

REVIEW ARTICLE

Mucormycoses

Mucormykosen

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Summary. Over recent years the clinical importance of mucormycosis has significantly increased. Most frequently mucormycosis occurs in neutropenic patients with haematological diseases. It is caused by fungi of the order *Mucorales*. The clinical patterns of the disease produced by different genera or species of *Mucorales* are virtually identical. *Rhizopus*, *Absidia*, *Rhizomucor* and *Mucor* are the organisms most commonly isolated from patients who suffer from mucormycosis. Diagnosis of mucormycosis is difficult as it is based on culture methods or microscopy of clinical specimens. The diagnosis is often only made after a delay or even post-mortem. Therapy includes surgical intervention if possible and is based on systemic amphotericin B (conventional or liposomal).

Zusammenfassung. Mucormykosen haben sich in den letzten Jahren zu einem klinisch bedeutsamen Krankheitsbild entwickelt. Die meisten Mucormykosen treten bei neutropenischen Patienten mit hämatologischen Erkrankungen auf. Die Infektion wird durch Pilze der Ordnung *Mucorales* verursacht, deren klinisches Erscheinungsbild sich nicht nach auslösender Gattung oder Spezies unterscheidet. Zu den

häufigsten Erregern der Mucormykose gehören die Gattungen *Rhizopus*, *Absidia*, *Rhizomucor* und *Mucor*. Die Diagnostik der Mucormykosen stellt nach wie vor eine Herausforderung für Kliniker und Mikrobiologen dar, da die Möglichkeiten, den Verdacht auf eine Mucormykose diagnostisch zu sichern, begrenzt sind und sich bisher nur auf den mikroskopischen oder kulturellen Nachweis stützen. Die Diagnose wird in einem hohen Prozentsatz spät oder erst postmortal gestellt. Die Therapie umfaßt die chirurgische Entfernung bei solitären Herden, falls der Patient operabel ist, und die medikamentöse Therapie mit konventionellem oder liposomalem Amphotericin B.

Introduction

Opportunistic fungal infections have increased in importance during recent decades. The growing number of immunocompromised patients who survive for longer periods of time, the widespread use of chemotherapeutic drugs and the more intensive chemotherapies have contributed to the high incidence of fungal infections. They are associated with a high mortality rate [1, 2]. *Candida* and *Aspergillus* species are the fungal pathogens most frequently isolated, depending on the type of disease and source of isolation. In patients with brain abscess after bone marrow transplantation, fungi are actually the most frequently isolated pathogens, accounting for up to 92% of all cases [3]. The mortality of fungal brain abscess in patients receiving bone marrow is high (97%). Apart from *Candida* and *Aspergillus* species, fungi of the order *Mucorales* have been

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increasingly recognized in immunocompromised patients [4, 5]. Among fungi of the order Mucorales the genera *Rhizopus*, *Absidia*, *Rhizomucor* and *Mucor* are the organisms most commonly isolated from clinical specimens [6]. They all belong to the family Mucoraceae. Less common are the genera *Cunninghamella*, *Mortierella*, *Saksenaia*, and *Apophysomyces*.

The term zygomycosis is often regarded as being synonymous with mucormycosis, but the class zygomycetes includes not only the order Mucorales but also the order Entomophthorales. As Entomophthorales are very uncommon in clinical specimens this review will refer to mucormycoses only [7, 8]. Mucorales are filamentous fungi. They are ubiquitous and are found in the soil, manure, plants and decaying material. They are aerogenous pathogens and hence most mucormycoses firstly afflict the paranasal sinus and lungs [5, 9, 10].

Epidemiology

After aspergillosis, mucormycosis is the second most common mycosis caused by filamentous fungi [3, 11]. Overall, genera of the order Mucorales represent the third leading cause of invasive fungal infection following *Aspergillus* and *Candida* species [3, 5, 10–12]. The number of species causing mucormycosis and the number of reported cases of mucormycosis in the literature have increased in recent years due to the increasing use and intensity of immunosuppressive therapy and have been diagnosed more accurately due to the increased awareness of clinicians and improved laboratory diagnosis. Obviously, differentiation according to genera and species is performed more frequently. *Rhizopus arrhizus* (*oryzae*) and *Absidia corymbifera* are mentioned as being the most frequent pathogens isolated from patients with mucormycosis [6]. Nevertheless, there is a lack of epidemiological studies that could prove this fact.

