Diabetes and mucormycosis: A complex interplay

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Abstract

Mucormycosis is a life-threatening invasive fungal infection that arises particularly in diabetic patients with or without other underlying conditions such as haematological malignancies or the need for solid-organ transplantation. Rhino-orbito-cerebral involvement is the primary site of mucormycosis, but the paucity of signs may be a cause of delayed diagnosis. Thus, any case of documented non-bacteriological sinusitis in diabetic patients, even without ketoacidosis, should prompt suspicion of a mucormycosis diagnosis. To optimize information for clinicians in charge of diabetic patients, this extensive review of the literature was carried out to provide an overview of mucormycosis specificities, epidemiology and pathophysiology in the setting of diabetes.

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Invasive fungal infections are often found in patients with severe underlying conditions. One of the most life-threatening invasive fungal infections, mucormycosis is seen in patients who have diabetes mellitus (DM). Mucorales are the major zygomycetes involved in mucormycosis in the Western world. Other zygomycetes such as the Entomophthorales can be found in immunocompetent patients in tropical areas, but not particularly in diabetic patients. As these widespread filamentous fungi are challenging to diagnose and eradicate, a better understanding of the pathophysiology of such infections might allow better patient management. Thus, the aim of the present review was to focus on the current knowledge of mucormycosis as seen in DM patients.

1. Epidemiology

More than 220 millions people worldwide were living with DM in 2011, and the World Health Organization (WHO) projects that the number of deaths due to DM is likely to double by 2030 [1]. This increasing prevalence has an unequal worldwide distribution, with at least 80% of DM-related deaths occurring in the low- and middle-income countries [1]. In the developed countries, DM is associated with obesity, high blood pressure
and sedentariness. Emerging countries such as India share the same risk factors but, in these cases, DM prevalence is higher among the educated, urban population than among the poor, uneducated, rural people [2]. Treatments with drugs, such as statins, have contributed greatly towards the reduction of cardiovascular diseases, the main causes of death in diabetic patients [3]. Statins are also possibly the most promising drugs for mucormycosis treatment [4–8].

The rising trend of mucormycosis associated with diabetes is commonly seen in uncontrolled DM and has a fatality rate ranging from 32% to 57% [9–12]. In the largest literature review of mucormycosis from 1885 to 2004, 36% (n = 337) of the 929 proven or probable cases also had DM [9]. More than half of these (n = 187/337) had type-2 diabetes, and 34% had documented ketoacidosis. Among the latter patients, mucormycosis revealed previously unrecognized type-2 diabetes in 43% [9]. A study in France evaluated a cohort of 101 patients with mucormycosis between 2005 and 2007, among whom DM was found in 32% [12]. In comparison, recent studies carried out in central Europe and Asia have found lower incidences of diabetes-associated mucormycosis, with DM involved in only 17% (n = 7/41) of mucormycosis cases [10]. A prospective study by the European Confederation of Medical Mycology (ECMM) of proven or probable mucormycosis cases, involving 13 European countries during 2005–2007, was also consistent with these findings [11]. Indeed, DM was diagnosed in 17% (n = 39) of the 230 cases found [11]. However, diabetes-associated mucormycosis has various distributions depending on the geographical area. In India, for example, uncontrolled DM was the most commonly found predisposing factor in 74% (n = 131) of the 178 cases identified between 2000 and 2004 [13]. In France, 531 cases of mucormycosis have been declared in the national hospital database between 1997 and 2006 [14]. In that study, the incidence of diabetes-associated mucormycosis had increased by 9% per year.

Type-2 diabetes is more frequently associated with mucormycosis, whereas type-1 accounted for 20% of the diabetes cases in the largest retrospective cohort so far [9]. In comparison, only 6% of the 178 patients in the Indian cohort had type-1 diabetes [13], as did 43% of the diabetic patients in the French retrospective cohort [12]. In the paediatric population, the association between type-1 diabetes and mucormycosis is less frequent, accounting for 13% of 157 mucormycosis cases published so far [15]. Of those cases, 10% presented with ketoacidosis. In the ECMM cohort study, only one mucormycosis case, a patient aged less than 14 years had type-1 diabetes [11].

