck, and even the glutei and the perineum. It is more common in young, male adults, between 20 and 40 years of age. Children can also be affected in up to 30% of cases². Many patients are low socioeconomic peasants or laborers. A distinctive characteristic of mycetoma is the formation of the parasitic elements called "grains", which are constituted by microorganisms, probably as part of a defense mechanism against the host immune system³. Given that a diversity of microorganisms can produce the disease, there is great morphologic variation found in the characteristics of the grains, which are related to the causative agent.

Method

A search was conducted in indexed international journals using the PubMed database (<http://www.ncbi>).

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nlm.nih.gov/pubmed) and the word “Eumycetoma” including articles published from January 1, 2000 to June 15, 2016, also relevant articles related to specific subjects that did not appear in the search being additionally included.

Objective

To analyze recent information in such a way that enabled a full review of the subject, including epidemiological, clinical, diagnostic and therapeutic aspects, research models and insights of interests for general practitioners, dermatologists and mycologists.

Results

Fifty-six articles were found, out of which a summary of the most relevant findings is next presented, and 16 articles containing associated and notable information are additionally cited.

Historical data

The term “mycetoma” stems from Greek *mykes* (fungus) and *oma* (tumor). In India, *Atharva Veda* refers it as *pada valmikan*, which means “foot anthill”⁴; however, the first official description of mycetoma, in 1842, is attributed to Dr. John Gill, who reported the “Madura foot” in India. Subsequently, Colebrook, from the same public health center, described more cases and referred to it as a distinctive disease of the region. Between 1860 and 1874, Vandyke Carter mentioned initially the damage caused both to soft tissue and bone, and subsequently detailed the presence of fungal structures in grains obtained from the affected area. At early 20th century, Pinoy classified the cases according to the causative microorganism⁵. In Mexico, in 1963, Latapi identified the grains in human eumycetomas, originally described in cattle actinomycosis by Bollinger in the 19th century⁶.

Epidemiology

Eumycetoma has a global distribution; it is found in arid zones with short rain seasons, in countries with tropical and subtropical climate located between the Cancer and Capricorn tropics, in the zone known as the mycetoma belt, and it predominates in the west of Africa, between Senegal and Sierra Leona, with Sudan standing out as endemic area⁷. In the Asiatic continent is reported more frequently in India and Yemen⁸, while

in Latin America its frequency is lower compared with actinomycetoma and occurs mainly in Mexico, Guatemala and Venezuela⁹⁻¹¹. In Mexico, eumycetomas account for 3.48% to 7.88% and actinomycetomas for 96.52% to 92.12%^{12,13}. In a review of 3933 cases of mycetoma in the Mexican Republic, which corresponded to an incidence of 73 new cases per year, 137 eumycetomas were found, with *Madurella grisea* (n = 39) being observed to have a slightly higher frequency than *Madurella mycetomatis* (n = 36), which is the most common causative agent in the world¹³. In Europe and Israel, the migration processes of population native to endemic areas have increased the number of reported cases in the past few years^{14,15}. In endemic areas, it occurs more commonly in people of low socioeconomic stratus who walk barefoot or wearing open shoes. In these references, mycetoma predominated in the male gender at a 4:1 ratio, probably for being more exposed to working activities such as agriculture. The disease is rare in children, but in endemic countries such as Sudan, numerous cases in children have been reported². Although eumycetoma is predominant in humans, is not exclusive of them and has been reported in other species such as dogs, horses and even buffaloes¹⁶.