The clinical importance of mucormycosis is underlined by the high mortality. Overall mortality is about 75–80%, the mortality rate of disseminated mucormycosis is more than 95% [5, 13]. In a retrospective study the approximate incidence of mucormycosis in patients with leukaemia was 1%. It can be supposed that the morbidity was higher than 1% as no comments referring to the rate of autopsy were made in this study, and pre-mortal diagnosis was made in 35% of documented mucormycosis only [5]. A considerable number of cases of mucormycosis

could have escaped post-mortal diagnosis due to a lack of autopsies.

Pathogenesis

The first step of infection constitutes the inhalation of fungal sporangiospores. Less often primary mucormycosis caused by the traumatic implantation of fungal elements occur in cutaneous lesions and burns [14–16]. Sometimes infections occur in association with intravenous drug abuse [17].

Inhaled spores may be deposited in the upper respiratory tract and paranasal sinus or may pass through to the pulmonary alveoli. In the immunocompromised organism spore germination occurs and as a consequence hyphal growth can start. The formation of mycelia characterizes the beginning of invasive mycosis. Control of the germination of spores is the critical step of infection which prevents mucormycosis. It was shown that diabetic as well as cortisone-acetate treated mice inoculated with spores were susceptible to infection of *Rhizopus* but normal mice were not. Spore germination did not occur in the lung tissue sections of normal mice. Alveolar macrophages prevented spores from germinating and activated spores induced strong fungicidal activity, but resting spores were not easily killed by macrophages or neutrophils [18, 19]. In cases of reduced immunocompetence, dysfunction or destruction of macrophages invasive infection occurs as fungal mycelia can invade the deeper tissue layers.

Mucorales have a high affinity for invading blood vessels (vasculotropism). The invasion of blood vessels leads to ischaemia and thrombosis in the involved organ as well as haematogenous dissemination and septic thromboembolism in other organs. In autopsies hyphae are frequently found near blood vessels (Fig. 1). Vasculotropism is of relevance, especially in neutropenic patients. Frequently, after invasion of a blood vessel in the primary organ of infection, fungaemia with haematogenous dissemination may occur and this often ends fatally due to a lack of circulating neutrophils in the blood [5, 9, 20–23].

As in other opportunistic fungal infections, neutropenia due to haematological disease (leukaemia, lymphoma or aplastic anaemia) or chemotherapy is one of the most important risk factors. In contrast, recovery of neutrophil granulocytes ($> 1000 \mu\text{l}^{-1}$) was shown to be a positive prognostic factor for survival in an univariate analysis by Pagano *et al.* [5]. Further factors associated with an increased risk of developing



Figure 1. Section of pulmonary blood vessel showing hyphal invasion of the vessel wall with secondary thrombosis (Grocott-Gomori, original magnification 25 ×).

mucormycosis include diabetic ketoacidosis, therapy with glucocorticoids, organ transplantation, HIV disease, drug abuse and haemodialysis [4, 23–31]. Infection without evidence of any immunodeficiency is rare [32].

Diabetes mellitus results in impaired chemotaxis and phagocytosis of neutrophils. Treatment with glucocorticoids results in transient T-cell sequestration, diminished synthesis of immunoglobulin, and decreased adherence of neutrophils. These are the major mechanisms responsible for the high incidence of mucormycosis in these patients. AIDS patients have been reported with this infection [33], but T-cell immunity is not considered to be an important factor leading to the infection. Most cases of mucormycosis occurred in patients with advanced HIV disease who had neutropenia or at least had experienced some periods of neutropenia preceding the onset of mucormycosis. More often AIDS patients reveal an additional risk factor, especially intravenous drug abuse. A large proportion of HIV patients with mucormycosis had a history of injection drug use that was often carried out in unhygienic conditions [33–39]. Treatment with deferoxamine, especially in patients undergoing haemodialysis, constitutes a risk factor that was not found in other invasive mycoses [8–10]. It was hypothesized that deferoxamine could function as a siderophore providing iron to promote the growth and sporulation of Mucorales [40]. However, not only deferoxamine therapy but also the iron overload due to a high rate of red cell transfusions without the concomitant use of deferoxamine seems to play a major role in the pathogenesis of mucormycosis [41, 42].

