Diagnosis and treatment of mucormycosis in patients with hematological malignancies: guidelines from the 3rd European Conference on Infections in Leukemia (ECIL 3)

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ABSTRACT

Mucormycosis is an emerging cause of infectious morbidity and mortality in patients with hematologic malignancies. However, there are no recommendations to guide diagnosis and management. The European Conference on Infections in Leukemia assigned experts in hematology and infectious diseases to develop evidence-based recommendations for the diagnosis and treatment of mucormycosis. The guidelines were developed using the evidence criteria set forth by the American Infectious Diseases Society and the key recommendations are summarized here. In the absence of validated biomarkers, the diagnosis of mucormycosis relies on histology and/or detection of the organism by culture from involved sites with identification of the isolate at the species level (no grading). Antifungal chemotherapy, control of the underlying predisposing condition, and surgery are the cornerstones of management (level A II). Options for first-line chemotherapy of mucormycosis include liposomal amphotericin B and amphotericin B lipid complex (level B II). Posaconazole and combination therapy of liposomal amphotericin B or amphotericin B lipid complex with caspofungin are the options for second line-treatment (level B II). Surgery is recommended for rhinocerebral and skin and soft tissue disease (level A II). Reversal of underlying risk factors (diabetes control, reversal of neutropenia, discontinuation/taper of glucocorticosteroids, reduction of immunosuppressants, discontinuation of deferroxamine) is important in the treatment of mucormycosis (level A II). The duration of antifungal chemotherapy is not defined but guided by the resolution of all associated symptoms and findings (no grading). Maintenance therapy/secondary prophylaxis must be considered in persistently immunocompromised patients (no grading).

Introduction

Invasive fungal infections (IFI) are an important cause of morbidity and mortality in immunocompromised patients with hematologic malignancies (HM) including those undergoing hematopoietic stem cell transplantation (HSCT). While invasive candidiasis and invasive aspergillosis still account for the majority of these infections, agents belonging to the class of the zygomycetes have emerged as increasingly relevant and highly lethal causes of IFI in many centers worldwide.[1-3] Zygomycosis includes infections due to fungi of the order Mucorales, as well as those due to fungi of the order Entomophthorales. However, as the latter are completely different infections, predominantly found in immunocompetent patients in tropical and subtropical areas, they are discussed in this manuscript. For this reason, the term 'mucormycosis' will be used instead of zygomycosis for infections caused by members of the order Mucorales.[4] These infections remain difficult to diagnose, and their management is complicated by their aggressive course and a paucity of data to guide treatment decisions. In an effort to summarize the existing information, and to provide guidance to clinicians faced with these life-threatening infections, we present evidence-based guidelines for the diagnosis and treatment of mucormycosis developed by multi-disciplinary experts at the third European Conference on Infections in Leukemia (ECIL 3).

These guidelines are also applicable to patients with other underlying diseases, such as diabetes mellitus, since most of the existing studies were performed on mixed populations (both hematologic and non-hematologic patients) and the approach to diagnosis and treatment is similar.

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The entity of mucormycosis was introduced at the ECIL 3 (25-26 September 2009, Juan-les-Pins, France) and brought together a panel of 57 expert hematologists, oncologists, microbiologists, infectious disease specialists and clinical trial investigators from across Europe. This is the first time the ECIL group addresses a topic where there are no randomized studies to be analyzed. However, the topic was chosen because of the increasing number of cases, new diagnostic tools and therapeutic approaches available and the need for the clinician to have practical guidelines that can be applied at the bedside. The guidelines were developed following an extended process of literature analysis, expert group discussion, panel debate and consensus.

Recommendations for the treatment of mucormycosis were rated according to the standard scoring system of the Infectious Diseases Society of America (IDSA) for rating recommendations in clinical guidelines as shown in Table 1. The group also had the option to provide no grading in cases where no recommendations could be given.