Clinical presentation

The described condition occurs in the skin and subcutaneous cellular tissue, but it can also compromise muscle fascia, tendons, muscles and bones. The lower limbs (Fig. 1), followed by the upper limbs, are the most affected regions; however, it has been reported in the trunk, abdominal wall, the jaw, paranasal sinuses, the orbit, the eyelids, the skull and the central nervous system, the vulva, the scrotum and surgical incisions¹⁷⁻¹⁹. The lesions are characterized by volume increase, which may be accompanied by the presence of fistulae and seropurulent exudate, with a slow and progressive evolution over months or years²⁰. In suppurative lesions, grains can be observed at plain sight. The lesions can exacerbate during pregnancy owing to the immunosuppression that accompanies gestation, as well as to the effect of estrogen on fungi, which possess hormone receptors on their membrane²¹. In the absence of diagnosis and appropriate treatment, eumycetomas produce significant morbidity secondary to disease progression, which can generate the following complications: bone destruction and deformity, lymphatic obstruction, secondary bacterial infection, pathologic fractures, nerve compression and paralysis, osteopenia and osteoporosis.

Figure 1. Clinical images. Increased volume, fistulae formation and exudate is observed in all lesions. **A** and **B**: arrows pointing at the black grains. **C**: easily visible multiple grains. **B** and **C**: longstanding lesion, where increased volume and fibrosis cause deformity.

Pathophysiology

The causative agents are found in the environment, including the soil, and many people are therefore exposed. However, most people don't develop mycetoma, since introduction of contaminated material is necessary by means of trauma; at Sudan endemic areas, acacia thorns have been associated with the development of the disease²². The inoculum amount, the host immune response and the hormone environment are factors regarded as important for the progression of the disease. Once the infectious agent penetrates, facilitated by a minor trauma, it organizes itself by forming grains inside the host. The grain consists of a dense package of fungal micelles imbibed in a material with cement properties of brown to black color⁸. The details of the chemical are not known, but it is known to contain lipids, proteins, DHN-type melanin, copper, zinc and calcium. The immune system tries to eliminate the grains by producing cytokine and enzymes, including chitinases. Both AMCase chitinase and chitotriosidase have been shown to bind to the chitin present in it. A polymorphism in the chitotriosidase gene was identified, the phenotype of which is manifested in enzyme inactivity, and it was associated with a higher risk of developing mycetoma with *M. mycetomatis*²³, which suggests susceptibility of the host. Furthermore, the efficient participation of the immune system was supported with epidemiological studies in endemic areas, where up to 80% individuals were reported to have antibodies, with the number of individuals who develop the disease being comparatively low. Evidence indicates that patients who develop the disease exhibit innate immunity failure^{24,25}. Several genes involved in neutrophil function were studied in patients infected with *M. mycetomatis*, with Hardy-Weinberg unbalance being found for single-nucleotide polymorphisms (SNP), in complement receptor 1, interleukin 8 (IL-8) and type 2 nitric oxide synthase (NOS2), which are correlated with IL-8 higher expression and NOS2

low secretion, whereas SNPs that induce IL-8 low expression and NOS2 high production were found in healthy controls²⁶. In general, cell immune response is important to eliminate fungal infections. Nasr et al.²⁷ demonstrated Th-1-type cellular immunity in patients with eumycetoma by *M. mycetomatis*, who had increased levels of Th-1-type cytokines (interferon gamma, tumor necrosis factor alpha, IL-1 β and IL-2) in comparison with healthy controls.

Diagnosis

Clinical presentation is important for diagnosis and, since it can be difficult, especially in initial lesions with few clinical data (mini-mycetoma), both mycology and imaging studies are indispensable. In longstanding lesions, grains can be commonly be observed at plain sight when smear of a fistula purulent exudate is obtained. Grains can be black in color if caused by *M. mycetomatis*, *Trematosphaeria grisea*, *Falciformispora senegalensis* (formerly *Leptosphaeria senegalensis*), *Falciformispora thompkinsii*, *Exophiala jeanselmei*, *Pyrenochaeta romeroi*, *Curvularia lunata* and *Phialophora verrucosa*, among other agents; or white if caused by *Scedosporium apiospermum*, *Aspergillus nidulans*, *Aspergillus flavus*, *Fusarium* sp., *Acremonium* sp., *Nostestudina rosatti* and *Microsporum audouinii*, among the most common⁶. Actinomycetomas can produce grains, usually of red or yellow color, but they can produce white grains as well, and microbiological is therefore essential to differentiate between eumycetoma and actinomycetoma²⁸. To verify the presence of the causative agent, direct examination should be carried out, as well as purulent exudate sampling for culture. The material obtained from the lesion is placed on a microscope slide, a drop of Lugol's iodine solution, saline or 10% potassium hydroxide is added, and the slide cover is placed. The grains' characteristics can guide the etiologic diagnosis. In case there are fistulae, the culture can be made with the obtained secretions; otherwise, the material is obtained by