There is not much known about genus- or species-specific differences concerning pathogenesis, diagnosis and therapy of mucormycosis. However, the genus *Mucor* is described less often in disseminated mycosis, but it is not clear whether this reflects a lower exposure to this genus or a lower intrinsic virulence of the genus *Mucor* [16].

Clinical manifestations

Mucormycosis is characterized by a rapidly progressive and usually fatal course. Common clinical manifestations include rhinocerebral, pulmonary, gastrointestinal and cutaneous involvement as well as dissemination. Less common are hepatolienal and renal involvement, which occurs most frequently in association with disseminated disease. Isolated renal mucormycosis seems to have a relatively favourable prognosis [26]. Both, *Aspergillus* species and fungi of the order Mucorales are primarily respiratory pathogens. The common manifestations of both mycoses involve the lung and the paranasal sinus [5, 9, 10, 43]. In patients with pulmonary mucormycosis, clinical findings mimic those described for aspergillosis. Major symptoms are cough, fever, thoracic pain, dyspnoea, and haemoptysis, but at the initial stage patients may be asymptomatic [12, 13]. In most cases central nervous system (CNS) involvement is a secondary manifestation following haematogenous dissemination [21, 25] or infection of the nose and paranasal sinus which extends into the brain. Additional involvement of the orbita is common [29, 44, 45]. Isolated involvement of the brain without detection of manifestations in other organs is rare [46]. Manifestation in the sinus maxillaris extends easily to the retro-orbital region and palatinum inducing unilateral ophthalmoplegia, mostly followed by blindness, proptosis, swelling of the eyelid, cornea oedema and tissue necrosis. CNS involvement may present as headache, meningism, paresis of cranial nerves, aphasia, hemiplegia, lethargy and coma. In rhinocerebral mucormycosis the frontal lobes are preferentially involved. Usually the onset of symptoms is very abrupt, the progression rapid and patients succumb within a few days [6, 20, 21, 29, 46].

The clinical appearance of cutaneous mucormycosis is generally non-specific and may arise as a painful erythematous induration of skin resembling common phlebitis. It may progress to a necrotizing lesion with a black eschar and an erythematous induration of the marginal area

[15, 27]. Insertion sites of peripheral or central venous lines are the preferred sites for cutaneous mucormycosis [5, 14, 27].

Patients with haematological diseases and neutropenia are at high risk from developing a disseminated mucormycosis. Dissemination occurs in up to 40% of mucormycosis in patients with haematological diseases [5, 22]. Commonly, dissemination arises from the lungs, which is the most common site of primary infection in neutropenic patients and may involve several organs.

Diagnosis

The only definite way to diagnose mucormycosis is to visualize the characteristic hyphae in tissue and materials such as sputum, exudates and scrapings and to grow the fungus in the laboratory [8]. Diagnosis is based on the morphological detection of hyphae. Mycelia and sporangiospores are rarely found *in vivo*. Fungi of the order Mucorales appear in tissue as irregularly shaped, broad (6–25 µm in diameter), non-septate hyphae with right-angle branching (Fig. 2). They can rarely be mistaken for *Aspergilli* or other *Hyalohyphomycetes*, as these fungi present septate hyphae with dichotomous branching at sharp angles. A reliable method for demonstrating the typical hyphae of Mucorales in wet smears is fluorescent staining with optical brighteners. Tissue sections may be stained for zygomycetous elements either using Grocott–Gomori methenamine–silver nitrate, periodic acid–Schiff reaction or similar fluorescent staining with optical brighteners. The latter procedure can be combined with immunofluorescence [47]. The staining reactions may be variable [48]. In most cases of mucormycosis, culture is