Epidemiology, microbiology, clinical presentation and diagnosis

Epidemiology

Although there are few epidemiological data on mucormycosis, it appears that the incidence of this complication has increased in HMs during the last decade.[2] In most studies, however, appropriate denominators are lacking and therefore a precise estimate of any trends in the incidence of the disease can not be made.

In the comprehensive literature review by Roden *et al.*, an increase in the proportion of immunocompromised patients became apparent in the 1980s and 1990s.[5] Patients with an HM or treated with HSCT represented 22% of the cases (17% and 5%, respectively). Similarly, in 157 pediatric cases, Zaoutis *et al.* reported 28 cases of mucormycosis in HMs and 9 in HSCTs (14% and 4%).[6] These analyses, which are based on the collection of cases reported in the literature, were biased by the fact that they were retrospective and included many cases from an era in which chemotherapy for HM had not yet been used.

In a recent study from France, the annual incidence rate of mucormycosis in patients with HM increased over time from 0.7 to 1.2 cases/million persons from 1997 through 2006 (+24% per year).[2] Before this, mucormycosis was often diagnosed at autopsy and its incidence in such studies ranged between 0.4% and 0.9% in patients with HMs.[7] Few epidemiological studies are available that may allow a better estimation of the incidence in this population. Among patients undergoing conventional treatments, patients with acute myeloid leukemia (AML) are at highest risk, with incidence rates ranging between 1% to 1.9% in single- or multi-center series.[8-11] In contrast, mucormycosis is rare in other acute or chronic HM, where a very low incidence (0.1%, 14 cases) has been reported by a recent study on 11,802 patients affected by different HMs.[12] The incidence in HSCTs is also lower than that observed in AML, ranging from 0.1% to 0.6%1;[13-15] the highest incidence in these patients was observed in association with graft *versus*-host disease (GVHD) (Table 2).

Microbiology and clinical presentation

Mucorales belong to the subphylum Mucormycotina, are ubiquitous in the environment and produce branched nonseptate mycelia (5-25 μm) with a chitinous wall. The most common species are *Rhizopus* spp, *Mucor* spp, *Rhizomucor* spp and *Lichtheimia* (formerly *Absidia*) spp. They are acquired either by inhalation or by direct inoculation of conidia.

In hematologic patients, the most prevalent site of infection is the lung.[5,17,18] Other common sites include the paranasal sinuses, the brain, skin, digestive tract, or disseminated disease with more than one affected site. As aspergillosis and mucormycosis share similar clinical and radiological presentations, several authors have attempted to outline clinical and radiological findings that are more frequent in mucormycosis. These include previous voriconazole prophylaxis, paranasal sinus involvement, diabetes mellitus, more than 10 pulmonary nodules, and pleural effusion.[19] These findings, although interesting, need prospective validation.[19] As several antifungal agents with activity against *Aspergillus* spp. are inactive against zygomycetes, mycological diagnosis is required.[20,21] However, there are clinical situations with a high level of suspicion for mucormycosis, as described above, where antifungal treatment aimed at Mucorales may be appropriate, even though definite diagnosis is not feasible.

Diagnosis

The diagnosis of mucormycosis is challenging and treatment should start as early as possible in order to decrease mortality.[22] No circulating antigen detection test (similar to galactomannan detection for invasive aspergillosis) is available for the diagnosis of mucormycosis, and although no sufficiently powered trials testing 1,3 beta-D-glucan in different types of mucormycosis have been performed, it is generally observed that 1,3 beta-Dglucan detection test is negative in Mucorales infections. However, these two tests help to rule out invasive aspergillosis, the most frequent differential diagnosis, or combined *Aspergillus* and *Mucorales* infections. So far, no standardized blood polymerase chain reaction (PCR) test is available. Therefore, analysis of bio-

Table 1. Infectious Diseases Society of America-United States Public Health Service grading system for ranking recommendations.

