Novel Perspectives on Mucormycosis: Pathophysiology, Presentation, and Management

Brad Spellberg, John Edwards, Jr., and Ashraf Ibrahim

Department of Medicine, Los Angeles Biomedical Institute at Harbor-UCLA Medical Center, Torrance, California, and David Geffen School of Medicine at UCLA, Los Angeles, California

INTRODUCTION

The zygomycoses are infections caused by fungi of the class Zygomycetes, comprised of the orders Mucorales and Entomophthorales. The Entomophthorales are rare causes of subcutaneous and mucocutaneous infections known as entomophthoromycosis, which largely afflict immunocompetent hosts in developing countries. In contrast, fungi of the order Mucorales are causes of mucormycosis, a life-threatening fungal infection almost uniformly affecting immunocompromised hosts in either developing or industrialized countries.

Fungi belonging to the order Mucorales are distributed into six families, all of which can cause cutaneous and deep infections (129). Species belonging to the family Mucoraceae are isolated more frequently from patients with mucormycosis than any other family. Among the Mucoraceae, Rhizopus oryzae (Rhizopus arrhizus) is by far the most common cause of infection (129). Other less frequently isolated species of the Mucoraceae family that cause a similar spectrum of infections include Rhizopus microsporus var. rhizopodiformis, Absidia corymbifera, Apophysomyces elegans, Mucor species, and Rhizomucor pusillus (61, 81, 129). Increasing cases of mucormycosis have been also reported due to infection with Cunninghamamella spp. (in the Cunninghamamellaceae family) (24, 78, 82, 161). To date, rare case reports have demonstrated the ability of species belonging to the remaining four families to cause mucormycosis (12, 68, 72, 89, 129).

PATHOGENESIS

Host Defenses

Both mononuclear and polymorphonuclear phagocytes of normal hosts kill Mucorales by the generation of oxidative metabolites and the cationic peptides defensins (33, 166, 169) (Fig. 1). Clinical evidence demonstrates that these phagocytes are the major host defense mechanism against mucormycosis. For example, neutropenic patients are at increased risk of developing mucormycosis. Furthermore, patients with dysfunctional phagocytes are also at higher risk for developing mucormycosis. Hyperglycemia and acidosis are known to impair the ability of phagocytes to move toward and kill the organisms by both oxidative and nonoxidative mechanisms (23). Additionally, corticosteroid treatment affects the ability of mouse bron-
choalveolar macrophages to prevent germination of the spores in vitro or after in vivo infection induced by intranasal inoculation (169). The exact mechanisms by which ketoacidosis, diabetes, or steroids impair the function of these phagocytes remain unknown.

Role of Iron in Pathogenesis

A recently identified important clinical feature is the increased susceptibility to mucormycosis of patients with elevated available serum iron. It has been known for two decades that patients treated with the iron chelator deferoxamine have a markedly increased incidence of invasive mucormycosis (16). However, it is now clear that iron chelation is not the mechanism by which deferoxamine enables mucormycosis infections. While deferoxamine is an iron chelator from the perspective of the human host, *Rhizopus* spp. actually utilize deferoxamine as a siderophore to supply previously unavailable iron to the fungus (15, 30). *Rhizopus* spp. can accumulate 8- and 40-fold-greater amounts of iron supplied by deferoxamine than can *Aspergillus fumigatus* and *Candida albicans*, respectively, and this increased iron uptake by *Rhizopus* spp. is linearly correlated with its growth in serum (15). Additionally, data from animal models emphasize the exceptional requirement of iron for *Rhizopus* pathogenicity since administration of deferoxamine or free iron worsens survival of animals infected with *Rhizopus* spp. but not *Candida albicans* (1, 16, 30, 158). Finally, animal models have demonstrated that other iron chelators, which are not used as siderophores by the fungus, do not similarly exacerbate mucormycosis infection (16).

Patients with diabetic ketoacidosis are at high risk of developing rhinocerebral mucormycosis (61, 81, 129). Multiple lines of evidence support the conclusion that patients in systemic acidosis have elevated levels of available serum iron, likely due to release of iron from binding proteins in the presence of acidosis (9). For example, sera collected from patients with diabetic ketoacidosis supported growth of *Rhizopus oryzae* in the presence of acidic pH (7.3 to 6.88) but not in the presence of alkaline pH (7.78 to 8.38). Acidic sera that supported the growth of *R. oryzae* were found to contain increased available serum iron (69 μg/dl versus 13 μg/dl for sera which did not support the growth of *R. oryzae*). Finally, simulated acidic conditions decreased the iron-binding capacity of sera collected from normal volunteers, suggesting that acidosis...
temporarily disrupts the capacity of transferrin to bind iron (9). Therefore, the increased susceptibility to mucormycosis of patients with diabetic ketoacidosis is likely due at least in part to an elevation in available serum iron during diabetic ketoacidosis.

**Fungal-Endothelial Interactions**

A hallmark of mucormycosis infections is the virtually uniform presence of extensive angioinvasion with resultant vessel thrombosis and tissue necrosis. This angioinvasion is associated with the ability of the organism to hematothegously disseminate from the original site of infection to other target organs. Hence, damage of and penetration through endothelial cells lining blood vessels is likely a critical step in the organism’s pathogenetic strategy. *R. oryzae* spores but not germcells (i.e., pregerminated spores) have the ability to adhere to subendothelial matrix proteins including laminin and type IV collagen in vitro (17). Similarly, we have recently found that *R. oryzae* spores adhere to subendothelial matrix proteins significantly better than do *R. oryzae* hyphae, however spores and hyphae adhere equivalently to human umbilical vein endothelial cells (64). The disparity of spore and germ tube adherence to subendothelial matrix proteins but equivalent adherence to endothelial cells indicates that *R. oryzae* adhesions to endothelial cells are likely distinct from the adhesins used to bind to subendothelial matrix proteins.