Depending on the study, DM was the single most predisposing factor in 9 to 23% of cases [9,11,12,16]. In the ECMM cohort, 18% (n = 8) of cases had another predisposing factor in combination with DM [11]. Among the solid-organ-transplant population and in patients with haematological malignancies, DM was shown to be an independent factor associated with the presence of mucormycosis [17,18]. In comparison to patients with invasive aspergillosis related to haematological malignancy, being diabetic was associated with an eightfold higher risk of mucormycosis [17]. An eightfold greater risk of mucormycosis was also noted in a matched case-control study of solid-organ-transplant patients with DM [18]. In addition, in a retrospective study over a 15-year period, an association with haematological malignancy was found in 17% of cases (n = 10/59) [19]. This rate was confirmed by a nationwide study in France, which showed that 18% of diabetes patients were among those with haematological malignancies [12]. However, the proportion was higher, ranging from 30% to 38%, in solid-organ-transplant patients [18,20].

Healthcare-associated mucormycosis may also arise in the diabetic population [16]. Indeed, DM was reported in 22% (n = 37/169) of such cases. The use of specific devices in the management of DM can, in fact, be a source of Mucorales infection. Insulin injections were implicated in two cases leading to infection with Cunninghamella bertholletiae and Rhizomucor pusillus, respectively [21,22]. In addition, the use of a subcutaneous insulin infusion pump was responsible for one case, and another was linked to the use of blood glucose self-monitoring equipment [21,23]. Other procedures, such as dental extractions [24,25], bandages [26,27], intramuscular injections [28], intravascular devices [29,30], peritoneal dialysis [31] and ophthalmic surgery [32,33] may also represent risky procedures for diabetes patients.

2. Pathophysiology

2.1. Immunity in diabetes

Innate immunity is altered in the diabetic population, as reflected by well-characterized polymorphonuclear (PMN) dysfunction [34]. Reduced PMN chemotaxis, impaired transmigration through vascular endothelium and reduced superoxide production are the three major components of PMN dysfunction [35]. Adaptive immunity is also impaired, albeit less well studied. It is known, for example, that in type-1 diabetics with poor metabolic control, inflammatory cytokine production, including interleukin (IL)-1, IL-6, tumour necrosis factor (TNF)-α and interferon (IFN)-γ, is reduced [36].

2.2. General view of Mucorales pathogenesis

Mucormycosis is rarely seen in non-immunocompromised hosts. In such cases, breach of the skin barrier (such as by surgery or trauma) is the main risk factor, leading to subsequent primary cutaneous mucormycosis. In diabetic patients and other immunocompromised situations such as solid-organ transplantation and haematological malignancy, the main portal of entry is an inhalation route rather than cutaneous inoculation or ingestion (Fig. 1).

Spores are the best way for Mucorales organisms to disseminate throughout the environment and to penetrate into the airways (nasal sinuses or lungs) or through a skin wound. As shown in a pulmonary mucormycosis murine model inoculated by an intranasal route, clearance of inhaled spores of R. pusillus in normal mice was slow, taking up to 30 days [37]. Once within the organism, Mucorales spores behave as in their natural environment: they swell, then germinate and progress to germ-tube formation with hyphal extension (Fig. 1). Hyphae are these...
organisms’ tissue-invasive form, and have a particular predilection for blood vessels, resulting in thrombosis and tissue necrosis [38]. It has also been shown that hyphae are able to damage endothelial cells in vitro in a dose- and time-dependent manner [39].

2.3. Role of phagocytic cells

Innate immunity plays a key role in mucormycosis, inhibiting spore germination and/or hyphal growth, and promoting their destruction [40,41]. As for adaptive immunity, its role is not so well clarified [42]. Different models both in vitro and in vivo have been developed to study the interaction between the diabetic host and Mucorales.

The immune response appears very early on, when spores invade tissues (Fig. 1). When Mucorales spores encounter an unimpaired immune system, phagocytic cells engulf the spores with the help of the alternative complement pathway [43]. Once inside the phagocytic cells, the spores cannot germinate, but how these phagocytic cells kill the spores has not been well understood until now [44]. In an experiment involving intradermal inoculation of Rhizopus oryzae spores in normal rabbits, leukocytic infiltration of the surrounding tissues appeared within less than 20 min [45]. At 24 h post-inoculation, the major change consisted of marked increases in macrophages and proliferating fibroblasts. In comparison, in diabetic rabbits, PMN margination was delayed, and massive fungal proliferation was associated with invasion of the deep tissues and large blood vessels at 24 h [45].