Table 1. Current taxonomic classification of the most common eumycetoma-causative species

Division	Subdivision	Class	Subclase	Order	Family	Genus	Species
Ascomycota	Pezizomycotina	Sordariomycetes	Sordariomycetidae	Sordariales		<i>Madurella</i>	<i>mycetomatis</i> <i>pseudomycetomatis</i> <i>fahalii</i>
		<i>Dothideomycetes</i>	<i>Pleosporomycetidae</i>	<i>Pleosporales</i>	<i>Melanommataceae</i>	<i>Trematosphaeria</i>	<i>grisea</i>
		<i>Sordariomycetes</i>	<i>Hypocreomycetidae</i>	<i>Microascales</i>	<i>Microascaceae</i>	<i>Pseudallescheria</i> <i>Scedosporium</i>	<i>boydii</i> <i>apiospermum</i>

Information obtained from Mycobank: <http://www.mycobank.org/quicksearch.aspx>

aspiration in closed lesions (cryptic eumycetomas) or from tissues obtained by incisional biopsy. Two temperatures should be used for culture growth: 26 °C to rule out dermatophytes, which are rather uncommon eumycetoma causative agents, and 37 °C to enable isolation of most human pathogens. Cultures should be preserved for up to 6 weeks, since some agents, such as *M. mycetomatis*, grow quite slowly. Culture in media for bacteria is indicated in order to establish the differential diagnosis with actinomycetoma⁶. In spite of all cautions, identifying the causative agent genus and species is not always possible, since cultures can be contaminated with bacteria or be negative. When the culture is negative, it should be assessed by an expert, since fungal structures identification is complex^{1,2,9}.

Characteristics of the grains and cultures of the most commonly reported species in the entire world are described next.

Madurella mycetomatis

It is the most widely reported species. On direct macroscopic examination, grains are black, globular or lobulated, from 0.5 to 5 mm, and one or more can be observed. On microscope, the grains are reddish brown in color, and the hyphae that make them up appear in color brown. On culture macroscopic observation, the colony is greyish, membranous, and subsequently it can be observed of yellow-brown color with dark pigmentation at the center. It grows at 26 °C, and optimally at 30 °C. Microscopically, it exhibits 3.5 to 5 µm pyriform conidia, which originate from simple or branched conidiophore fungi. In corn flour medium, it can develop microconidia²⁹.

Madurella pseudomycetomatis*, *Madurella tropica* and *Madurella fahalii

These recently-described species should be differentiated from *M. mycetomatis*. These are dematiaceous

fungi that fail to produce conidia or ascospores and, by definition, belong to the *Madurella* genus. They produce greyish-brown color colonies; on the back, orange, brown or grey pigmentation is observed. Microscopically, pigmented, sterile septate hyphae that can produce chlamydospores are appreciated. They have optimal growth at 30 °C. There are no known teleomorph forms. *Madurella fahalii* is differentiated for not presenting diffusible pigment. These species are identified by molecular biology using the ITS sequence, the beta tubulin gene and the RNA polymerase II subunit gene³⁰.

***Trematosphaeria grisea* (formerly *Madurella grisea*) (Table 1)**

In the patient, it appears with black, globular or lobulated grains, of 0.5 to 1 mm in diameter, which can initially be soft and subsequently rock-hard. When cultured, the colonies exhibit optimal growth at 27-30 °C (maximum 40 °C), and can develop reddish-brown pigmentation (Fig. 2 a and b). Microscopically, hyaline or brown septate hyphae, of 1 to 3 µm, with thick walls, are observed. The conidia measure 2-4 µm. Conidiophores are hyaline and short. Environmental isolates can develop pycnidia (asexual fruiting bodies) after 8 weeks' incubation, which are observed as small black dots with white droplets on top of the culture. The sexual form is unknown³¹.