We also found that germings of *R. oryzae* damage endothelial cells in vitro. This damage is independent of serum factors and requires phagocytosis of *R. oryzae* by endothelial cells (64). Surprisingly, *R. oryzae* viability was not required for endothelial cell damage, but phagocytosis was required for dead *R. oryzae* to cause damage (64). In a subsequent pilot study, intravenous administration of four doses of heat-killed *R. oryzae* blastospores resulted in a 40% mortality in diabetic mice (unpublished observations). The precise mechanisms by which dead *R. oryzae* mediates tissue injury remain unclear. Nevertheless, the clinical implication is that simply killing *R. oryzae* once it has already established a presence in tissue may not prevent subsequent tissue injury, perhaps in part explaining the lack of efficacy of cidal antifungal agents during clinical disease.

**CLINICAL PRESENTATION**

**General Principles**

As mentioned earlier, the clinical hallmark of mucormycosis is vascular invasion resulting in thrombosis and tissue infarction/necrosis. Mucormycosis virtually always occurs in patients with defects in host defense and/or with increased available serum iron, although rare cases have been reported in apparently normal hosts (83, 85, 119). In most cases, the infection is relentlessly progressive and results in death unless treatment with a combination of surgical debridement and antifungal therapy is initiated promptly.

**Epidemiology and Disease Manifestations**

Mucormycosis is less common than other opportunistic fungal infections, such as those caused by *Candida* and *Aspergillus* spp. One population-based study estimated the incidence of mucormycosis to be 1.7 cases per million people per year, which translates to approximately 500 cases per year in the United States (126). In autopsy series, the prevalence of mucormycosis has ranged from 1 to 5 cases per 10,000 autopsies, making the infection 10- to 50-fold less common than invasive *Candida* or *Aspergillus* infections (56, 154, 178). Finally, in patients at higher risk, such as those undergoing allogeneic bone marrow transplantation, the prevalence of mucormycosis has been described to be as high as 2 to 3% (90, 96).

Based on clinical presentation and the involvement of a particular anatomic site, mucormycosis can be divided into at least six clinical categories: (i) rhinocerebral, (ii) pulmonary, (iii) cutaneous, (iv) gastrointestinal, (v) disseminated, and (iv) miscellaneous. Of note, these categories of invasive mucormycosis tend to occur in patients with specific defects in host defense (Table 1). For example, diabetes in ketoacidosis typically develop the rhinocerebral form of the disease, and much more rarely develop pulmonary or disseminated disease (75, 99, 107, 118).

The mechanism for ketoacidosis preferentially causing susceptibility to the rhinocerebral form of the disease remains unclear. As mentioned earlier, patients in ketoacidosis, or indeed any systemic acidosis, have increased available iron in serum due to dissociation of iron from sequestering proteins in acidic conditions (9). However, the predominant presentation of mucormycosis in the setting of deferoxamine therapy is disseminated disease (37, 46, 123, 151, 160), indicating that increased available iron cannot, by itself, explain the preferential occurrence of rhinocerebral disease in ketoacidosis. Furthermore, while it is known that hyperglycemia and acidosis negatively impact neutrophil chemotaxis and phagocytic activity (23), these observations cannot explain the preferential occurrence of rhinocerebral disease in diabetic ketoacidosis because neutropenic patients more commonly develop pulmonary mucormycosis than rhinocerebral disease (98, 150).

Much more readily understood are the risk factors for invasive skin and soft tissue infections caused by the agents of mucormycosis. These infections occur in patients with disrupted cutaneous barriers, as a result of either traumatic implantation of soil, maceration of skin by a moist surface (5, 119), or even via direct access through intravenous catheters or subcutaneous injections (4, 73, 125). These cases illustrate an alarming trend in the epidemiology of mucormycosis. Mucormycosis, formerly virtually always community acquired and often in the setting of diabetic ketoacidosis, is rapidly becoming a nosocomial infection in patients with malignancy or undergoing organ or hematopoietic cell transplantation (108). Given the increasing incidence of diabetes and cancer in the

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increasingly obese and elderly United States population, it is not surprising that a recent review found a marked increase in reported cases of mucormycosis over the last two decades (45). As mentioned, there has also been a shift from community onset to nosocomial onset of disease. Nosocomial mucormycosis has been associated with iatrogenic immunosuppression (79, 108, 130) and a variety of procedures or devices used in hospitals, including antifungal prophylaxis (69, 130), bandages or medication patches (43, 119), intravenous catheters (4, 11, 74, 87), and even tongue depressors (50, 54, 92) (see below).

At transplant centers there has also been an alarming rise in the incidence of mucormycosis (69, 140). For example, at the Fred Hutchinson Cancer Center, Marr et al. have described a doubling in the number of cases from 1985 to 1989 to 1995 to 1999 (94). Similarly, Kontoyianis et al. have described a greater than doubling in the incidence of mucormycosis in transplant patients over a similar time span (79). In patients undergoing hematological stem cell transplantation, mucormycosis develops at least as commonly in nonneutropenic periods as in neutropenic periods. For example, two major transplant centers have recently reported that more than half the cases of mucormycosis occurred more than 90 days after transplantation (90, 94).

Major risk factors for mucormycosis in the transplant setting include underlying myelodysplastic syndrome (possibly due to iron overload from repeated blood transfusions) and graft-versus-host disease treated with steroids (90, 94, 116, 140). Administration of antithymocyte globulin may also pose a risk for mucormycosis (140). Although less than half of these patients are neutropenic at the time of disease onset, prolonged neutropenia is a risk factor for mucormycosis in this setting (130), as are diabetes mellitus and steroid use (130). The role of antifungal prophylaxis in predisposing patients to developing mucormycosis is increasingly being described, as discussed further below. Prophylaxis with either itraconazole (130) or voriconazole (65, 66, 69, 96, 163) has been implicated in predisposing to mucormycosis.

**Rhinocerebral mucormycosis.** Rhinocerebral mucormycosis continues to be the most common form of the disease, accounting for between one-third and one-half of all cases of mucormycosis (122). About 70% of rhinocerebral cases (occasionally referred to as craniofacial) are found in diabetic patients in ketoacidosis (99). More rarely, rhinocerebral mucormycosis has also occurred in patients who received a solid organ transplant or those with prolonged neutropenia (2, 45, 118, 119, 122, 179). Recently, rhinocerebral disease has been an increasing problem in patients undergoing hematopoietic stem cell transplantation (94, 102). These cases have largely been associated with steroid use for graft-versus-host disease.

