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Rhino-Orbital-Cerebral Mucormycosis

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Abstract This review focuses on sinus, sino-orbital, and rhinocerebral infection caused by the Mucorales. As the traditional term of "rhinocerebral" mucormycosis omits the critical involvement of the eye, the more comprehensive term as rhino-orbital-cerebral mucormycosis (ROCM) is used. The most common underlying illnesses of ROCM are diabetes mellitus, hematological malignancies, hematopoietic stem cell transplantation, and solid organ transplantation. Sporangiospores are deposited in the nasal turbinates and paranasal sinuses in immunocompromised patients. Qualitative and quantitative abnormalities of neutrophils, monocytes and macrophages increase the risk for development of mucormycosis. Altered iron metabolism also is a critical factor in the pathogenesis of patients with diabetes mellitus who are at risk for ROCM. Angioinvasion with thrombosis and tissue necrosis is a key pathophysiological feature of human Mucorales infection. The ethmoid sinus is a critical site from which sinus mucormycosis may extend through the lamina papyracea into the orbit, extraocular muscles, and optic nerve. The brain may be seeded by invasion of the ethmoidal and orbital veins, which drain into the cavernous sinuses. Diplopia and ophthalmoplegia may be the earliest manifestations of cavernous sinus syndrome before changes are apparent on diagnostic imaging modalities. Negative diagnostic imaging does not exclude cavernous sinus mucormycosis. Mucormycosis of the maxillary sinus has a constellation of clinical features that are different from that of ethmoid sinus mucormycosis. A painful black necrotic ulceration may develop on the hard palate, indicating extension from the maxillary sinus into the oral cavity. Orbital apex syndrome is an ominous complication of mucormycosis of the orbit. Once within the orbital compartment, organisms may extend posteriorly to the optic foramen, where the ophthalmic artery, ophthalmic nerve and optic nerve are threatened by invasion, edema, inflammation and necrosis. Early diagnosis of sinus mucormycosis is critical for prevention of extension to orbital and cerebral tissues. Optimal therapy requires a multidisciplinary approach that relies on prompt institution of appropriate antifungal therapy with amphotericin B, reversal of underlying predisposing conditions, and, where possible, surgical debridement of devitalized tissue. Outcomes are highly dependent upon the degree of immunosuppression, site and extent of infection, timeliness of therapy, and type of treatment provided. New modalities for early diagnosis and therapeutic intervention are critically needed for improved outcome of patients with ROCM.

Keywords Sinusitis · Rhinocerebral mucormycosis · Cerebral mucormycosis · Orbital mucormycosis

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Introduction

Infections caused by members of the order Mucorales are termed "mucormycosis." Among the different invasive mycoses, mucormycosis has emerged as a life-threatening

infection associated with severe morbidity and high mortality [1]. The challenges of this infection are magnified only further by the paucity of diagnostic tools and therapeutic options.

Mucormycosis is characterized by a rapidly evolving course of angioinvasion and tissue necrosis in immunocompromised hosts [2, 3•, 4]. The terms "Zygomycosis" and "Zygomycetes" are currently being reassessed as advances in molecular taxonomy are revealing new mycological relationships [5•]. Thus, the term "mucormycosis" is used throughout this review.

Mucormycosis manifests as pulmonary, cutaneous, disseminated, gastrointestinal, osteoarticular, sinus, sino-orbital and rhinocerebral forms disease. This review will focus principally on sinus, sino-orbital, and rhinocerebral infection as a disease process. As the traditional term of "rhinocerebral" mucormycosis omits the critical involvement of the eye, we will refer to the more clinically comprehensive term as rhino-orbital-cerebral mucormycosis (ROCM).

Medical Mycology

Among causes of microbiologically documented cases of mucormycosis, *Rhizopus oryzae* is the most commonly reported single species [4]. Other medically important *Rhizopus* species include *Rhizopus rhizopodiformis* and *Rhizopus microsporus*. After genus *Rhizopus*, the genus *Mucor* is the second most commonly reported. *Rhizopus* spp. and *Mucor* spp. are then followed by *Cunninghamella bertholetiae*, *Apophysomyces elegans*, and *Leictheimia corymbifera* (formerly, *Absidia corymbifera*) in frequency of reporting. These patterns may be evolving with the emergence of *Leictheimia corymbifera* as a relatively common cause of mucormycosis in France [6].