***Pseudallescheria boydii*/Scedosporium apiospermum complex**

Until recently, *Pseudallescheria boydii* and *Scedosporium apiospermum* were regarded as the teleomorph (asexual) and anamorph (asexual) states, respectively, of the same fungus, but currently they are reclassified as different species: *S. apiospermum*, the heterothallic teleomorph form of which is called *Pseudallescheria apiosperma*, whereas *Scedosporium boydii* homothallic teleomorph form is *Pseudallescheria*

Figure 2. Cultures, **A** and **B**: *Trematosphaeria grisea*. **B**: incipient sclerotia are observed. **C**: *Pseudallescheria boydii* complex.

Figure 3. Histologic images of a grain produced by *P. boydii* complex. **A**: H&E staining, where a multilobulated grain is observed (10x). **B**: inflammatory infiltrate and fistulae formation stand out. **C** and **D**: PAS and Gomori Grocott special stainings (respectively), where hyphae are observed inside the grain.

boydii (Table 1). For differentiation, ITS region sequences, the beta tubulin gene and calmodulin are required^{32,33}.

The eumycetoma-causative agent most commonly reported in Europe is *S. apiospermum*, especially in immunosuppressed subjects¹⁴. In infected patients, fungi of this complex produce yellowish-white, lobulated or globular grains of 1 to 2 mm in diameter. It grows quickly in Sabouraud-glucose agar at 25 °C, but it can tolerate 37 °C and even up to 42 °C. Initially white colonies turn dark grey or smoked brown color in the surface, while the back is observed with brownish grey or black areas. Microscopically, it exhibits hyaline, flexuous hyphae of 2 to 5 µm, and brown color-pigmented conidia. The teleomorph forms develop in corn flour agar or dextrose-potato agar, and show oval or globular asci containing eight ascospores. When the ascus wall breaks, it releases elliptic, dark ascospores of 4 to 8 µm in diameter³¹⁻³³.

Histopathological diagnosis

Anatomopathological study is considered to be the next step when direct examination fails⁶. Characteristically, grains are observed surrounded by a suppurative-type granuloma, located in the dermis or in

subcutaneous cellular tissue. Around the suppuration zone, there is a palisade of histiocytes; outside this palisade, a mixed inflammatory infiltrate composed of neutrophils, plasmatic cells and histiocytes can be observed, accompanied by progressive fibrosis. Special staining, such as Gomori Grocott, periodic acid-Schiff (PAS) stain and Gram staining, can help to distinguish the grain varieties. In eumycetomas, large, segmented filaments of 2 to 4 µm in diameter, with rod-shaped, bulgy hyphae and chlamydospores can be observed (Fig. 3 and 4); this image contrasts with that of actinomycetomas, where thin, gram-positive filaments of 1 µm or less in diameter are revealed. In the specific case of *M. mycetomatis*, 0.5 to 3-mm, round, oval or trilobulated grains are described, which are composed of a central zone and a more compact external layer. Two filamentous and vesicular types of grain are described. *M. mycetomatis* grains stained with hematoxylin-eosin (H&E) contain a homogeneous pink material (cement-type), while the hyphae are observed in the periphery. With the Fontana-Mason staining, the presence of melanin is demonstrated at the grain periphery and hyphae, which acquire a blackish-brown color (Fig. 5). The presence in the grain structure of heavy metals, proteins and lipids,

Figure 4. Histologic images of a grain produced by *Acremonium* sp. **A:** H&E staining, where a grain surrounded by inflammatory infiltrate and fistula formation is observed (10x). **B:** at higher augmentation, neutrophils in the inflammatory infiltrate stand out (40x). **C:** PAS staining (10x). **D:** PAS staining, where hyphae are observed inside the grain (40x).

which are elements that presumably hinder antifungal agents penetration, has also been demonstrated^{34,35}.