The initial symptoms of rhinocerebral mucormycosis are consistent with either sinusitis or periorbital cellulitis (32, 149) and include eye or facial pain and facial numbness, followed by the onset of conjunctival suffusion, blurry vision, and soft tissue swelling (75, 118, 153). Fever is variable and may be absent in up to half of cases (149); white blood cell counts are typically elevated, as long as the patient has functioning bone marrow (153). If untreated, infection usually spreads from the ethmoid sinus to the orbit, resulting in loss of extraocular muscle function and proptosis. Marked chemosis may also be seen. The infection may rapidly extend into the neighboring tissues. Onset of signs and symptoms in the contralateral eye, with resulting bilateral proptosis, chemosis, vision loss, and ophthalmoplegia, is an ominous sign that suggests the development of cavernous sinus thrombosis.

Upon visual inspection, infected tissue may appear normal during the earliest stages of spread of the fungus. Infected tissue then progresses through an erythematous phase, with or without edema, before onset of a violaceous appearance, and finally the development of a black, necrotic eschar as the blood vessels become thrombosed and tissue infarction occurs (57, 119). Infection can sometimes extend from the sinuses into the mouth and produce painful, necrotic ulcerations of the hard palate (119).

Cranial nerve findings represent extensive infection and signal a grave prognosis. Progressive vision loss and ultimately blindness may result either from involvement of the optic nerve or from arteriolar invasion resulting in infarction (58, 109, 143, 153) or from cavernous sinus thrombosis. Cranial nerves five and seven may also be affected, resulting in ipsilateral loss of facial sensation and palsy and papillary dilation (32, 118, 153). Infection can also spread posteriorly from either the orbit or sinuses to the central nervous system. A bloody nasal discharge may be the first sign that infection has invaded through the terbinates and into the brain. When there is extensive central nervous system involvement, the angioinvasive nature of the fungus may result in cavernous sinus thrombosis and internal carotid artery encasement and thrombosis with extensive resulting cerebral infarctions (7, 88, 104, 153). Occasional cerebral vascular invasion may lead to hematogenous dissemination of the infection (84, 122), with or without development of mycotic aneurysms (135).

A high index of suspicion is required to make the diagnosis of rhinocerebral mucormycosis, as evidenced by the fact that autopsy series have found up to half of cases are diagnosed postmortem (78, 101, 154). Imaging techniques may be suggestive of mucormycosis but are rarely diagnostic. Indeed, the initial imaging study is frequently negative or has only subtle findings. The most common finding on computerized tomography (CT) scanning of the head or sinuses is subtle sinus mucosal thickening or thickening of the extracranial muscles. It is also common to detect no abnormalities in the bones of the sinuses despite clinical evidence of progressive disease (149). However, when present, the finding of bony erosion of the sinuses is strongly suggestive of the diagnosis in the appropriate clinical context (e.g., patient in diabetic ketoacidosis with proptosis). It should be emphasized that it is very uncommon to visualize an organized retroorbital mass.

Although evidence of infection of the soft tissues of the orbit may sometimes be seen by CT scan, magnetic resonance imaging is more sensitive (40). Still, as with CT scans, patients with early rhinocerebral mucormycosis may have a normal magnetic resonance imaging, and surgical exploration with biopsy of the areas of suspected infection should always be performed in high-risk patients. It is critically important to emphasize that if mucormycosis is suspected, initial empirical therapy with a polyene antifungal should begin while the diagnosis is being confirmed, rather than waiting while a protracted series of diagnostic tests are completed.

Given the limitations of imaging studies, diagnosing mucormycosis almost always requires histopathologic evidence of fungal invasion of the tissues. Culturing organisms from a
potentially infected site is rarely sufficient to establish the diagnosis of mucormycosis because the causative agent is ubiquitous, may colonize normal persons, and is a relatively frequent laboratory contaminant. Additionally, the organism may be killed during tissue grinding (168), which is routinely used to process tissue specimens for culture. Thus, a sterile culture does not rule out the infection (149). Furthermore, waiting for the results of the fungal culture may delay the institution of appropriate therapy.

There are no reliable serologic, PCR-based, or skin tests for mucormycosis. Therefore, the diagnosis should be made by biopsy of infected tissues. The biopsy should demonstrate the characteristic wide, ribbon-like, aseptate hyphal elements that branch at right angles. The organisms are often surrounded by extensive necrotic debris. Other fungi, including Aspergillus, Fusarium, or Scedosporium spp, may look similar to the Mucorales on biopsy. However, these molds have septae, are usually thinner, and branch at acute angles. The genus and species of the infecting organism may be determined by culture of the infected tissue. However, the organism is rarely isolated from cultures of blood, cerebrospinal fluid, sputum, urine, feces or swabs of infected areas.

**Pulmonary mucormycosis.** Mucormycosis of the lung occurs most commonly in leukemic patients who are receiving chemotherapy or in patients undergoing hematopoietic stem cell transplants. Indeed, the pulmonary form of the disease is the most common form found in neutropenic and stem cell-transplant patients (94, 102). These patients typically have severe neutropenia and are frequently receiving broad-spectrum antibiotics for unremitting fever (150), while in patients undergoing allogeneic stem cell transplantation, the disease often occurs postengraftment and is strongly associated with graft-versus-host disease. Patients with diabetic ketoacidosis can also develop pulmonary mucormycosis, although infections in these patients are less common and less fulminant and follow a more subacute course than is typically seen in patients with neutropenia (132, 150).

Pulmonary mucormycosis may develop as a result of inhalation or by hematogenous or lymphatic spread. Symptoms of pulmonary mucormycosis include dyspnea, cough, and chest pain (150). In a recent series of 32 cases of pulmonary mucormycosis, fever was present in the majority of patients (150). Angioinvasion results in necrosis of tissue parenchyma, which may ultimately lead to cavitation and/or hemoptysis, which may be fatal if a major blood vessel is involved (49, 171).