Members of the order Mucorales are identified to the genus or species level according to colonial morphology, microscopic morphology, and growth temperature. Most medically important Mucorales are thermotolerant, and are able to grow at temperatures at or above 37 °C. They grow rapidly on virtually any carbohydrate substrate. They are commonly found in soil, and in decaying organic matter, as well as numerous unprocessed food items.

In order to optimize growth, clinical specimens should be inoculated onto appropriate media such as Sabouraud glucose agar and incubated at room temperature. Grinding or homogenization of tissue specimens may destroy the delicate hyphae rendering cultures negative. Recovery in culture is enhanced if tissue is sliced into small pieces before inoculation onto media. Close collaboration between clinicians and the microbiology laboratory is essential to ensure proper handling of the specimen. Colonies typically appear within 24 to 48 h unless residual antifungal agents, such as AmB are present which can suppress growth. Most mucoraceous species fill a culture dish within 3–5 days and demonstrate a grayish white, aerial mycelium with a wooly texture. The colonies readily separate from the agar surface.

Microscopic characterization of non-septate hyphae, rhizoids, columellae, sporangia, and sporangiospores help to define genus and species within the order Mucorales. As a detailed microbiological description of the Mucorales is beyond the scope of this chapter, the reader is referred to a more in-depth review elsewhere [7•].

Identification of the Mucorales to the genus or species level carries valuable epidemiological, therapeutic, and prognostic implications. For example, *Rhizopus oryzae* is the most common Zygomycete recovered from clinical specimens but tends to be resistant to posaconazole; *Mucor circinelloides* is less commonly isolated but more susceptible to posaconazole. *Cunninghamella* tends to have higher MICs to amphotericin B (AmB) and a higher associated overall mortality [8, 9].

Pathogenesis and Host-Pathogen Interactions

Inhalation of sporangiospores from environmental sources is the principle mode of acquisition of the fungal elements of the Mucorales. Following inhalation, sporangiospores are deposited in the nasal turbinates, paranasal sinuses, and pulmonary alveoli where they can precipitate allergic sinusitis and interstitial pneumonitis in immunocompetent hosts [7•, 8–10] or invasive sinus infection and pneumonia in immunocompromised patients [11]. Among diabetic and other immunocompromised patients the inhaled sporangiospores germinate to form hyphae that invade tissues causing locally destructive sinus, orbital, and pulmonary infections.

Tissue macrophages and neutrophils, which serve as functional effector cells, are needed in sufficient quantity to mount an effective response to the sporangiospores and hyphal elements of the Mucorales. Macrophages ingest and then kill sporangiospores by non-oxidative mechanisms, thereby preventing their germination to hyphae [12, 13•]. If germination of sporangiospores evades or escapes this first line of defense, functional neutrophils are required to damage hyphae and prevent their invasion of surrounding tissue [14].

Qualitative and quantitative abnormalities of neutrophils, monocytes and macrophages increase the risk for development of mucormycosis. Infections typically occur when several layers of innate host defenses are simultaneously impaired due to intrinsic or iatrogenic abnormalities. For example, in patients with diabetes mellitus, monocytes and macrophages fail to suppress germination of sporangiospores. Diabetic ketoacidosis is associated with impairments in neutrophil function including chemotaxis, adherence, and oxidative burst [15–19].

Altered iron metabolism also is a critical factor in the pathogenesis of patients with diabetes mellitus who are at risk for ROCM. There is an increased availability of unbound serum iron, which can then be utilized by the organism, in ketoacidosis, and possibly in other metabolic acidoses [20–23]. As patients with adult onset Type II diabetes mellitus without metabolic acidosis are increasingly observed as presenting with mucormycosis, other mechanisms for releasing free iron are hypothesized [24]. The excessive glycosylation of proteins, such as transferrin and ferritin, also result in decreased affinity for iron and its availability as the free ion to *Rhizopus oryzae* and other Mucorales. The crucial role of iron in the pathogenesis of mucormycosis is further underscored by an increased susceptibility to infection with chronic deferoxamine therapy and in iron overload states [25–29]. Deferoxamine acts as an iron chelator in humans but as a siderophore for *Rhizopus oryzae,* thereby facilitating iron uptake and enhancement of hyphal growth.

Angioinvasion with thrombosis and tissue necrosis is a key pathophysiological feature of human Mucorales infection. In vitro*,* living and even non-viable germinated *R. oryzae* sporangiospores adhere to and are phagocytosed by endothelial cells resulting in endothelial damage [30]. The resulting entholelial injury may activate the coagulation pathway allowing or thrombosis to develop at the site of hyphal invasion of blood vessels.