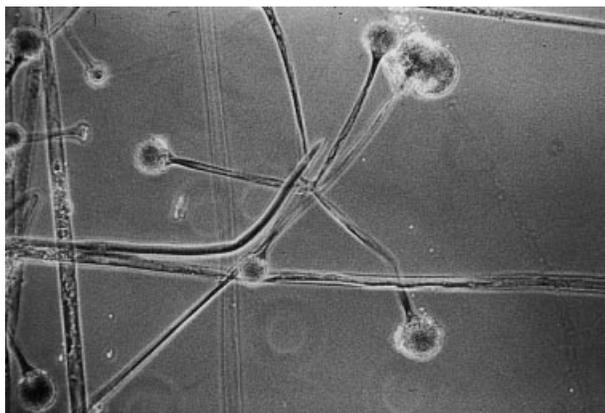


Figure 2. Non-septate hyphae and sporangia of a fungus of the order Mucorales, in this case *Absidia corymbifera* (original magnification 150×).

the only method leading to species identification. Members of the Mucorales grow well on most of the routine mycological culture media, provided that they do not contain cycloheximide. The use of antibiotics such as gentamicin and chloramphenicol in primary isolation media is recommended to prevent growth of bacteria. Sabouraud glucose agar with antibiotics is usually used as the primary isolation medium. Mechanical homogenization of tissue may impair the growth of the brittle hyphae and should be avoided [48]. Due to the vasculotropism of Mucorales, hyphal infiltrations are frequently found near blood vessels in the organ of primary infection and in the area of septic thromboembolization in the terminal vascular bed of other organs if dissemination has occurred (Fig. 3) [8, 20]. Serological tests detecting mucormycosis have not been established for routine clinical use.

In the case of suspected mucormycosis a biopsy from the suspected site of infection has to be obtained, although this may be difficult regarding the underlying disease of the patient. Cancer patients, especially patients with leukaemia often present pancytopenia including severe thrombocytopenia due to underlying disease or chemotherapy. In these cases resection of the infectious site or even a biopsy is often not feasible or the diagnostic procedure can only be performed after a delay. Frequently, apart from bronchoalveolar lavage (BAL) and blood cultures, no further invasive diagnostic procedure can be performed in patients with pancytopenia if pulmonary mycosis is suspected. Blood cultures are of great importance in the diagnosis of fungal infection even though rarely yielding positive results in mucormycosis [49]. Cultures of both bronchoalveolar lavage fluid and blood have

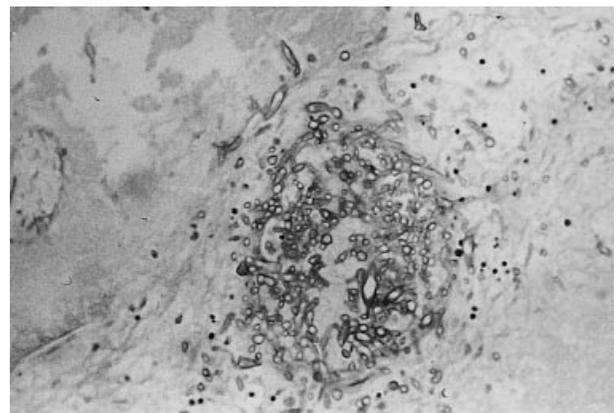


Figure 3. Septic thromboembolism of an arterial vessel in the brain, produced by disseminated mucormycosis (PAS, original magnification 60×).

a low sensitivity [50]. The investigator should be aware of possible artifactual contaminations of clinical specimen by agents of mucormycosis, as has been reported by Verweij *et al.* [51].

In CNS mucormycosis, moderate pleocytosis occurs, the protein and sugar values are elevated and are usually not diagnostic [9].

The premortal diagnosis of mucormycosis is difficult. Diagnosis has been made only infrequently ante mortem [4, 22, 52]. In a retrospective study on 116 leukaemia patients with documented pulmonary filamentous fungal infections, the diagnosis was made ante mortem in only 12 patients (10%) [12]. In a retrospective study of 37 patients with haematological malignancies and histologically documented mucormycosis, the diagnosis was made ante mortem in 35% of patients [5]. Molecular-biological methods detecting invasive mycoses by polymerase chain reaction (PCR) focus on the detection of the most common opportunistic fungi such as *Aspergillus* and *Candida* species. Some promising trials have been performed with experimental PCR assays that showed high sensitivity and specificity for detecting fungal DNA. But they are not yet routinely applied for reasons such as contamination with ubiquitous fungal DNA, false-positive results, and high costs [53–55]. The application of PCR assays, particularly pan-fungal PCR, for the diagnosis of mucormycosis is complicated by the fact, that Zygomycetes are genetically quite distinct from the common fungal opportunists and thus may be missed [56].