Radiographically, a variety of findings may be present, including, in descending order of frequency: lobar consolidation, isolated masses, nodular disease, and cavitation (70, 98, 150). Wedge-shaped infarcts of the lung may also be seen, particularly following thrombosis of the pulmonary vessels due to fungal angioinvasion (93). High-resolution chest CT scan is the best method of determining the extent of pulmonary mucormycosis and may demonstrate evidence of infection before it is seen on the chest x-ray. One suggestive finding is expansion of a mass or consolidation across tissue planes, in particular towards the great vessels in the mediastinum (127). Unfortunately, sputum culture is highly unreliable. In two case series, sputum and bronchial alveolar lavage cultures were negative in 18 of 19 cases of biopsy-proven pulmonary mucormycosis (127, 150). Therefore, biopsy with histopathological assessment remains the best modality to diagnose pulmonary mucormycosis.

If pulmonary infection is not treated, hematogenous dissemination to the contralateral lung and other organs frequently occurs. Patients with untreated pulmonary mucormycosis usually die from disseminated disease before respiratory failure occurs. The notable exception is the rare patient with massive hemoptysis (103, 113). The overall mortality of pulmonary mucormycosis is approximately 50 to 70% but is >95% if the pulmonary mucormycosis is part of a disseminated process (45, 150).

**Cutaneous mucormycosis.** As mentioned, patients who are at high risk of developing cutaneous mucormycosis are those with disruption of the normal protective cutaneous barrier. The agents of mucormycosis are typically incapable of penetrating intact skin. However, burns, traumatic disruption of skin, and persistent maceration of skin enable the organisms to penetrate into deeper tissues. A typical case results from traumatic implantation of soil, for example, as a result of a motor vehicle accident or penetrating injury with plant material (e.g., a thorn) (4, 119). In diabetic and immunocompromised patients, cutaneous lesions may also arise at insulin injection or catheter insertion sites (73, 125). Contaminated surgical dressings have also been implicated as a source of cutaneous mucormycosis (43, 100). Cutaneous mucormycosis has also occurred in the context of contaminated tape used to secure an endotracheal tube in a ventilated patient (5).

Cutaneous disease can be very invasive locally and penetrate from the cutaneous and subcutaneous tissues into the adjacent fat, muscle, fascia, and even bone. Secondary vascular invasion may also lead to hematogenously disseminated infection of the deep organs. Cutaneous and subcutaneous disease may lead to necrotizing fasciitis, which has a mortality approaching 80% (18, 80, 115, 124). However, isolated cutaneous mucormycosis (i.e., not disseminated disease) has a favorable prognosis and a low mortality if aggressive surgical debridement is done promptly (4).

**Gastrointestinal mucormycosis.** Mucormycosis of the gastrointestinal tract is rare. It mainly occurs in patients who are extremely malnourished (especially infants or children) and is thought to arise from ingestion of the fungi. In particular, gastrointestinal mucormycosis has been seen in premature neonates, often in association with widespread disseminated disease (6, 27, 71, 76, 128, 137). Necrotizing enterocolitis has been described largely in premature neonates (35, 71, 106, 128, 139, 157, 174, 177) and more rarely in neutropenic adults (146, 152). Rare cases of gastrointestinal mucormycosis have been described in association with other immune-compromising conditions, including AIDS (19), systemic lupus erythematosus (55), and organ transplantation (77, 95, 97, 138).

The stomach, colon, and ileum are the most commonly involved sites. Cases of hepatic mucormycosis have also been associated with ingestion of herbal medications (111). Because this infection is acute and rapidly fatal, it is often diagnosed postmortem. The symptoms are varied and depend on the site affected. Nonspecific abdominal pain and distention associated with nausea and vomiting are the most common symptoms. Fever and hematochezia may also occur. The patient is often
thought to have an intra-abdominal abscess. The diagnosis may be made by biopsy of the suspected area during surgery or endoscopy. Recently, a iatrogenic outbreak of gastric mucormycosis occurred due to contamination of the wooden applicators used to mix drugs that were poured down the patients' nasogastric feeding tubes (92). These patients presented with massive gastric bleeds. The diagnosis was made by culture of gastric aspirates and culture of the box of wooden tongue depressors. This experience further underscores the alarming trend of increasing iatrogenic/nosocomial onset for mucormycosis.

**Disseminated mucormycosis.** Hematogenously disseminated mucormycosis may originate from any primary site of infection. Pulmonary mucormycosis in severely neutropenic patients has the highest incidence of dissemination. Less commonly, dissemination can arise from the gastrointestinal tract, the sinuses, or cutaneous lesions, the last occurring particularly in burn patients. The most common site of dissemination is the brain, but metastatic lesions may also be found in the spleen, heart, skin, and other organs. Cerebral infection following dissemination is distinct from rhinocerebral mucormycosis and results in abscess formation and infarction. Patients present with sudden onset of focal neurological deficits or coma. The mortality associated with dissemination to the brain approaches 100% (144). Even without central nervous system involvement, disseminated mucormycosis has a mortality of >90% (45). In patients undergoing hematopoietic stem cell transplantation, the 1-year mortality is >95% due to a combination of underlying disease, graft-versus-host disease, and the infection (57, 94).

Recent case series have described frequent dissemination in the context of voriconazole prophylaxis of transplant patients. In five recent case series, a total of 18 cases of disseminated mucormycosis have occurred in patients post-allogeneic hematopoietic stem cell transplantation who were receiving voriconazole either prophylactically or therapeutically for other infections (65, 66, 96, 140, 163). An additional patient who had leukemia and was treated with combination voriconazole plus caspofungin for proven *Aspergillus* pneumonia, who subsequently developed disseminated mucormycosis, has been described (13). Finally, three patients who developed mucormycosis while receiving voriconazole for empirical neutropenic fever (not in the transplant setting) or for prophylaxis postrenal transplantation have been described (112, 163).