The innate line of host response against filamentous fungi including Zygomycetes consists of polymorphonuclear leukocytes (PMNs) and mononuclear phagocytes. These effector cells have the ability to recognize challenging sporangiospores and hyphae through a pattern recognition system consisted of both soluble and membrane-bound molecules. The most studied pattern recognition receptors (PRRs) are Toll-like receptors (TLRs) bound on the surface of phagocytes. These receptors recognize pathogen-associated molecular patterns (PAMPs) on the surface of fungal spores or hyphae and generate molecular signal intracytoplasmically. The significance of Zygomycetes recognition for the development of infection has been demonstrated in a mucormycosis model of *Drosophila melanogaster* flies [31]. While *A. fumigatus* is recognised by both TLR2 and TLR4 [32], hyphae of *R. oryzae* are recognized only by TLR2 [33].

The signal is transduced to the nucleus where it is followed by up- or down-regulation of a great number of cytokine and chemokine genes, most notably interleukin (IL)-6 and tumor necrosis factor (TNF)-α. These molecules play a critical role on the pro-inflammatory and antifungal activities of phagocytes in response to Zygomycetes [34]. Indeed, their release is higher in response to *Rhizopus oryzae* than to all *Aspergillus* spp. including *Aspergillus fumigatus* [35] suggesting a more pronounced pro-inflammatory response to this fungus than to *Aspergillus*. Furthermore, in the *Drosophila* model, *R. oryzae* down-regulates certain genes related to immune response as compared to *A. fumigatus* [31]. The genes that are differentially regulated by the two fungal pathogens may provide pathways by which innate immunity responds to these different fungal infections.

Human phagocytes ingest *R. oryzae* sporangiospores less efficiently than *A. fumigatus* conidia [36]. Similarly, they damage hyphae of *R. oryzae* less efficiently than *A. fumigatus* [33]. Among different species of Zygomycetes, there are differences in hyphal damage. For example, *R. oryzae* and *R. microsporus* are equally susceptible to PMNs; whereas, *Absidia corymbifera*, a less virulent species, elicits more oxidative metabolites by PMNs and is damaged much more by phagocytes [37]. By comparison, *Cunninghamella bertholetiae*, a cause of less frequent but more aggressive, refractory and fatal infection, is more resistant to PMN-induced hyphal damage than *Rhizopus* spp. In addition, it induces significantly decreased IL-8, but increased TNF-α release from PMNs compared to *Rhizopus* spp. [38].

Epidemiology

The annual estimated incidence of mucormycosis in the United States is 1.7 cases/million [39]. Mucormycosis is a relatively uncommon infection, which occurs in approximately 10-fold and 50-fold less frequently, respectively, than invasive aspergillosis or candidiasis. The male to female ratio of affected patients is approximately 2:1, suggesting a male predisposition to this infection [4]. The pathophysiological basis for this predilection of mucormycosis for males is not known.

The most common underlying illnesses of ROCM are diabetes mellitus, hematological malignancies, and hematopoietic stem cell transplantation (HSCT), and solid organ transplantation [4]. Among these non-diabetic patient populations, the possible risk factors for mucormycosis include prolonged neutropenia, corticosteroid use, and graft vs. host disease (GVHD). Patients with hematological malignancy, solid organ or hematopoietic stem cell transplantation (HSCT) comprise an increasingly greater proportion of patients with mucormycosis since the 1980s [4]. This trend coincides with improved control of hyperglycemia in diabetics [40] and a concurrent increase in the incidence of mucormycosis at many large cancer centers [3•, 41, 42]. Among other biologically plausible hypotheses accounting for this evolving pattern are (a) improved posttransplantation survival rates, changes in transplant procedures, (b) use of more aggressive immunosuppressive regimens that include high doses of corticosteroids, (c) voriconazole prophylaxis creating a mycological vacuum in which the Mucorales may emerge and (4) a direct modulating effect of voriconazole on the fungal pathogen to increase virulence [43•]. Laboratory and epidemiological

data from several studies support the hypotheses that voriconazole use and high intensity immunosuppression have contributed to this increase [44–49]. As voriconazole is increasingly used prophylactically in HSCT recipients it likely exerts selective pressure for growth of resistant fungi, such as Mucorales, in high-risk patients. That a large multicenter, blinded clinical trial comparing voriconazole with fluconazole for prophylaxis in HSCT recipients did not demonstrate a difference in breakthrough mucormycosis [50] may be related to the moderate risk for invasive fungal infections in this population and to the protocol-defined use of empirical antifungal therapy with lipid formulation of amphotericin B (LFAmB).