Radiological diagnosis

Imaging techniques are used to determine the extent of the lesions since, as previously mentioned, eumycetoma can affect deep tissues and even the bones (bone involvement should be ruled out in especially longstanding lesions). Standard radiography can be useful to define lesion limits and whether there is bone involvement³⁶. Abd El Bagi³⁷ proposed a radiological classification to define the degree of compromise according to alterations found in radiological images; it goes from stage 0, where the lesion is limited to the penetration site, with soft tissue inflammation and without bone compromise, to stage VI, which shows multidirectional dissemination with complete destruction of bone structures. However, radiological changes are only evident in advanced disease,

Figure 5. *M. mycetomatis* grains stained with Fontana-Masson staining show melanin at their periphery, which exhibits a blackish-brown color.

and adequately determining soft tissue compromise is not possible with plain radiograph; in addition, imaging differential diagnosis includes bacterial osteomyelitis, sarcoma and bone cysts, among other pathologies.

In ultrasound, the presence of hypoechoic lesions containing small hyperechoic foci is considered characteristic of mycetoma; they are associated with the

presence of grains, capsule and peripheral inflammation. In general, they are better visualized in eumycetoma than in actinomycetoma, since the latter usually has less fistulae, which hamper hyper-reflective echoes' signal³⁸.

Magnetic resonance is a non-invasive technique that facilitates soft tissue and bone destruction visualization, and it is therefore useful to assess the mycetoma extent. Grains appear as round-shaped, low-intensity, small lesions of 2 to 5 mm in diameter (which remind of a dot), in clusters surrounded by a high-intensity ring (resembling a circle) that represents the peripheral granulomatous reaction. This characteristic image is known as the "dot in a circle sign"³⁹⁻⁴², and it was subsequently recognized in ultrasound-obtained images, where it is observed as a hyperechoic area (dot) surrounded by hypoechoic tissue (circle)^{43,44}. El Shamy et al. proposed a scale to assess mycetoma seriousness using magnetic resonance findings. The degree of compromise is assessed in three planes: skin, muscle and bone. Cutaneous and subcutaneous findings include skin destruction, abscess formation and fistulae with or without presence of grains. In muscle, edema and microabscesses and macroabscesses formation can be observed. In bones, it is possible for bone marrow edema, cavitation and bone destruction to be found. Lesions are classified as mild, moderate or severe according to the sum of the scores obtained for each plane⁴⁵.

Molecular diagnosis

Culture and molecular techniques are not generally available in endemic areas; however, the treatment of eumycetoma can vary depending on the causative agent, and sometime, exact identification only can be achieved with molecular techniques. The tools used include amplification by polymerase chain reaction (PCR), followed by sequencing of the region of interest such as, for example, the region comprising the ribosomal genes and the intergenic regions (ITS)⁴⁶. PCR products can also be used for identification by other, less sensitive, but less expensive techniques, such as restriction fragment length polymorphism⁴⁷. More recent versions, such as real-time PCR, have similarly been employed to identify eumycetoma-causative species using tissue samples or isolates of the environment^{48,49}. Recently, two techniques were proposed, recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP), which are suggested as alternatives to phenotypical

methods. Both methods demonstrated high specificity and sufficient sensitivity, and are appropriate for the detection of *M. mycetomatis*; however, RPA is simpler and has higher potential to be implemented in endemic areas⁵⁰.

Other genes used for eumycetoma-causative fungal species include beta tubulin, nuclear ribosomal RNA large and small subunits (nucLSU and nucSSU, respectively), RNA polymerase second subunit in size and elongation factor alpha⁵¹.