It has been pointed out that the increase in frequency of mucormycosis in transplant patients preceded the availability of voriconazole and that therefore the precise role of voriconazole in predisposing patients to mucormycosis is unclear (69). For example, increasingly intensive immunosuppressive regimens and broader availability of allogeneic transplantation (e.g., to patients of increasing age) may be playing a role. Furthermore, the increasing use of peripheral stem cell transplants (in lieu of bone marrow-derived cells), nonmyeloablative conditioning regimens, and unrelated donor and/or HLA-mismatched transplants have increased the incidence of graft-versus-host disease. As mentioned, graft-versus-host disease and its treatment with corticosteroids are strongly linked with the risk of mucormycosis in the transplant setting (90, 94, 116, 140). Indeed, most of the reported cases of mucormycosis occurring during voriconazole therapy have occurred in patients receiving corticosteroids for graft-versus-host disease (66, 96, 140).

Nevertheless, the uniformity of the reports of mucormycosis in patients receiving voriconazole infections implicates a link between the drug and the disease. Voriconazole has broad activity against *Aspergillus*, *Candida*, and *Scedosporium* spp. and the dematiaceous fungi, but has no clinically relevant activity against the agents of mucormycosis (34, 117, 120, 147). Therefore, it is possible that the predisposition to mucormycosis is due to selective inhibition of other fungi, which allows the agents of mucormycosis to colonize the patient. As well, it is also possible that voriconazole is preventing early-onset deadly infections caused by other species of fungi (i.e., *Candida* and *Aspergillus*), thereby allowing highly immunocompromised patients, who in the past would have died earlier posttransplant, to live long enough to become infected with the agents of mucormycosis. It should be reiterated that a similar phenomenon has been described with itraconazole prophylaxis, the use of which is also an independent risk factor for development of mucormycosis in this setting (130). Finally, there have been two case reports of breakthrough mucormycosis in patients receiving either caspofungin (133) or caspofungin plus voriconazole (13) for other infections.

The diagnosis of disseminated disease is difficult because patients are usually severely ill from multiple diseases and virtually always have negative blood cultures. If there is evidence of infarction in multiple organs, the diagnosis of mucormycosis should be considered. However, aspergillosis is commonly associated with an identical clinical picture. When disseminated mucormycosis is suspected, a careful search should be made for cutaneous lesions that can be biopsied for diagnostic purposes.

**Miscellaneous forms.** Agents of the Mucorales may cause infection in virtually any body site. Brain involvement in the absence of sinus infection, endocarditis, and pyelonephritis occur occasionally, mainly in intravenous drug abusers (156, 162, 164, 176). Other reports have described mucormycosis in bones (91, 121), mediastinum (25, 86), trachea (8, 175), kidneys (172), and peritoneum associated with dialysis (4, 136). Other unusual forms of infection include superior vena cava syndrome (51) and external otitis (110). Although mucormycosis is not commonly seen in AIDS patients, there have been a number of case reports of this infection in this patient population (41, 105, 134).

**TREATMENT**

**General Principles**

Four factors are critical for eradicating mucormycosis: rapidity of diagnosis, reversal of the underlying predisposing factors (if possible), appropriate surgical debridement of infected tissue, and appropriate antifungal therapy. Early diagnosis is important because small, focal lesions can often be surgically excised before they progress to involve critical structures or disseminate (107). Unfortunately, there are no serologic or PCR-based tests to allow rapid diagnosis. As mentioned, autopsy series have reported that up to half the cases of mucormycosis are diagnosed postmortem (78, 101, 154), un-
underscoring the critical need to maintain a high index of clinical suspicion and to aggressively pursue diagnostic biopsy. Correcting or controlling predisposing problems is also essential for improving the treatment outcome. In diabetic ketoacidotic patients, hyperglycemia and acidemia should be corrected. Discontinuation of deferoxamine or immunosuppressive therapy, particularly steroids, should be strongly considered when the diagnosis of mucormycosis is made.

Given the rapidly progressive nature of rhinocerebral mucormycosis and the marked increase in mortality when the fungus penetrates the cranium, any diabetic patient with a headache and visual changes is a candidate for prompt evaluation with imaging studies and nasal endoscopy to rule out mucormycosis. Furthermore, to reiterate a concept that is frequently poorly grasped by clinicians inexperienced with mucormycosis, the initial imaging study is frequently negative or has subtle findings. Radiographic findings lag behind clinical progression in this disease, and a negative imaging study does not provide a rationale to delay more aggressive diagnostic maneuvers (e.g., endoscopy with biopsy) if clinical suspicion is high. The appearance of tissue at endoscopy may also lag behind invasion, as the mucosa can appear pink and viable during the initial phase of fungal invasion. Therefore, if the suspicion for disease is high, blind biopsies of sinus mucosa and/or thickened extraocular muscles are warranted to make the diagnosis.

Finally, time is of the essence in the management of mucormycosis. Because patients with rhinocerebral disease may initially present with normal mental status and appear clinically stable, the urgency for establishing the diagnosis is frequently underappreciated. The key concept is that initial spread of the fungus to the brain may be relatively asymptomatic. Once the fungus has penetrated the cranium or entered the major intracranial vasculature, mortality increases substantially. Additionally, starting the patient on an antifungal is not definitive therapy, since surgery may be a key addition to the treatment strategy. The sensitivity of the organisms varies considerably, so that a patient on amphotericin B alone may be receiving completely ineffective therapy during the diagnostic period. Minutes and hours count, and if the clinical suspicion is high, the workup should proceed on an emergent basis even if the patient currently appears clinically stable. Indeed, delayed diagnosis has been associated with a dramatically worse outcome (75). One strategy to expedite the workup is to rely upon frozen sections to guide further diagnostic and therapeutic decisions rather than waiting for fixed and stained histopathology from a biopsy. Use of frozen sections in this setting has been shown to shorten the time to diagnosis and has been associated with improved outcomes in two recent case series (48, 53).

Role of Surgery

Mucormycosis is frequently rapidly progressive, and antifungal therapy alone is often inadequate to control the infection. The numerous agents of mucormycosis have a broad range of susceptibilities to antifungal agents; some strains may be highly resistant to amphotericin B. Furthermore, the hallmark angioinvasion, thrombosis, and tissue necrosis of this disease result in poor penetration of anti-infective agents to the site of infection. Therefore, even if the causative organism is susceptible to the treating antifungal agent in vitro, the antifungal may be ineffective in vivo. Finally, surgery is necessary due to the massive amount of tissue necrosis occurring during mucormycosis, which may not be prevented by killing the organism (63). Surgical debridement of infected and necrotic tissue should be performed on an urgent basis.