Category, grade	Definition
Strength of recommendation	
A	Good evidence to support a recommendation for use
B	Moderate evidence to support a recommendation for use
C	Poor evidence to support a recommendation
Quality of evidence	
I	Evidence from ≥ 1 properly randomized, controlled trial
II	Evidence from ≥ 1 well-designed clinical trial, without randomization; from cohort or case-controlled analytical studies (preferably from >1 center); from multiple time-series; or from dramatic results from uncontrolled experiments
III	Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees

logical specimens from clinically involved sites is mandatory for diagnosis. Every effort should be made to obtain tissue biopsies for histopathology and culture. Unfortunately, this is often difficult in patients with hematologic malignancies because of severe thrombocytopenia. If biopsy is not possible, all available specimens, such as sputum, should be used for direct examination, as well as culture. In case of sinusitis, sinus biopsies are required. Ear, nose and throat (ENT) endoscopy should always be performed and repeated in order to re-evaluate the response to treatment. In case of pulmonary involvement, if sputum smear analysis is negative, broncho-alveolar lavage or pulmonary biopsies (endoscopic, computed tomography (CT)-guided or surgical) should be performed depending on the radiological findings obtained by CT scans.[23] Lass-Florl *et al.* showed a high efficiency of CT guided percutaneous lung biopsy for differentiation of aspergillosis from mucormycosis in hematologic patients.[24] However, it should be noted that no patients with less than 50 x 10^9/L platelets were included in this study. Whatever the initial clinical site involved, a sinus and chest CT should be performed in addition to brain imaging, especially if there are suggestive signs and symptoms. This is important, because the therapeutic approach is different in case of cerebral lesions.

The material taken from biopsies should be carefully managed so as not to be crushed because zygomycetes are fragile, and culture may thus remain negative. Growth is rapid and usually occurs during incubation for 24 h at 25-37°C. Culture of a sterile site confirms mucormycosis infection and allows precise genus and species identification. Blood cultures are almost always negative and their positivity should evoke the suspicion of contamination. Similarly, agents of mucormycosis are rarely present in the cerebrospinal fluid even during central nervous system infections.

Demonstration of hyphae in clinical samples by direct microscopy is important because it is rapid and highly suggestive of disease. Specimens can be observed after treatment with potassium hydroxide, staining with an optical brightener (calcofluor white), or with Gomori methamine-silver.[23] Hyphae are hyaline, non- or pauci-septate, ribbon-like with a large diameter (5-25 μm). Width is irregular with branching angles of 90°. When hyphae are fragmented, a definitive diagnosis of mucormycosis can be difficult by direct examination and culture is required to confirm the diagnosis.[23] Tissue can be stained with Gomori methamine-silver or Periodic-acid Schiff. Hyphae may be observed within necrotic tissue with signs of angioinvasion and infarction; neutrophilic infiltrates or granuloma formation may be present in patients who are not granulocytopenic or with more chronic infection, respectively. Occasionally, immunohistochemistry with commercially available antizygomycete antibodies may help in the diagnosis.[25]

When cultures are negative, molecular identification from tissue samples can confirm the histological diagnosis. However, at present, there is no standardized method available. Fresh or frozen samples are preferred; however, based on recent inter-laboratory experimental and clinical data, formalin-fixed paraffin-embedded tissues may also be used.[26,27] Molecular identification of agents of mucormycosis can help to confirm diagnosis and identify the fungus to the genus and species level. Different techniques have been reported: DNA probes targeting 18S subunit, ITS1 sequencing after polymerase chain reaction (PCR) with pan-fungal primers, 18S-targeted semi-nested PCR and real-time PCR targeting cytochrome b gene.[28]

Antifungal drugs used for the treatment of mucormycosis

The summarized ECIL-3 recommendations for the treatment of mucormycosis are presented in Tables 3 and 4. The therapeutic approach to mucormycosis is multimodal, including antifungal agents, surgical debridement, and correction of the underlying condition predisposing the patient to the disease. Control of underlying conditions is critical in mucormycosis. Rapid correction of metabolic abnormalities is mandatory in uncontrolled diabetes. Corticosteroids should be discontinued, if feasible, and other immunosuppressive drugs should be tapered as much as possible.