There is also evidence that hyperglycaemia and acidosis can impair the ability of phagocytes to move towards and kill spores through oxidative and non-oxidative mechanisms [46]. These findings have been confirmed in both human and murine experiments. In vitro, bronchoalveolar macrophages from diabetic mice had a reduced ability to inhibit spore germination and to attach to Rhizopus spp. hyphae [37] whereas, in contrast, contact with bronchoalveolar macrophages from non-diabetic mice was able to cause damage to the R. oryzae spores and hyphae. Serum from diabetic mice also promoted spore germination and impaired the attachment of macrophages to spores [37]. In this model, the amount of available serum iron from the healthy and diabetic mice did not differ. In another study, bronchoalveolar macrophages incubated with serum samples from diabetic rats as well as from DM patients were less able to inhibit spore germination [41].

The role of PMN is also an essential part of the anti-Mucorales defense [47]. In cases of impaired PMN function such as in a diabetic mouse model, intracerebral mucormycosis developed following R. oryzae intrasinus inoculation, and 90% of the mice died within 11 days [48]. In histopathological lesions in diabetic mice, the spores were surrounded by foci of inflammation with an influx of macrophages and PMN [37]. Once hyphae developed, interactions with PMN promoted IL-8 and TNF-α secretions, which may then be amplified by stimulation of IFN-γ and granulocyte–macrophage colony stimulating factor (GM-CSF) [49]. When the hyphae were too large to be phagocytosed by mononuclear cells, damage to hyphae was mainly due to oxidative burst activity induced by PMN [50]. However, this capacity differs from one filamentous fungus to another: for example, the PMN capacity to induce oxidative damage is reduced against R. oryzae compared with Aspergillus fumigatus [51]. The presence of oxidative burst activity and the role of phagocytosis cells were confirmed in vivo in a Drosophila model [52]. In this model, D. melanogaster S2 phagocytic cells internalized fewer spores and caused less hyphal damage to Rhizopus spp. compared with Aspergillus spp. [52]. In addition,
**2.4. Role of iron and pH**

The role of ketoacidosis was highlighted in the late 1970s in diabetes patients [53], and was studied both in vitro [46] and in vivo from the 1980s onwards through the development of the diabetic ketoacidosis murine model [54]. It is known that *R. oryzae*, the most common Mucorales found in diabetes-associated mucormycosis, is unable to grow in human serum in vitro due to the sequestration of iron by iron-binding proteins [55]. However, *R. oryzae* acquires iron in iron-limited environments thanks to high-affinity iron permease, which enhances its growth [56]. The gene supporting this permease—called “*FTR1*”—is expressed in infected ketoacidotic mice [57]. Decreasing serum pH, as seen in ketoacidotic conditions, allows fungal growth by disrupting the iron-binding capacity of transferrin (Fig. 2). Also, reducing the number of *FTR1* gene copies reduces *R. oryzae* virulence in ketoacidotic mice [57]. Thus, iron metabolism, known to be modified in diabetes, plays a central role in Mucorales virulence and particularly that of *R. oryzae*. Once in contact with endothelial cells in vitro, endocytosis of *R. oryzae* spores occurs [58]. The use of the iron chelator phenanthroline inhibits endocytosis, whereas ketoacidosis inhibits the action of the iron chelator and enhances spore endocytosis [58]. In addition, a novel host receptor —78 kDa glucose-regulated protein (GRP-78)—that mediates invasion and damage of human endothelial cells by *R. oryzae* has recently been identified [58]. Mice with ketoacidosis exhibit increased expression of GRP-78 in the sinuses, lungs and brain compared with normal mice [58]. Overall, there is a close interplay between diabetes-induced inhibition of innate immunity and changes in the iron-metabolism pathways/pH during the early phase of Mucorales invasion.

**2.5. Platelets**

Together with innate immunity, platelets also play a role in damaging Mucorales hyphae in a time-dependent manner, and reduce fungal growth by their capacity to hamper germination and hyphal development [59]. However, platelet function is altered in diabetes patients through structural and functional modifications of the platelet-membrane properties and alterations of nitric-oxide metabolism [60].