In rhinocerebral mucormycosis, early surgical excision of the infected sinuses and appropriate debridement of the retro-orbital space can often prevent the infection from extending into the eye, thereby obviating the need for enucleation and resulting in extremely high cure rates (>85%) (107). Repeated surgical exploration of the sinuses and orbit may be necessary to ensure that all necrotic tissue has been debrided and the infection has not progressed. Published case series continue to support the need for surgical debridement to optimize outcomes. For example, in a case series totaling 49 patients with rhinocerebral mucormycosis, the mortality was 70% in cases treated with antifungal agents alone versus 14% in cases treated with antifungal agents plus surgery (75, 118). Similarly, in a combined series of rhinocerebral, cutaneous, and pulmonary mucormycosis, 11 of 17 (65%) patients treated with surgery plus antifungal agents survived the infection, compared to zero of seven (0%) patients treated with antifungal agents alone (119). Clearly there is the potential for selection bias in these case series, as patients who do not undergo surgery may have fundamental differences in severity of illness or comorbidities. Nevertheless, the observational clinical data support the concept that surgical debridement is necessary to optimize cure rates.

In patients with pulmonary mucormycosis, surgical treatment plus antifungal therapy also greatly improves outcome compared to the use of antifungal therapy alone (10, 79, 116, 127, 150). In one series, the mortality of patients treated with antifungal agents alone was 68%, versus 11% in patients treated with antifungal agents plus surgery (150).

Finally, localized (nondisseminated) cutaneous mucormycosis treated with aggressive surgical debridement and adjunctive antifungal therapy has a mortality of <10% (4, 73). A similar experience has been described with isolated renal mucormycosis (172). However, because surgical debridement of necrotic tissue is frequently highly disfiguring, if the patient survives the acute phase of the disease, major reconstructive surgery may be necessary.

Antifungal Therapy

A major obstacle for clinicians to choose among the current available antifungal agents in treating mucormycosis is the lack of available clinical trials (Table 2). Prospective interventional study of mucormycosis has been impractical for several reasons. First, although the disease is unusually deadly, it occurs at a lower frequency relative to other opportunistic infections. By extrapolation from studies of Aspergillus infection (52), which is more common, dozens to possibly even hundreds of trial sites and multiple years would be required to accrue sufficient patients to adequately power a standard phase III superiority study. Such a study would undoubtedly cost tens of millions of dollars, and mucormycosis cases represent an insufficient po-
potential market to spur any pharmaceutical company to sponsor such a study.

An additional barrier to clinical trials of mucormycosis is the abysmal rate of success of monotherapy. Because of this low success rate, it might be considered unethical to randomize patients in a clinical trial to any “less intensive” regimen (i.e., standard-dose versus high-dose monotherapy, monotherapy versus combination therapy, etc.). For these reasons, prospective interventional trials have not been performed. Lacking any significant clinical trial data, physicians have been forced to rely upon anecdotal case reports, limited retrospective reviews, and unpublished observations in determining the first-line therapy for mucormycosis. Such reports are intrinsically subject to publication and observer bias and allow no comparison of the relative efficacies of various treatment strategies. For these reasons, animal models of mucormycosis are essential to provide well-controlled comparative analyses of antifungal therapies.

Several murine models have been developed to study mucormycosis in vivo, including intravenous (59), intranasal (169), and intrasinus (167) diabetic mice models. Additionally, neutropenic (148), corticosteroid- (169), and deferoxamine-treated mouse models (1) and a deferoxamine-treated guinea pig model (15, 16) have been reported. More rarely, immunocompetent mice have been studied (28). A variety of species have been utilized in these models, including \textit{R. oryzae}, \textit{R. microsporus}, and \textit{Mucor} and \textit{Ab- sidia} spp. There is no clear advantage to any one of these models in evaluating the efficacy of different antifungal regimens, and none of the models completely accurately recapitulates the normal route of infection (inhalation) of the majority of mucormycosis infections. Nevertheless, given the lack of controlled clinical trials for mucormycosis, these models are essential to evaluating the relative merits of different antifungal strategies.

\textbf{Polyenes.} There have been no prospective randomized trials to define the optimal antifungal therapy for mucormycosis. Until recently, only members of the polyene class, including

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Antifungal</th>
<th>Pros</th>
<th>Cons</th>
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<tr>
<td>\textbf{Established therapies}</td>
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<tr>
<td>Amphotericin B deoxycholate (AmB)</td>
<td>50 years experience Cidal</td>
<td>Toxicity Resistance seen in individual isolates</td>
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<tr>
<td>Liposomal amphotericin B (LAmB)</td>
<td>Less toxic than AmB Improved CNS penetration (47) High-dose LAmB (15 mg/kg/day) superior to AmB (1 mg/kg/day) in murine model (59) Superior to AmB in retrospective clinical study (45)</td>
<td>Most expensive polyene</td>
<td></td>
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<tr>
<td>Amphotericin B lipid complex (ABLC)</td>
<td>Less toxic than AmB</td>
<td>Inferior CNS penetration vs. LAmB in one rabbit study (47) Not superior to placebo or AmB in murine model even at high doses (up to 30 mg/kg/day) (62, 141) No comparative clinical data published</td>
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<td>\textbf{Investigational/adjunctive therapies}</td>
<td></td>
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<tr>
<td>Itraconazole</td>
<td>Superior toxicity profile Successful case reports (36, 125)</td>
<td>Poor activity in animal models despite in vitro susceptibility (28, 29, 159) Breakthrough mucormycosis described during prophylactic itraconazole (130)</td>
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<td>Posaconazole</td>
<td>More effective than itraconazole in animal models (28, 148) Successful case reports (65, 155) Possible combination with polyene therapy, but no data available</td>
<td>Not yet FDA approved Static in vitro Activity inferior to AmB in murine models (28, 148)</td>
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<tr>
<td>Caspofungin</td>
<td>Very low toxicity Synergistic with ABLC in murine model (141) FDA approved (not for mucormycosis)</td>
<td>Virtually no clinical data for mucormycosis Minimal activity as monotherapy in murine model (60)</td>
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<tr>
<td>Iron chelation</td>
<td>Theoretical benefit in combination with antifungals</td>
<td>No data available No effective agents are FDA approved</td>
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<tr>
<td>Hyperbaric oxygen</td>
<td>Nontoxic Successful case reports (22, 42)</td>
<td>Not widely available No controlled studies</td>
<td></td>
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<tr>
<td>Cytokine therapy</td>
<td>In vitro activity (44) Successful case reports (3)</td>
<td>Expensive Toxicity profile unclear</td>
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amphotericin B deoxycholate and its lipid derivatives, had been demonstrated to have activity against the agents of mucormycosis. Furthermore, the various species that cause mucormycosis have a broad range of susceptibilities to amphotericin. Therefore, the recommended dose of amphotericin B deoxycholate has been 1 to 1.5 mg/kg/day (61, 81, 145), which results in a very high toxicity rate. Unfortunately, given the underdeveloped state of the molecular biology of the Mucorales, virtually nothing is known about the mechanisms of drug resistance in these organisms. The molecular basis of drug resistance in these organisms is an area that is highly meritorious of future research.