Among the more recent therapeutic developments in mucormycosis treatment are: the lipid formulations of

Table 2. Incidence of mucormycosis among hematologic patients treated with conventional chemotherapy and those who underwent transplant procedures.

Reference	Years	Population	Cases	%
Conventional therapy				
Pagano *et al.*[8]	1987-1995	3148 acute leukemia	37	1
Nosari *et al.*[9]	1987-1999	653 acute leukemia	13	1.6
Kontoyiannis *et al.*[10]	1989-1998 1989–1993 1994–1998	624 autopsy in HMs 88207 cancer patients 82490 cancer patients	12 7 17	1.9 0.008 0.02
Pagano *et al.*[12]	1999-2003	11802 HM	14	0.1
HSCT				
Marr *et al.*[13]	1985-1999	5589 HSCT	29	0.5
Kontoyiannis *et al.*[15]	2001-2006	16200 HSCT	77	0.4
Pagano *et al.*[12]	1999-2003	1249 alloHSCT 1979 autoHSCT	1 0	0.08
Garcia Vidal *et al.*[14]	1998-2002	1248 HSCT	8	0.6
Neofytos *et al.*[1]	2004-2007	alloHSCT autoHSCT	12 8	
Xhaard *et al.*[16]	2003-2008	4138 alloHSCT	23	0.56

amphotericin B, which are now the drugs of choice; the new triazole posaconazole, with promising efficacy as salvage treatment; the iron chelators deferasirox and deferiprone; the echinocandins in combination with amphotericin B (AmB) and recombinant growth factors such as granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF). Because of the relative rarity of mucormycosis, prospective, comparative studies of antifungal agents and strategies have not been conducted. Therefore, the management of mucormycosis is still based on the results of case series and case reports, animal model studies, and *in vitro* susceptibility data.

Polyenes

Amphotericin B

Amphotericin B (AmB) has shown excellent activity against the Mucorales in several *in vitro* studies.[20,21,57-59] In the most comprehensive study presented so far, AmB was the most active antifungal agent with the majority of strains displaying MICs near the suggested breakpoint of ≤1 μg/mL (Table 5). Only some strains of *Cunninghamella* sp. had higher MICs.[20] In another comparative study of 37 clinical isolates of Zygomycetes, the 90% minimum inhibitory concentrations (MIC90) of AmB ranged from 0.03 to 2 μg/mL.[21]

Amphotericin B deoxycholate (d-AmB) is the only antifungal agent that has been approved by the US Food and Drug Administration for primary treatment of mucormycosis. However, this formulation has significant toxicity, and has been replaced by the lipid formulations of AmB that include liposomal AmB (L-AmB), AmB lipid complex (ABLC), and AmB colloidal dispersion (ABCD). The lipid formulations of AmB are less nephrotoxic than d-AmB that allows for the administration of larger daily dosages and long-term administration with less nephrotoxicity; the rate of infusion-associated reactions, however, is variable.[35]

Animal models support the use of lipid formulations of AmB. In a diabetic murine model, Ibrahim *et al.* showed that high-dose L-AmB (15 mg/kg) treatment was significantly more effective than conventional AmB (1 mg/kg) or lower dose L-AmB in disseminated mucormycosis due to Rhizopus oryzae, nearly doubling the survival rate.[60] The same investigators compared the efficacies of L-AmB and ABLC in diabetic ketoacidotic, as well as neutropenic mice, with disseminated mucormycosis, and found that ABLC was as effective as L-AmB in neutropenic but not ketoacidotic mice. In addition, low-dose ABLC was less effective than L-AmB at reducing brain fungal burdens in both models,[61] and the superior GDF penetration of L-AmB in the CNS compared to ABLC has been shown in a rabbit model of Candida meningoencephalitis.[62] Different findings were reported in another study in which the pharmacodynamics of ABLC and L-AmB were compared in a murine model of pulmonary mucormycosis.[63] The two drugs demonstrated different kinetics, with ABLC achieving higher concentrations in the lung tissue when administered at a dose of 5 mg/kg, while when given at 10 mg/kg both drugs achieved similar concentrations.