The epidemiological trends of ROCM may differ by country [51]. Diwakar and colleagues reported that among 461 cases of mucormycosis in India, ROCM, occurring in 269 (58 %), were the most common manifestation. Cutaneous disease in 66 cases (14 %) was the second most common form. The etiologic agents encountered were *Rhizopus oryzae*, *Apophysomyces elegans*, *Saksenaea vasiformis*, *Cunninghamella bertholetiae*, *Lecthemia corymbifera*, *Basidiobolus ranarum* and *Conidiobolus coronatus*, which are a different pattern than that of other non-oryzae, non-Rhizopus spp. seen in countries of more temperate climates. The most common probable risk factor in India for ROCM was uncontrolled diabetes mellitus, in contrast to cases from North America and Europe, where transplant recipients and patients with hematological malignancies are increasingly reported with mucormycosis.

Clinical Manifestations

The clinical manifestations of mucormycosis may be classified as sinus (localized or extended to the orbit and/or brain), pulmonary, cutaneous, gastrointestinal, miscellaneous, and disseminated infection. Sinus infection is the most commonly reported presentation [4]. Patients with diabetes mellitus most commonly present with sinus disease but seldom with pulmonary infection; whereas, neutropenic patients frequently develop pulmonary infection, as well as sinus disease [4].

Sinus Mucormycosis

Sinus infection is a common clinical manifestation of mucormycosis [52, 53]. Approximately two-thirds of cases of sinus mucormycosis occur in diabetics, often with ketoacidosis; however, sinus infection may also occur in association with other forms of immunosuppression, including neutropenia, HSCT, and SOT. Nasal endoscopy may reveal necrotic ulcers along the nasal mucosa or turbinates. Infection may remain contained within the paranasal sinuses or progress into the orbit (sino-orbital) and/or brain parenchyma (rhinocerebral), constituting a medical and surgical emergency. This progression of sinus mucormycosis with extension to adjacent structures may be rapid.

The ethmoid sinus is a critical site from which sinus mucormycosis may extend through the lamina papyracea into the orbit, extraocular muscles, eye, and optic nerve. The brain may be seeded by invasion of the ethmoidal and orbital veins, which drain into the cavernous sinuses. Invasion of the cavernous sinus may involve one or more cranial nerves III, IV, VI, V1 and V2. Thus, diplopia and dysconjugate gaze are early manifestations of cavernous sinus involvement. As these cranial nerve deficits may precede detection of abnormalities on diagnostic imaging modalities, including CT and MRI scans, meticulous physical examination is important in assessing extension of mucormycosis beyond the sinuses. Infection of the ethmoid sinus may penetrate anteriorly though bone and soft tissue to create an eschar over the infected area.

Mucormycosis of the maxillary sinus has a constellation of clinical features that are different from that of ethmoid sinus mucormycosis. A painful black necrotic ulceration may develop on the hard palate, indicating extension from the maxillary sinus into the oral cavity. The maxillary sinus also may become infected by Mucorales in normal hosts from dental extraction of subjacent molars [54].

Sinus mucormycosis may present as nasal congestion, dark blood-tinged rhinorrhea or epistaxis, sinus tenderness, retro-orbital headache, fever, and malaise [55]. More advanced sinus infection may present as facial or periorbital swelling and numbness, blurred vision, lacrimation, chemosis, diplopia, proptosis, and loss of vision in the affected eye [56, 57]. Infection also can extend to adjacent bone and ultimately to the skull base.

Progression to the central nervous system occurs via the optic nerve or from the ethmoid sinuses by way of the cavernous sinus. Abnormal mentation often signifies cerebral involvement. Vision loss, ophthalmoplegia, corneal anesthesia and facial anhidrosis may indicate cavernous sinus thrombosis [58, 59], which may be further complicated by internal carotid artery thrombosis with contralateral hemiplegia [60, 61].

Sinusitis with acute onset of blurred vision or diplopia in a diabetic or otherwise immunocompromised patient should prompt careful clinical and radiological evaluation for mucormycosis, as well as rapid therapeutic intervention. The clinical manifestations of cavernous sinus thrombosis may precede radiological findings in the central nervous system. Thus, medical interventions and surgical consultation should not be delayed when cavernous sinus thrombosis is clinically evident but not yet apparent in diagnostic imaging studies. Negative diagnostic imaging does not exclude cavernous sinus mucormycosis.