Fortunately, a series of new therapies that have the potential to impact the outcomes of mucormycosis have or may soon become available. The lipid formulations of amphotericin are significantly less nephrotoxic than amphotericin B deoxycholate and can be safely administered at higher doses for a longer period of time. However, the use of increased dosing for lipid-based amphotericin also increases costs enormously. For example, in contrast to US$5 per day for a 1-mg/kg daily dose of amphotericin B deoxycholate, 5 to 15 mg/kg of lipid-based amphotericins can cost between US$500 and US$3,000 per day (142). Nevertheless, several case reports and case series of patients with mucormycosis have documented successful outcomes with either liposomal amphotericin B or amphotericin B lipid complex (21, 38, 170, 173).

In our murine model of disseminated *R. oryzae* infection in mice in diabetic ketoacidosis, high-dose liposomal amphotericin B (15 mg/kg/day) was considerably more effective than amphotericin B deoxycholate (1 mg/kg/day), nearly doubling the survival rate (59). Further in support of the first-line role of liposomal amphotericin are the results of a recent retrospective review of 120 cases of mucormycosis in patients with hematological malignancies (45). Treatment with liposomal amphotericin was associated with a 67% survival rate, compared to 39% survival when patients were treated with amphotericin B deoxycholate (*P* = 0.02, $\chi^2$). Given the retrospective nature of this study, there is clear potential for several types of bias to affect the outcome. Nevertheless, based on the combination of these retrospective clinical data, the historically poor success rates with amphotericin B deoxycholate, and the available animal data showing superiority of liposomal amphotericin B over amphotericin B deoxycholate, there is a developing consensus that high doses of lipid formulation amphotericin are the preferred initial antifungal therapy for patients with mucormycosis.

Recent data are useful for guiding the choice of liposomal amphotericin B versus amphotericin B lipid complex. A study in rabbits (47) demonstrated that liposomal amphotericin B penetrated brain parenchyma at levels more than fivefold above those of amphotericin B lipid complex. In fact, the brain levels of amphotericin B lipid complex were lower than the levels of amphotericin B deoxycholate, despite the fact that amphotericin B lipid complex was administered at a fivefold-higher dose. Furthermore, in contrast to liposomal amphotericin B, amphotericin B lipid complex (5, 20, or 30 mg/kg/day) did not improve survival compared to placebo or amphotericin B deoxycholate in our murine model of disseminated *R. oryzae* infection (62, 141). Finally, in contrast to the recent review of the effect of liposomal amphotericin B in clinical mucormycosis, no comparable data set has been published reviewing the effect of amphotericin B lipid complex in this setting.

Until direct comparisons of the efficacy of liposomal amphotericin B versus amphotericin B lipid complex are published, definitive conclusions regarding their relative efficacies for mucormycosis cannot be made. For now, the pharmacokinetic data, animal model data, and retrospective clinical data all support the first-line use of high-dose liposomal amphotericin B for mucormycosis, particularly for cases of central nervous system disease, with amphotericin B lipid complex serving as a reasonable second-line agent. Therefore, a rational approach to the treatment of life-threatening mucormycosis infections is emergent surgical consultation followed by immediate initiation of liposomal amphotericin B at 10 to 15 mg/kg/day.

**Azoles.** Itraconazole is the only marketed azole drug that has in vitro activity against Mucorales (147). There are case reports of successful therapy with itraconazole alone (36, 125). However, as mentioned above, itraconazole prophylaxis has been described as a risk factor for breakthrough mucormycosis (130). Furthermore, animal studies revealed that itraconazole was completely ineffective against *Rhizopus* and *Mucor* spp. even though the isolates were susceptible in vitro (28, 29, 159). In contrast, itraconazole did have activity in vivo against a hypersusceptible strain of *Absidia* (MIC, 0.03 μg/ml). Therefore, itraconazole should not be considered a first-line agent against mucormycosis, but its use may be considered as adjunctive therapy in selected situations where highly susceptible fungi have been cultured.

Voriconazole, a recently approved second-generation broad-spectrum triazole, is not active against the Mucorales in vitro (147). Conversely, posaconazole and ravuconazole, investigational triazoles, have promising in vitro activity against the agents of mucormycosis (120, 147). In experimental animal models of disseminated mucormycosis, posaconazole is more efficacious than itraconazole but less efficacious than amphotericin B deoxycholate (28, 148). There are increasing reports of salvage posaconazole therapy for refractory mucormycosis. Successful outcomes have been seen in patients with rhinoencephalic mucormycosis in conjunction with amphotericin (65), and in a heart/kidney transplant patient who failed on amphotericin therapy (155). Further data are needed to determine whether posaconazole, alone or in combination with amphotericin, may be useful for the treatment of mucormycosis.