Advances in molecular identification and phylogeny

The use of molecular diagnostic in phylogeny and taxonomy revealed errors in the classification of isolates previously identified with traditional methods. One recent phylogenetic analysis demonstrated that *M. mycetomatis* and *M. grisea* belong to different orders; currently, the *Madurella* genus is comprised by *M. mycetomatis*, *M. pseudomycetomatis*, *M. tropica* and *M. fahalii*, which belong to the *Sordiales* order, whereas *M. grisea* is member of the *Pleosporales* and went on to be denominated *Trematosphaeria grisea*^{29,30,52,53}. In addition, four CBS sterile mycelium dematiaceous strains obtained from patients with mycetoma, originally identified by traditional methods as *M. mycetomatis*, showed significant differences in the ITS region, thus being demonstrated that they belong to different species; none of these strains was native to Africa²⁸. Subsequently, inclusion of the *Madurella* genus in the *Chaetomiaceae* family was demonstrated; member of this family are commonly found in dung and manure, which drove to conclude that the manure present in endemic areas can be a niche for *Madurella* and play an essential role in eumycetoma acquisition⁵⁴.

Molecular identification is also used to infer ecological data based on the phylogenetic relationships with species for which there is more information available. Most strains obtained from human eumycetomas were found to correspond to species that are related to marine fungi, which suggests an association between virulence and halotolerance³¹.

Auxiliary methods

Immunologic methods, such as enzyme-linked immunosorbent assay (ELISA), which detect the presence of specific antibodies, are regarded as; however, they are used for epidemiological studies rather than for clinical diagnosis.

Other tools to identify the causative agent include immunohistochemistry and matrix assisted laser desorption ionization time-of-light mass-spectrometry (MALDI-TOF); however, the complexity and costs of these techniques limit their availability^{55,56}.

Differential diagnosis

Many infectious and non-infectious diseases can be mixed up with eumycetoma. The first difference should be established with actinomycetomas, which in general terms are more aggressive, produce more inflammation and fistulae, dissemination to lymphatic vessels is common, and bone compromise is early, but with small cavities. In turn, eumycetomas produce few fistulae, bone compromise is usually late and with large cavities, and lymphatic dissemination is occasional; on microscopic examination, the lesion is well defined and has capsule¹. However, clinical examination is not sufficient to establish a distinction between these pathologies, which is why the microorganism should be isolated in order to confirm the mycetoma etiology. Among infectious diseases, cutaneous tuberculosis, atypical bacteria infections with atypical bacteria, chromomycosis, sporotrichosis, cutaneous blastomycosis, leishmaniasis, hyalohyphomycosis, botryomycosis and cellulitis should be considered as differential diagnoses. Eumycetoma initial lesions can also be confused with foreign body granulomas and different neoplasms, both benign and malignant⁶.

Treatment

Eumycetomas treatment includes debridement of affected tissues plus antifungal therapy, usually for one year, which can be extended to 18-24 months. Azoles are the most widely used drugs, owing to their availability and low cost in comparison with other antifungals. Itraconazole (400 mg/day) is considered first-choice, whereas ketoconazole (400-800 mg/day) is less used due to its hepatotoxicity. However, high treatment-resistance rates are reported, with recurrence ranging from 20% to 90%⁵⁷. This might be related to identification, by traditional methods, of different species that possess different antifungal resistance profiles, such as *M. mycetomatis*. For example, *M. farhii* (CBS 129176) showed resistance to 5-flucytosine, fluconazole, itraconazole and caspofungin, and *M. tropica* (CBS 201.38) was resistant to 5-fluorocytosine and caspofungin^{29,30}. The use of broad-spectrum strong triazoles, such as posaconazole (200-800 mg/

day) and voriconazole, yields high rates of cure (80%) against *Madurella* and *S. apiospermum*, with remission being observed during follow-up 2 years after treatment conclusion, but its high cost precludes its use in endemic regions⁵⁸⁻⁶¹. According to Dupont et al., in 20% of resistance cases diclofenac (100 mg/day) can be an efficacious concomitant treatment, they report a eumycetoma case produced by a multi-drug resistant *M. mycetomatis*, where addition of non-steroidal anti-inflammatory drug (NSAID) was associated with clinical, radiological and functional improvement. As regards this clinical observation, we found that in vitro and in vivo studies demonstrated that other NSAIDs, such as ibuprofen, potentiate the azoles fungistatic activity; although the mechanism of action is not known to this moment, it is believed that it might be associated with ibuprofen inhibitor effect on ATP-dependent transport pumps, which are related to fungal resistance⁶⁴. On the other hand, studies in *Candida albicans* demonstrated that NSAIDs reduce the formation of biofilm, which favors drug access to the fungus⁶⁶. In addition, in bacterial-origin osteomyelitis, NSAIDs decrease bacterial cytotoxic effect on osteoblasts⁶⁶.