**3. Clinical features of mucormycosis in diabetic patients**

The association between DM and sinus-cavity involvement has been extensively described in the literature, but its pathogenesis has not been as well characterized [9,13,38]. Basically, the host factors and probably pathogen-related factors are intricate. There could be a strong link between DM, sinus involvement and *Rhizopus* spp. Indeed, sinus involvement is the preferential site in diabetic patients [11], and *Rhizopus* spp. more frequently lead to rhino-orbital-cerebral involvement [13].

**Of the 929 mucormycosis cases described by Roden et al. [9], sinus involvement was the most common form of infection, accounting for 39% of cases. The majority of patients with DM (222/337, 66%) in that review had sinus involvement consisting of lesions in the nose and paranasal sinuses, with frequent involvement of the maxillary sinus, orbit, cavernous sinuses and brain parenchyma [9]. Independent predictive factors for sinus mucormycosis were type 1 (OR: 4.04; 95% CI: 2.36–6.90) and type-2 (OR: 6.35; 95% CI: 3.89–10.36) diabetes [9], which is consistent with the data obtained by others [11–13,61].**

In the recent ECMM cohort, DM correlated with rhinocerebral involvement: of the 21 patients with rhinocerebral mucormycosis, 11 had DM (52%) [11]. In the Retrozygo study from France, 70% of diabetic patients had rhinocerebral involvement [12] and, in a recent series of 90 rhino-orbital-cerebral mucormycosis in solid-organ-transplant patients, 46% were diabetic [62]. In that cohort, having cerebral involvement was associated with higher mortality rates. In addition, uncontrolled
type-2 diabetes and diabetic ketoacidosis were significantly associated with rhino-orbito-cerebral involvement [13]. In a retrospective series of 41 cases of rhinocerebral mucormycosis, 83% were diabetics and 42% (n = 13/31) of these diabetic patients were acidic at presentation [61]. Of the 11 patients with type-2 diabetes without ketoacidosis, rhino-orbito-cerebral involvement was the most frequent site of mucormycosis [63].

Concerning clinical lesions, nasal obstruction or congestion with noisy breathing, headache, odontalgia, sinusitis with low-grade fever and unilateral facial swelling, maxillary pain and hyposmia or anosmia have been described [64]. Central-nervous-system invasion presenting as seizures, coma and/or cranial nerve palsy or even hemiplegia are common presentations. Atypical clinical presentations with facial nerve palsy caused by extension of the disease into the infratemporal fossa have also been reported [65]. Furthermore, multiple cranial nerve palsies are possible [66]. Through contiguous extension, osteomyelitis of the maxilla and orbit leading to preseptal and orbital cellulitis may be observed with ophthalmoplegia and ptosis, which were the most frequent signs in this series [64,67]. In other case reports, patients with uncontrolled DM had rapidly deteriorating vision loss that was either unilateral or bilateral, with or without other nasal and orbit-related symptoms [68,69]. At this stage of infection, meticulous examination of the nasal cavity, turbinates and palate need to be performed for necrotic eschars. Mucosal necrosis presenting as a blackened eschar is a classical finding that should be sought on the hard palate and nasal fossa. In cases of orbital involvement, necrosis of the eyelid may be encountered. These black eschars are indicators of rapidly invasive disease.

Pulmonary involvement is the second most common location in patients with DM. Of 337 diabetic patients with mucormycosis, 16% had pulmonary involvement [9]. In the French study, 13% of DM patients presented with isolated lung involvement [12] while, in other series, 7–14% of such patients had pulmonary involvement [11–13]. Clinical symptoms are nonspecific and include fever, dyspnoea, chest pain and haemoptysis [70]. Interestingly, diabetic patients have a predilection for developing endobronchial lesions compared with patients without DM [71]. Mucorales can invade lung vessels and cause distant vascular lesions [72]. The clinical presentation may be either acute (within 30 days or less) or subacute [71,73]. Diabetic patients with pulmonary mucormycosis have an identical survival rate to that of solid-organ-transplant patients and those with haematological malignancies (45–60% vs 25%, respectively) [71].