**Echinocandins.** Caspofungin, the first member of the novel echinocandin class of antifungal drugs to be marketed in the United States, has minimal activity against the agents of mucormycosis when tested in vitro by standard techniques (31, 39). However, the accuracy of current in vitro testing of caspofungin activity against molds remains unclear. It is now known that *R. oryzae* expresses the target enzyme for caspofungin (60), and in the murine model of disseminated mucormycosis, caspofungin did have limited activity against *R. oryzae* (60). Furthermore, we found that the combination of caspofungin (1 mg/kg/day) plus amphotericin B lipid complex (5 mg/kg/day) was synergistic (141). While either therapy alone mediated no survival benefit, the combination significantly improved survival (50% survival for the combination versus 0% for placebo, caspofungin alone, or amphotericin B lipid complex alone).

Clinical experience with caspofungin in the setting of mu-
Corneal mucormycosis is extremely limited. In a report of a patient with necrotizing pancreatitis and abdominal mucormycosis, the addition of caspofungin to liposomal amphotericin did not improve the outcome, but the patient had already clinically progressed on liposomal amphotericin prior to initiation of caspofungin (165). As mentioned, there have also been reports of breakthrough mucormycosis in patients receiving either caspofungin alone (133) or caspofungin plus voriconazole (13). Conversely, the related experimental echinocandin micafungin has been added on a salvage basis to a patient failing antifungal therapy for craniofacial mucormycosis (67). The patient began responding to therapy shortly after the addition of micafungin and was ultimately cured. These data suggest that echinocandins may have a role as a second agent, especially in combination with a polyene, in serious cases of mucormycosis. More study of the utility of echinocandins in this setting is needed.

**Novel Iron Chelators**

The central role of iron metabolism in the pathogenesis of mucormycosis suggests the possibility of utilizing effective iron chelators as adjunctive antifungal therapy. In fact, two experimental iron chelators have been studied in vitro against *R. oryzae* (16). In contrast to deferoxamine, the other iron chelators did not allow the organism to take up iron and did not support its growth in vitro in the presence of iron. Furthermore, while deferoxamine significantly worsened disseminated *R. oryzae* infection in guinea pigs, one of the other chelators had no impact on the *in vivo* infection and one of them more than doubled the mean survival time (16). The latter agent is approved for use in India and Europe and is available on a compassionate-use basis for iron overload in the United States and Canada. The potential for this iron chelator to serve as adjunctive therapy in combination with other antifungal agents is under active investigation.

**Other Adjunctive Therapies**

Case reports have suggested that hyperbaric oxygen may be a beneficial adjunct to the standard surgical and medical antifungal therapy of mucormycosis, particularly for patients with rhinocerebral disease (22, 42). It is hypothesized that hyperbaric oxygen might be useful for treating mucormycosis in conjunction with standard therapy because higher oxygen pressure improves the ability of neutrophils to kill the organism (26). Additionally, high oxygen pressure inhibits the germination of fungal spores and growth of mycelia in vitro (131). Whether hyperbaric oxygen actually improves the outcome of patients with mucormycosis remains to be established through appropriately controlled prospective clinical trials.

The role of adjunctive cytokine therapy for mucormycosis has been understudied. Cytokines that activated phagocytic activity, such as gamma interferon and granulocyte-macrophage colony-stimulating factor, increase the ability of phagocytes to kill agents of mucormycosis *in vitro* (44). A recent case report suggested a favorable outcome in a leukemic child with rhinocerebral mucormycosis following the addition of gamma interferon and granulocyte-macrophage colony-stimulating factor to the regimen (3). Further studies of cytokines that activate host phagocyte function are warranted for this disease.

**PROGNOSIS**

Previously, cases of rhinocerebral mucormycosis were almost consistently fatal (84). Although the mortality rate of rhinocerebral disease remains high, the infection can be cured when diagnosed early and treated with aggressive surgery and antifungal agents (discussed further below) (20, 114, 119). Recent series have described a mortality of approximately 40% in diabetics with rhinocerebral mucormycosis (107, 118) and a similar survival rate for rhinocerebral disease in patients with hematological malignancies (45). Of note, the prognosis is much better if the disease has not penetrated beyond the sinus prior to surgical debridement; in local sinonasal disease, the mortality has been reported to be <10% (107). The nature of the underlying disease and the reversibility of the immune dysfunction are also important determinants of survival. One study showed that 75% of patients with rhinocerebral disease who had no underlying immune compromise survived, while 60% of those with diabetes and only 20% of patients with other immunocompromised states were cured (14).

The overall survival rate of patients with mucormycosis is approximately 50%, although survival rates of up to 85% have been reported more recently. Much of the variability in outcome is due to the various forms of the disease. Rhinocerebral mucormycosis has a higher survival rate than does pulmonary or disseminated mucormycosis because the rhinocerebral disease can frequently be diagnosed earlier and the most common underlying cause, diabetic ketoacidosis, can be treated readily (114). In contrast, pulmonary mucormycosis has a high mortality (~65% at 1 year) (94) because it is difficult to diagnose and it frequently occurs in neutropenic patients. For example, in one large study, only 44% with pulmonary mucormycosis were diagnosed premortem, and the overall survival rate was only 20% (150). In a separate study in which 93% of the infections were diagnosed premortem, the survival rate was 73% (114). Mortality in patients with disseminated disease approaches 100%, in large part because surgical removal of infected tissues is not feasible and in part because these patients tend to be the most highly immunocompromised (e.g., allogeneic stem cell transplantation).

**CONCLUSIONS**

Mucormycosis is an increasingly common infection in immunocompromised patients. The central role of iron in the organism’s pathogenesis has only recently been appreciated. The interaction between the Mucorales and endothelial cells is also beginning to be understood. Both of these pathogenetic features of disease may be amenable to novel therapeutic intervention in the future. Currently, novel regimens for the treatment of mucormycosis include combination lipid-based amphotericin plus either an echinocandin or itraconazole or both. As well, compassionate-use posaconazole is currently available, and its potential for combination therapy with a polyene, caspofungin, or both is meritorious for study. In the future, novel iron chelator therapy may be useful as an adjunct to standard antifungal therapy. Finally, prompt diagnosis, reversal of predisposing conditions, and aggressive surgical debridement remain cornerstones of therapy for this deadly disease.
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