Treatment

All patients with suspected fungal infection require immediate treatment. Successful treatment of mucormycosis is based on three principles: control of the underlying disease, extensive surgical resection of the infectious focus or debridement of necrotic tissue and medical treatment with antimycotic agents. Especially in neutropenic patients, the prognosis of mucormycosis remains dismal if no haematopoietic regeneration occurs during the course of the fungal infection.

Surgical treatment has a high impact on the outcome of mucormycosis. It was reported by Tedder *et al.* [13] that the overall mortality of mucormycosis was 60%, compared with 11% in patients who underwent surgical treatment, respectively. This observation was supported by other studies [5, 26]. Although surgical treatment seems to dramatically ameliorate the clinical outcome, in most patients with pulmonary mucormycosis the procedure may not be feasible

due to thrombocytopenia, the frequent presence of multiple pulmonary infiltrates or early dissemination [5, 12, 13, 22]. Patients with thrombocytopenia are generally not suited for operation if the thrombocyte count cannot be maintained at a high level by peri-operative platelet transfusions. Multiple infectious foci in one organ or disseminated disease are also contraindications and are associated with a worse outcome. In haematological patients about 40% of mucormycoses are disseminated and the mortality of disseminated mucormycosis is 96% [5].

Amphotericin B (either conventional or liposomal) is the only agent that has been applied successfully in mucormycosis.

Overall, randomized studies concerning the antimycotic treatment of mucormycosis are lacking. The use of amphotericin B may be limited by its common adverse effects, especially hypokalaemia, renal impairment and infusion-related intolerance reaction. Penetration of the blood–brain-barrier by amphotericin B is poor. However, an intracerebral infection may alter the blood–brain barrier resulting in a better penetration of the drug into the brain tissue. The survival rate of neutropenic patients with mucormycosis involving CNS is extremely low [57].

Liposomal amphotericin B or amphotericin B colloidal dispersion could represent promising alternative drugs in the treatment of mucormycosis. Liposomal amphotericin B can be applied in higher doses ($3\text{--}5\text{ mg kg}^{-1}\text{ day}^{-1}$ or even higher) without significant increase of toxicity compared with conventional amphotericin B [58–61]. Data from animal experiments indicate that liposomal amphotericin B penetrates brain parenchyma better than the conventional drug and results in greater drug concentrations in the brain, especially in higher doses ($5\text{ mg kg}^{-1}\text{ day}^{-1}$) [62].

The poor prognosis of cerebral mucormycosis seems to justify an aggressive treatment regimen including excision of brain lesions combined with intravenous amphotericin B [17, 63–65]. One patient was reported with cerebral mucormycosis who was cured by excision of brain abscesses and intravenous amphotericin B as well as interstitial and cerebrospinal fluid administration of amphotericin B using an Ommaya reservoir [64]. In other cases, mucormycosis was successfully treated with adjunctive hyperbaric oxygen [65]. However, whether additional interstitial and cerebrospinal fluid administration of amphotericin B or adjunctive hyperbaric oxygen results in a better outcome of cerebral mucormycosis remains unknown.

As only a few patients with mucormycosis have been treated with azole antifungals, the precise efficacy of these drugs is not known in the treatment of mucormycosis [5, 66]. In the study of Pagano *et al.* [5] four patients were treated with azoles, two with itraconazole and two with fluconazole, but without success. All of the nine successfully treated patients received amphotericin B as medical treatment and five of these patients underwent additional surgical intervention.

Conclusion

Mucormycosis, although a rare disease, is the second most frequent mycosis caused by moulds in immunocompromised patients. Mucormycosis occurs most frequently in neutropenic patients with haematological diseases. The mortality is high. More rapid and accurate diagnostic methods and the availability of more effective antifungal drugs may help to improve the prognosis of mucormycosis in future. The development of PCR-based diagnostic tools for the detection of Mucorales might permit a more timely diagnosis of systemic mucormycosis than that obtained with conventional diagnostic methods.

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