After rhinocerebral and pulmonary forms, the skin is the third most common mucormycosis localization, with 10% of DM patients having skin involvement in the cohort reported by Roden et al. [9]. To complete this study after 2004, Skiada et al. [74] collected all reports of cutaneous mucormycosis in the literature. Of their 78 cases, 14% were diabetic. These data are consistent with those of the ECMM cohort, in which 14% of diabetes patients had soft-tissue involvement [11]. Nevertheless, the data vary widely across studies and geographical areas. In the French Retrozygo study, none of the diabetic patients had cutaneous involvement [12] whereas, in India, 9% did [13]. Clinical findings consist of either surgical wound or skin breach infections. Expansion into the surrounding tissues presents as erythematous and/or ulcerative necrotic lesions that spread extensively to end up as necrotizing cellulitis and sometimes osteomyelitis [74]. Although the link between upper and/or lower airways mucormycosis and airborne contamination is difficult to establish, its presence at other locations, such as skin involvement, should lead to a suspicion of healthcare-associated mucormycosis [16].

4. Diagnosis

One of the most common differential diagnoses of mucormycosis is aspergillosis, as both fungal infections share common symptoms and evolve dramatically if left untreated [75]. Prompt recognition of a fungal infection and, thus, a precise fungal identification are essential for selecting appropriate treatment, which differs between aspergillosis and mucormycosis because of the lack of sensitivity of zygomycetes to a number of antifungal drugs [76]. The European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) has recently revised the definitions of invasive fungal infections [77]. To establish a diagnosis of proven mucormycosis in the context of a compatible infectious process, tissue biopsies and/or positive cultures obtained from sterile sites are required [78,79]. A probable mucormycosis diagnosis requires the association of host factors (DM can be considered a risk factor for mucormycosis), clinical and/or radiological signs compatible with mucormycosis, and direct examination or a positive culture isolated from a sample obtained from a pathological site.

4.1. Radiological findings at the main infected sites

For rhinocerebral lesions, non-enhanced paranasal sinus computed tomography (CT) scans show sinus mucosa thickening and early bone destruction (Fig. 3A), while magnetic resonance imaging (MRI) is mandatory for assessing extension into the cavernous sinus and identifying cerebral involvement [80]. MRI allows an accurate study of orbital and intradural expansion, and may even display cavernous sinus thrombosis, or thrombosis or aneurysm of the internal carotid artery. Findings of non-enhancing paranasal sinus tissue on MRI indicate devitalization that is consistent with extensive necrosis of mucosal tissue [81].

As for pulmonary mucormycosis, chest X-rays or CT scans can reveal consolidation, nodules or masses with the halo sign or reversed halo sign, either singly or as multiples and either unilateral or bilateral (Fig. 3B). Cavitations and pleural effusion may also be found [70]. Positron emission tomography using 18F-fluorodeoxyglucose (18FDG-PET) is another technique that has proved useful for more accurate evaluation of mould infection extent, as it can detect small infectious foci before the onset of the anatomical abnormalities assessed by conventional radiological tools [82]. However, 18FDG-PET is not recommended for the evaluation of cerebral involvement because of spontaneous glucose uptake in the brain. Also, as 18FDG-PET uses glucose...
injection, strict surveillance of glycaemia is required in diabetic patients.

4.2. Microbiological diagnosis

To diagnose skin involvement, biopsies are necessary whereas, in cases of sinus involvement with nasal discharges, scraping of the nasal mucosa, sinus aspirates or tissue specimens from the affected area should be obtained [83]. In pulmonary mucormycosis, flexible fibreoptic bronchoscopy may be helpful for obtaining deep samples as well as bronchoalveolar lavage (BAL), as sputum lacks sensitivity in non-neutropenic patients [71,73]. In cases of negative BAL findings, CT-guided biopsy should be performed [84]. All biopsy samples and other specimens should be transported rapidly to the laboratory without being refrigerated [83]. Also, the mycology laboratory should be made aware of the possibility of mucormycosis so that the specimens are not crushed before culture.