Computerized tomography (CT) and magnetic resonance imaging (MRI) of the sinuses are important modalities for

delineating the extent of infection and can guide surgical debridement [62–64]. The principle radiographic findings of mucormycosis of the sinuses are opacification of the paranasal sinuses, fluid levels, bone destruction and osteomyelitis [65–67].

Orbital Mucormycosis

Orbital mucormycosis most commonly develops by extension from the ethmoidal sinus through the lamina papyracea, from the roof of the maxillary sinus, and from direct traumatic penetration into the orbital compartment.

Extension from the ethmoid sinus through the lamina papyracea may directly involve the contiguous medial rectus muscle. This invasion results in diplopia, dysconjugate gaze, and impaired medial adduction movement of the globe of the eye. Damage to the medial retus muscle may simulate an oculomotor nerve palsy. Extension through the floor of the orbit from the maxillary sinus may entrap the inferior oblique and inferior rectus muscles. Depending upon the extent of involvement of each muscle, infection of the inferior oblique will impair upward lateral gaze while damage to the inferior rectus will compromise downward gaze. Given the proximity of the two muscles, both are usually involved. Impairments of theses extraocular muscles may also simulate an oculomotor nerve palsy. Once organisms have penetrated into the orbital compartment, they may invade other extreocilurlar muscles. Direct traumatic inoculation from penetrating injury varies considerably depending upon the degree of infection within the orbit.

Orbital apex syndrome is an ominous complication of mucormycosis of the orbit [68, 69]. As a detailed understanding of the anatomy of the orbit is important to accurately understand this complication, the reader is referred to a recent review of these structures [70]. Once within the orbital compartment, organisms may extend posteriorly to the optic foramen, where the ophthalmic artery, ophthalmic nerve and optic nerve are threatened by invasion, edema, inflammation and necrosis. In approximately 40 % of patients, the ethmoidal air cells are adjacent posteriorly to the optic nerve such that invasion across the ethmoid sinus may directly encroach on this critical site. Orbital apex syndrome is most commonly manifested as painful oculomotor palsy, which ultimately ensues to proptosis and impaired visual acuity. Although mucormycosis orbital apex syndrome progress rapidly with painful ophthalmoplegia [71], the process also may be more indolent [72].

Cerebral Mucormycosis

Development of cerebral mucormycosis as a complication of sino-orbital mucormycosis is a life-threatening development. Invasion of brain tissue may develop through the ethmoidal and orbital veins that drain into the cavernous sinuses, by invasion along the optic nerve, and by direct extension into the cranial cavity from the frontal, ethmoid and sphenoid sinuses. The initial process is insidious. Early clinical manifestations may include headache, confusion, localizing pain. Nonetheless, these symptoms already may be associated with cerebral invasion. Diplopia and ophthalmoplegia may be the earliest manifestations of cavernous sinus syndrome before changes are apparent on diagnostic imaging modalities. More advanced findings include focal neurologic deficits, cranial nerve deficits, hemiparesis, and seizures.

Diagnosis

Early diagnosis of sinus mucormycosis is critical to prevention of extension to orbital and cerebral tissues [73]. The importance of early diagnosis and therapeutic intervention of mucormycosis also was recently demonstrated by Chamilos and colleagues, which found that a delay in initiation of AmB in patients who were later found to have invasive mucormycosis was associated with a significant increase in overall mortality [74]. As the symptoms, signs, and radiographic manifestations of sinus mucormycosis are nonspecific, a definitive diagnosis requires direct identification of characteristic hyphae and/or recovery of the organism in culture from specimens obtained from the site of infection. Direct examination of paranasal sinus secretions may be diagnostic. Recovery of Mucorales from a susceptible host with sinusitis should be considered as compelling evidence for infection.

Samples for direct microscopy by wet mount, cytopathological studies, or histopathologic examination may be collected by nasal endoscopy, radiographically guided percutaneous needle aspirate, and direct biopsies of infected lesions. Although obtaining biopsy material from deep tissue sites is frequently difficult in patients with thrombocytopenia or coagulopathies, histopathology is specific and reliably establishes the diagnosis of mucormycosis.

Direct Examination and Histopathology

Direct microscopic examination is performed on all materials sent to the clinical laboratory. Sinus aspirates should be submitted for examination by clinical microbiology and cytopathology laboratories. Hyphae of the Mucorales are typically broad (6 to 16 μm in diameter), ribbon-like and irregularly shaped, non-septate (coenocytic) or sparsely septate, with branches often arising non-dichotomously in “right angles.” Hyphae of the Mucorales may be difficult to observe on an unenhanced KOH wet mount and may not stain well with conventional Gram stain. The use of chitin-binding stains,

such as calcofluor, fungiflour, or blancofluor, may be used with a fluorescent microscope to identify hyphal elements on KOH wet mounts [75].