With regard to the use of other antifungals, in vitro, terbinafine alone or in combination with ketoconazole or itraconazole showed no synergistic effect or better response than azoles in the treatment of eumycetomas caused by *M. mycetomatis*, and in infections with *S. apiospermum* and *S. boydii*, terbinafine showed no benefits, and its use is therefore not indicated, at least in most eumycetomas⁶⁷. In disseminated cases or which don't respond to initial treatment, intravenous amphotericin B is used at a total dose of 2 to 4 g, alone or combined with voriconazole or micafungin⁶⁸.

In conclusion, the best treatment for eumycetoma includes antifungals plus surgery; these drugs are indicated prior and after surgical excision. During surgery, subcutaneous nodules must be eliminated intact to prevent dissemination of the grains to the adjacent tissue⁶⁹. The prolonged duration of treatment, together with the low socioeconomic stratus most patients belong to the low income group of most patients, favors therapeutic schemes non-compliance, which increases resistance, and is reflected in higher likelihood of complications and radical surgical treatments, such as amputation^{1,2}.

Experimental models

To develop better therapeutic strategies, study models mimicking the infectious agent status inside the

patient are required. Similarly to other fungal agents, eumycetoma-causative fungi produce a protective structure that surrounds the hypha. The presence of grains is key in eumycetoma, but their formation and sensitivity to antifungals is not yet fully understood. Partially, this is so because grains cannot be induced *in vitro*, with a mammal host being required to elicit their formation. In the past, mice and monkeys were used to stimulate the formation of grains simulating mycetomas by *M. mycetomatis*, with large inoculum concentrations being required and with variable rates of success and reproducibility difficulties for other authors. When the grain was obtained in animal models, it resembled the one formed in humans, characterized by mycelia embedded in a cement-like material surrounded by neutrophils⁷⁰. Given that both in humans and in animal models there are neutrophils found surrounding the grain, these cells are considered to be important in its formation. However, neutrophils cannot currently be regarded as the only effector cell involved, given that exposure of *M. mycetomatis* to neutrophils does not elicit the formation of grain, that were development *in vitro*. As an alternative to induce grain formation, the larva of the greater wax moth (*Galleria mellonella*) was used; the grains produced in this larva resemble those found in humans and mammals, and this infection model might be used in the study of grain formation and therapeutic responses to antifungal agents in *M. mycetomatis*³.

Finally, other fungi described in eumycetomas, such as *Aspergillus*, *Scedosporium* and *Fusarium* species, also recognized as opportunistic infections causative agents, were studied using *Drosophila melanogaster* as host, and the treatment with voriconazole was found to protect the flies against the infection, except in those infected with *Scedosporium prolificans*, which showed no improvement^{71,72}.

Conclusions

Although eumycetoma has a low prevalence in Europe and Latin America, knowledge about this mycosis is essential, since ignorance on the subject leads to diagnostic delay and inadequate patient management, which constitutes a health problem, especially in rural areas. On the other hand, the current process of human migration from Africa to Europe will probably increase the presence of this mycosis in the Old Continent, and it should therefore be considered among the diagnostic possibilities. Correct identification of the causative agent is imperative in order for In order

to start appropriate treatment as soon as possible. Currently, molecular biology techniques, such as PCR and sequencing, enable correct identification when traditional methods fail. Given the presence of resistant strains against first-line therapeutic agents, it is important for antifungal sensitivity tests to be carried out.

Conflicts of interests

None to be declared.

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