Specific stains generally used for fungal identification should also be applied (such as Gomori methenamine–silver or periodic acid–Schiff) to assess the presence of tissue damage related to hyphae, whereas culture is always useful for identification and antifungal drug susceptibility testing. In diabetic patients, R. oryzae is the most common zygomycete found in countries where identification tools are available [13]. However, no specific studies looking at the distribution of other pathogens in the diabetic population have so far been conducted, and it is known that Mucorales distribution varies from one country to another, mainly depending on the climate. Thus, in India, Apophysomyces elegans is the second most common pathogen...
found in diabetics [13] whereas, in Europe, the second most common pathogens identified in mucormycosis cases are *Mucor* spp. [11] and, in France, it is *Lichtheimia* spp. (Lanternier, Retrozygo study; data not shown). A positive culture for Mucorales in a patient with no clinical symptoms or a positive blood culture should be interpreted with caution because of possible environmental contamination [85]. Indeed, blood cultures are almost always negative in patients with disseminated disease.

Molecular tools may help to identify species, as phenotypic identification by culture remains difficult [86]. Even in embedded paraffin-fixed tissues, a precise identification can be obtained by polymerase chain reaction (PCR), but its sensitivity needs to be optimized before using it routinely [87].

5. Treatment

5.1. Correction of risk factors

Reversal of the underlying condition, whenever possible, is the success factor for mucormycosis treatment. The presence of two risk factors of mucormycosis was associated with a 3.7 higher risk of mortality compared with one or no risk factor [12]. However, the prognosis for mucormycosis in DM is better than in other settings [88]. Compared with those who have haematological malignancies, mortality is lower among diabetes patients [11,12]. In a review of 145 cases of rhinocerebral mucormycosis, the best outcomes were obtained in DM patients, most likely due to prompt reversal of ketoacidosis and control of hyperglycaemia [89].

5.2. Surgical treatment

Another cornerstone of mucormycosis treatment is prompt and aggressive surgery [90]. The rationale for surgery is to obtain localized control of the disease as much as possible. This is achieved by extensive surgical debridement of necrotic tissue. Drilling into the underlying bone is another critical technique for removing intraosseous lesions and necrotic bone. The purpose of surgery is to leave living tissue as margins to allow antifungal treatment to be delivered locally and to promote normal-looking mucosa to colonize the surgical cavity. Revision surgery and multiple operations may also be required. At the same time, the possible spread of mucormycosis should be monitored by early and repeated imaging.

In cases of rhinocerebral involvement, prompt and aggressive surgery is required to surgically control the disease in the nasal cavity and sinuses, thus limiting orbital and cerebral invasion (Fig. 4). However, such operations may lead to aesthetic and functional sequelae [91]. Nevertheless, as orbital exenteration may be life-saving, it should be considered for an actively infected orbit even after intracranial spread has occurred [92]. Furthermore, lung surgery can stop or prevent haemorrhagic complications. The mortality rate in patients with pulmonary mucormycosis treated surgically was 11%, which was significantly lower than the 68% in those treated medically [93].

5.3. Antifungal treatment

What data there are come from case reports or small series using various evaluation criteria. Most antifungals, including echinocandins and voriconazole, have no activity against Mucorales, whereas polyenes and posaconazole do [94]. Animal models have contributed to the development of new indications for antifungal treatments and new antifungal combinations (Table 1). All diabetic ketoacidotic mouse models of mucormycosis were implemented by the same team [95–102]. Although amphotericin B (AmB) deoxycholate remains the classical treatment, the first-line antifungal therapy is now based on a lipid formulation of AmB [103]. This liposomal amphotericin B (LAmB) formulation has better penetration into cerebral tissue than other agents and, accordingly, may have potentially better efficacy in cases of rhinocerebral involvement [104]. It is also

Fig. 4. Localized control of nasal and paranasal mucormycosis achieved with surgery. A. Coronal view of a craniofacial CT scan shows surgical debridement of all paranasal sinuses and the nasal septum to achieve focal control of the disease. B. Craniofacial T1-weighted MRI (coronal view) shows the absence of disease extension into the orbit and adjacent anterior skull base.
Table 1
Contribution of animal model studies to the development of antifungal treatment.