McDermott and colleagues recently reported the use of calcofluor stained tissue as a rapid technique for intraoperative diagnosis and assessment of clean resected margins in lieu of frozen sections by pathology [76]. Molecular diagnostic assays for rapid and early detection of mucormycosis are being developed but remain investigational at this time [77].

The Mucorales are usually distinguishable histologically from other filamentous fungi, such as *Aspergillus* spp, *Fusarium* spp, *Pseudallescheria boydii,* which typically appear as slender dichotomously branching septate hyphae. Distinction by direct examination may allow AmB and other potentially life-saving therapeutic interventions to be initiated.

Treatment and Prevention

Optimal therapy requires a multidisciplinary approach that relies on prompt institution of appropriate antifungal therapy with amphotericin B (AmB), reversal of underlying predisposing conditions, and, where possible, surgical debridement of devitalized tissue. Outcomes are highly dependent upon the degree of immunosuppression, site and extent of infection, timeliness of therapy, and type of treatment provided [4, 78, 79].

Antifungal Therapy with Amphotericin B

Amphotericin B is the drug of choice for primary treatment of mucormycosis. The efficacy of AmB has been reproducibly demonstrated in both laboratory (in vitro and in vivo) investigations and in clinical studies [80–89]. Although interpretive breakpoints for determination of in vitro susceptibility to AmB have not been determined, apparent in vitro resistance, with elevated minimal inhibitory concentrations (MICs), may be observed in clinical isolates, especially among *Cunninghamella* species [90–93]. These in vitro properties are consistent with the poor prognosis of mucormycosis caused by *Cunninghamella bertholetiae*, where among 34 reported cases; overall mortality was 76 % [4].

Underscoring the important therapeutic role of AmB in treatment of mucormycosis, a recent review of mucormycosis demonstrated that by multivariate analysis antifungal therapy and surgery were significantly associated with survival, that survival was similar (61 vs. 69 % respectively; $p>0.05$) for patients treated with conventional ($n=532$) or lipid formulation AmB ($n=116$), and that mortality was nearly uniform (97 %) for those who received no treatment at all. ($n=241$) [4]. Among the lipid formulations of AmB, including colloidal dispersion (ABCD), lipid complex (ABLC) and liposomal (LAmB), all have been used for treatment of mucormycosis [4, 89, 94–110]. As with many antifungal agents and mycoses, the optimal dosage for AmB and its formulations against mucormycosis has not been determined. Although there are no randomized comparative trials in the treatment of ROCM, the lipid formulations of AmB appear to be at least as active as deoxycholate AmB and have less nephrotoxicity.

If a lipid formulation of AmB is available, the initial dosage of this class may be 5.0 mg/kg/day. ABLC at 5 mg/kg/day has been used as salvage treatment with complete or partial responses in 17/24 (71 %) patients [109]. Dosages of LAmB as high as 15 mg/kg/day have been tolerated without substantial renal or infusion-related toxicity [111]; however, as plasma concentrations in that study peaked at 10 mg/kg/day justification of higher daily dosages does not seem tenable. Therapy with LAmB at 10 mg/kg/day has been reported [112], but such dosing has not been systematically compared with a standard dosage of 5 mg/kg/d. Daily dosages beyond 5 mg/kg (e.g. 7.5 or 10 mg/kg) may be considered on an individual basis, especially if there is CNS involvement. The length of therapy also should be individualized according to a patient's response and underlying condition. In the absence of definitive comparative studies of duration, continuation of antifungal therapy until complete resolution of signs and symptoms, particularly in immunocompromised patients seems reasonable.

Selection of the highest dosage possible of AmB does not necessarily result in a more favorable pharmacodynamic outcome compared to a lower and less toxic dosage. The dosage of AmB for treatment of mucormycosis has ranged from 0.5 to 1.5 mg/kg/d. A dosage of 0.75 mg/kg/d to 1.0 mg/kg/d is used by many physicians; however, these dosages may incur dose-limiting nephrotoxicity. Selection of a dosage is based upon published reports as well as careful assessment of individual patient tolerability of adverse effects. Where possible, a lipid formulation of AmB is preferable to deoxycholate AmB. However, in resource-challenged environments, deoxycholate AmB may be the only option available.

Antifungal Therapy with Posaconazole

Among the antifungal triazoles, fluconazole, voriconazole and itraconazole have little or no activity in vitro against the Mucorales [90, 91, 112–114]. Sporadic success has been reported with itraconazole for *Basidiobolus, Rhizopus,* and cutaneous *Cunninghamella* infections [115–117].

By comparison, posaconazole has more activity in vitro against the Mucorales than do the other aforementioned triazoles [90, 91, 113, 114]. Laboratory animal studies, however, demonstrate variable activity against the Mucorales, depending upon the species. Experimental infection produced by *Mucor* spp. are most responsive to posaconazole and those caused by *Rhizopus* spp. are unresponsive in most studies

[113, 118–120]. In contrast, individual case reports and several case series report that posaconazole is active in salvage therapy of patients with mucormycosis who are refractory to and/or who are intolerant of AmB therapy [121–125]. A recent review concluded that based upon the current laboratory and clinical data, orally administered posaconazole may be useful as salvage therapy, but cannot be recommended as primary therapy for mucormycosis [126]. Further studies are needed to understand the pharmacokinetics and pharmacodynamics of this agent, particularly in the parenteral formulation, in order to better understand its potential role in primary treatment of mucormycosis.

Combination Antifungal Therapy with Echinocandins

By targeting different biochemical pathways, combination antifungal therapy may increase efficacy of AmB regimens. Although the echinocandins per se have minimal activity against the Mucorales, when combined with AmB in a murine model of disseminated mucormycosis they augment antifungal activity and improve survival [87, 88]. A retrospective clinical study of patients with mucormycosis was consistent with these experimental observations; i.e., patients receiving an echinocandin plus AmB had improved response rates [89]. A prospective clinical trial is warranted in order to ascertain whether the combination of AmB and echinocandin versus AmB alone is more effective as primary therapy in treatment of mucormycosis.

Combination Antifungal Therapy with Deferasirox

Desferrioxamine increases the risk for development of disseminated and localized mucormycosis by serving as a false siderophore [45]. By comparison, hydroxypyridinone iron chelators, such as deferasirox, do not act as siderophores for the Zygomycetes and subsequently are not associated with increased susceptibility to mucormycosis. Instead, deferasirox protects mice from mucormycosis through deprivation of iron from *Rhizopus oryzae* [127]. A recent case report of a patient with rhinocerebral mucormycosis illustrates the potential benefit of this combination therapy with deferasirox [128]. A randomized trial assessing the safety and efficacy of deferasirox with AmB versus AmB alone is necessary in order to further define the role of iron chelation as primary therapy for mucormycosis.

Hyperbaric Oxygen

Hyperbaric oxygen (HBO) is sometimes used as adjunctive therapy in management of mucormycosis. The laboratory foundation for treatment of mucormycosis with HBO includes the early observations that extended exposure to high pressures (10 atm absolute, ATA) of 100 % oxygen is fungicidal for some Mucorales and other fungi in vitro [129–131]. Shorter exposures and lower oxygen pressures retain a fungistatic effect on *Rhizopus spp.* and are achievable with clinically relevant doses of HBO. The antifungal effects of HBO are likely related to generation of oxygen-based free radicals. HBO also reduces tissue hypoxia and acidosis, and enhances neutrophil function and fibroblastic collagen production. However, results of the laboratory animal studies are inconsistent, there are no randomized trials, and the potentially beneficial clinical effects of HBO are confounded by concomitant surgery and antifungal therapy. Favorable outcomes have been reported when HBO is used in conjunction with surgery and amphotericin B for sinus, cutaneous, and soft tissue mucormycosis [129–134].

Among the potential adverse effects of HBO are pneumothorax, seizures, nausea, tinnitus, and visual abnormalities. As the chamber pressures of HBO were 2 to 2.5 ATA of 100 % oxygen in most reported cases, central nervous system toxicity is uncommon at these doses. However, because of the uncontrolled nature of the clinical observations, HBO mode of therapy cannot be recommended for routine primary treatment of mucormycosis.

Reversal of the Underlying Host Impairments

Correction of a patient's underlying host impairments is a critical condition for successful management of mucormycosis. For example, hyperglycemia and metabolic acidosis (particularly diabetic ketoacidosis) should be aggressively corrected. Among transplant recipients and patients with hematological malignancies, reduction or temporary discontinuation of corticosteroids or other immunosuppressive agents, should be considered until the infection is controlled. When mucormycosis complicates deferoxamine therapy, that drug should be discontinued. As with other infections in neutropenia, response to therapy of mucormycosis hinges upon neutrophil recovery. With the exception of the single patient with severe aplastic anemia treated with GCSF, in a review of 26 neutropenic ($\leq$500 cells/mm$^{3)}$ patients with histologically documented disseminated mucormycosis occurring from 1959 to 1994, infections were uniformly fatal [135]. Granulocyte transfusions may also provide a temporizing strategy while supporting neutropenic patients with until recovery from neutropenia [136].