<table>
<thead>
<tr>
<th>Publication year</th>
<th>Tested molecules</th>
<th>Results</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>Monotherapy</strong></td>
<td></td>
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<tr>
<td>2003</td>
<td>LAmB (5 or 15 mg/kg/day) vs AmB (1 mg/kg/day)</td>
<td>High survival rate with high-dose LAmB</td>
<td>[100]</td>
</tr>
<tr>
<td>2005</td>
<td>Caspofungin (1 mg/kg b.i.d.)</td>
<td>Improved survival with a small inoculum</td>
<td>[101]</td>
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<tr>
<td>2006</td>
<td>Deferiprone (100 or 200 mg/kg/day) vs LAmB (15 mg/kg/day)</td>
<td>Deferiprone at 100 mg/kg was as effective as LAmB at improving survival and decreasing brain fungal burden</td>
<td>[97]</td>
</tr>
<tr>
<td>2008</td>
<td>LAmB vs ABLC (7.5 and 15 mg/kg/day)</td>
<td>LAmB increased survival and decreased brain fungal burden</td>
<td>[102]</td>
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<tr>
<td><strong>Combination therapy</strong></td>
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<tr>
<td>2005</td>
<td>ABLC (5 mg/kg/day) ± caspofungin (1 mg/kg/day)</td>
<td>Improved survival, but not organ clearance</td>
<td>[96]</td>
</tr>
<tr>
<td>2007</td>
<td>Deferasirox (2, 6 or 20 mg/kg/day) ± LAmB (15 mg/kg/day)</td>
<td>Deferasirox alone or in combination with LAmB improved survival with a dose-response effect, and reduced tissue fungal burden</td>
<td>[98]</td>
</tr>
<tr>
<td>2008</td>
<td>LAmB (5 mg/kg/day) ± micafungin (1 or 3 mg/kg/day) or anidulafungin (1 or 10 mg/kg/day)</td>
<td>LAmB + micafungin 1 mg/kg and LAmB + anidulafungin 10 mg/kg improved survival and reduced tissue fungal burden</td>
<td>[95]</td>
</tr>
<tr>
<td>2011</td>
<td>Deferasirox (20 mg/kg/day) + LAmB (15 mg/kg/day) + micafungin (1 mg/kg/day)</td>
<td>Triple therapy improved survival and reduced tissue fungal burden compared with mono- and bitherapy</td>
<td>[99]</td>
</tr>
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AmB: amphotericin B; LAmB: liposomal amphotericin B; ABLC: AmB lipid complex.

NB: All experiments used diabetic ketoacidotic mice infected by *Rhizopus oryzae* strains.

less nephrotoxic than AmB deoxycholate. In a study by Lewis et al. [105] wherein AmB lipid complex (ABLC) and LAmB were compared in a murine model of pulmonary mucormycosis, both achieved higher concentrations in lung tissues when administered at a dose of 10 mg/kg.

Several retrospective studies have assessed AmB deoxycholate or LAmB as first-line mucormycosis treatments. One such study conducted between 1987 and 2001 in 51 patients with haematological malignancies found a 23% (9/39) response-rate in patients treated with AmB deoxycholate (1 mg/kg per day), and a 58% response-rate in those treated with LAmB at 3 mg/kg per day [19]. Another small retrospective study saw a response rate of 22% (2/6) in patients treated with AmB deoxycholate at 1 mg/kg per day, while one out of two patients treated with LAmB at 3–5 mg/kg per day responded [106]. Recently, a large retrospective study including all zygomycosis cases in the literature reported a 61% survival rate with AmB deoxycholate and 69% with LAmB [9].

Two retrospective studies have evaluated two other forms of lipid derivatives of AmB as a second-line treatment for mucormycosis. In one, ABLC resulted in a 71% success rate as salvage therapy for mucormycosis [107]. The other study evaluated an AmB colloidal dispersion (2–6 mg/kg per day) and observed a 60% objective response rate [108]. These results suggest that lipid derivatives of AmB are probably more effective and better tolerated than AmB deoxycholate in mucormycosis.

In addition, higher dosages of LAmB are often suggested for this indication. Indeed, the LAmB area under the curve (AUC) is maximum at 10 mg/kg per day; this regimen can saturate mononuclear cells, thereby allowing greater accumulation of AmB in the lungs, a major target site in mucormycosis [22]. Furthermore, a clinical response has been described with high-dose LAmB after failure of conventional dosages [48,49]. In one recent study, 28 patients who had been treated with LAmB as the first-line therapy for invasive mucormycosis between 1998 and 2005 experienced an overall mortality rate of 61% [109]. However, to determine whether or not high-dose LAmB is a good treatment regimen, the results of a prospective French phase-II multicentre study (the Ambizygo trial) to evaluate the maximum effective and tolerated dose of LAmB (up to 10 mg/kg per day) as the first-line treatment of mucormycosis in adults and children are currently being awaited [103].

Although the place of antifungal drug combinations has yet to be defined for first-time therapy, there is potential benefit with the combination of lipid polyenes and echinocandins as a second-line therapy. However, it should be borne in mind that echinocandins have no activity in vitro [110], but may have a class effect in vivo [95]. Indeed, a few studies in vivo have demonstrated the synergistic activity of caspofungin plus ABLC and micafungin/anidulafungin plus LAmB combinations in the treatment of disseminated mucormycosis in diabetic ketoacidotic mice [95,96]. Also, in a recent retrospective clinical study, 41 cases of proven rhino-orbital-cerebral mucormycosis, 34 (83%) of which were diabetic, were treated with AmB formulations alone (n = 34) or in combination with caspofungin (n = 7) [61]. The authors emphasized the significant success of the combination therapy in all six of the evaluable patients 30 days after discharge compared with the monotherapy. The survival rate was also significantly better with the combination therapy.

Posaconazole may also be effective in mucormycosis patients who are refractory or intolerant to LAmB, including kidney-impaired patients, and may help to facilitate these patients’ discharge [111]. In a series of 91 patients who received posaconazole at a dose of 800 mg/day as a salvage treatment, one-third were diabetic [112]. Of these patients, the response to therapy 12 weeks after its initiation was 60%. It should be noted, however, that posaconazole can only be administered orally and has drug–drug interactions. In addition, steady state was only obtained after seven to ten days of treatment, so therapeutic drug monitoring should also be performed [113].
A few promising drugs have been tested either in vitro or in vivo and found to have intrinsic antifungal effects. The first, deferasirox, is an iron chelator mainly used in transfusion-related iron overload. Data in vitro suggest that both deferiprone and deferasirox can inhibit *R. oryzae* growth, whereas defereroxamine does not [97,98]. In murine models, deferasirox has occasionally been used in combination with other antifungal drugs [98,99]. In combination with LAmB, it significantly improved diabetic ketoacidotic mouse survival vs a placebo and as monotherapy [99]. Furthermore, in the same murine model, triple therapy using LAmB, micafungin and deferasirox significantly improved survival rates, and reduced tissue fungal burden compared with mono- and bithera [99].

In humans, deferasirox may have the same beneficial effects. Indeed, in a review of eight cases of proven mucormycosis in which all patients were diabetics except one, deferasirox was used in combination with antifungal drugs in three cases as salvage therapy [114]. Seven of these eight cases survived with minimal adverse effects (rash). Unfortunately, a double-blinded, randomized, placebo-controlled, phase II clinical trial—the Deferasirox-AmBisome Therapy for Mucormycosis (DEFEAT Mucor) study—failed to demonstrate a benefit of adjunctive deferasirox therapy to LAmB for mucormycosis [115].

In addition, a novel antifungal drug belonging to the azole family, isavuconazole, has shown activity against Mucorales. Tested against 345 Mucorales isolates, isavuconazole revealed partial antifungal activity [116]. It was also tested *in vitro* against *A. elegans* strains from diabetes patients and showed minimum inhibitory concentrations of 2–4 μg/mL, which were higher than those of posaconazole (0.5–1 μg/mL) [117].

Without doubt, delaying mucormycosis treatment may be fatal [118]. Treatment has to be started as soon as possible after diagnosis, and its duration adapted individually with at least 3 months of therapy for sinusitis in DM patients [119]. To monitor responses to antifungal therapy, 18FDG-PET may be useful for invasive fungal infections, but needs further investigation in cases of mucormycosis [82].

6. Conclusion

DM is a well-recognized risk factor for mucormycosis. Physicians need to be careful when facial symptoms arise in a diabetic patient. A simple purulent nasal discharge may reflect an aggressive fungal infection, but Mucorales identification is often difficult. It is also important to note that ketoacidosis enhances the risk of mucormycosis, although most cases of mucormycosis are seen in poorly balanced diabetes patients without ketoacidosis. Diagnostic delay is the most important cause of morbidity and mortality in DM patients. Nevertheless, the prognosis of mucormycosis in this population is better than in patients who have haematological malignancies.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References