Immune recovery may be accelerated by treatment with granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon-γ (IFN-γ). G-CSF and GM-CSF stimulate production of neutrophils and/or monocytes and enhance their antifungal activity [37, 137, 138]. IFN-γ directly enhances the antifungal activity of host effectors cells and induces development of Th1 responses, which further augments innate

defenses against fungi [139]. Ex vivo incubation of neutrophils derived from transplant recipients with G-CSF enhances the oxidative respiratory burst against *Rhizopus* sporangiospores [140]. Similarly GM-CSF and IFN-γ augment the antifungal activity of neutrophils against *R. oryzae*, *Rhizopus microsporus*, and *Absidia* (*Mycocladus* or *Lichtheimia*) *corymbifera* [37].

Both G-CSF and GM-CSF reduce the depth and duration of chemotherapy-induced neutropenia and diminish the frequency of infections and are included in many antineoplastic protocols for hematological malignancies and HSCT [141–143]. While individual reports of adjuvant therapy for mucormycosis with G-CSF, GM-CSF and IFN-γ have been published [135, 144, 145]. As a general principle, the use of recombinant cytokines G-CSF or GM-CSF, for acceleration of recovery from neutropenia is biologically sound and supported by randomized trials. However, the efficacy of recombinant cytokines in non-neutropenic patients with mucormycosis has not been evaluated through adequately powered randomized controlled trials. Their use in such conditions should be individualized for each patient.

Granulocyte transfusions are an alternative approach for augmentation of innate phagocytic host defenses against invasive fungal infections [137, 146]. Early studies of this therapeutic modality were limited by difficulties in collecting adequate doses of leukocytes from healthy steroid-mobilized donors. Treatment of donors with G-CSF with or without corticosteroids increases the yield of neutrophils, allowing for as much as a 10 to 100-fold increase in yield of transfused cells and sustained concentrations of circulating leukocytes [147–150]. At this time efficacy data for neutrophil transfusion in mucormycosis are limited and potential benefits should be weighed against known complications, including respiratory distress, alloimmunization, and anaphylaxis. Nonetheless, granulocyte transfusions with cytokine augmentation may provide critical support to a neutropenic host with a life-threatening infection until recovery from neutropenia ensues [151]. In resource-challenged environments, granulocyte transfusions and recombinant cytokines may not be available as adjunctive modalities.

Surgical Therapy

Surgical debridement is fundamental for successful management of most cases of ROCM. Infection is associated with angioinvasion and extensive necrosis, and thus antifungal therapy alone may be inadequate for control of infection. Surgery should be considered early in the course of treatment with the goal of removing all necrotic tissue. Repeated debridements are frequently necessary and the extent of surgery should ideally be guided by evaluation of frozen tissue sections examined histologically or by fresh homogenized specimens stained by calcofluor [75, 152].

Compared with antifungal therapy alone, survival is enhanced with a combined medical/surgical approach [4, 78, 153, 154]. The type of surgical procedure is dictated by the extent of the patient's infection. For maxillofacial infection, there is increasing emphasis on using less disfiguring surgical procedures while simultaneously controlling the infection with medical interventions [75, 126, 128, 155].

Prevention of Mucormycosis

The most important strategy for prevention of ROCM consists of maintaining adequate host defenses. These include but are not limited to maintaining adequate blood glucose in diabetics, shortening durations of neutropenia, and using corticosteroids and other immunosuppressive agents judiciously. Instructing patients to avoid aerosols of soil, dust and debris may reduce exposure to large respiratory inocula of sporangiospores. Similarly, maintaining appropriate environmental control measures within health care facilities may also help to prevent acquisition of organisms.

Control of environmental transmission during hospital construction and renovation can be established with floor to ceiling impervious barriers. Air conditioning and ventilation systems should be monitored for microbial contamination. While the use of high-efficiency particulate air (HEPA) filters in hospital rooms of profoundly immunosuppressed patients reduce the risks of development of aspergillosis and mucormycosis, financial constraints preclude many centers from routinely using these devices in resource-constrained settings. Whether AmB formulations or posaconazole can prevent ROCM is conceptually plausible but has not been definitively demonstrated in clinical studies.

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