The various open studies in which the efficacy of the lipid forms of AmB against mucormycosis has been estimated are shown on Table 6.[16,29-34,36-38,40,41,64]

In the largest review of cases of mucormycosis by Roden *et al.*, the response rate of the 532 patients who had been treated with d-AmB was 61%, while the response of the 116 who had received lipid formulations of AmB was 69%.[5]

Table 3. ECIL-3 recommendations for first-line treatment of mucormycosis.

Management should include antifungal therapy, control of underlying conditions and surgery	AII
Antifungal therapy	
AmB deoxycholate[4,29,30]	CII
Liposomal AmB[16,29-34], 5-10 mg/kg[35,36]	BII[1]
ABLC[37,29], 5-7.5 mg/kg[35]	BII1
ABCD[42,45]	CII
Posaconazole[16,17] 400 mg bid	CIII[2]
Combination therapy[39]	CIII
Control of underlying condition[5,42,43]	AII[3]
Surgery	
-rhino-orbito-cerebral[44-47]	AII
-soft tissue[48,49]	AII
-localized pulmonary lesion[50,51]	BII
-disseminated[22]	CIII[4]
Hyperbaric oxygen	CIII

[1]Liposomal amphotericin B should be preferred in CNS infection and/or renal failure. [2]No data to support its use as first-line treatment. May be used as an alternative when amphotericin B is absolutely contraindicated. [3]Control of underlying condition includes control of diabetes, hematopoietic growth factor if neutropenia, discontinuation/tapering of steroids, reduction of immunosuppressive therapy and discontinuation of deferroxamine. [4]Surgery should be considered on a case by case basis, using a multi-disciplinary approach.

Table 4. ECIL-3 recommendations for second-line and maintenance treatment of mucormycosis.

Second-line treatment: first-line treatment intolerance or failure[1]	
Posaconazole 400 mg bid[52,53]	BII
Combination lipid AmB and caspofungin[39]	BII
Combination lipid AmB and posaconazole	CIII
Combination with deferasirox NOT recommended[104]	AI
Maintenance therapy Posaconazole	BIII[2]

[1]For guidelines regarding what is defined as treatment failure references 55 and 56 may be used. [2]Overlap of a few days (at least 5) with first-line therapy to obtain appropriate serum levels. Monitoring of serum levels might be indicated.

Table 5. Comparative activity of amphotericin B, posaconazole and itraconazole against 216 clinical isolates of 10 Mucorales.

	Amphotericin B % with MIC ≤1 μg/mL	Posaconazole % with MIC ≤0.5 μg/mL	Itraconazole % with MIC ≤0.5 μg/mL
Rhizopus sp (101)	100	80	62
Rhizopus arrhizus (20)	100	64	50
Rhizopus microsporus (12)	100	78	60
Mucor sp. (41)	94	70	57
Mucor circinelloides (6)	100	0	0
Rhizomucor sp.(5)	100	67	67
Lichtheimia sp. (3)	100	100	50
Lichtheimia corymbifera (9)	100	100	100
Cunninghamella sp. (13)	63	75	29
Apophysomyces elegans (6)	100	83	80

Modified from Almyroudis 2007.[20]

The outcome, however, of mucormycosis depends on several factors, including the site of infection, the immune status of the host and the use of surgery or other adjunctive treatments. Furthermore, the results of various studies cannot be directly compared because there are significant differences in their design. In a review of 120 mucormycosis cases in patients with HMs, the survival rate was 67% (10 of 16) in patients treated with L-AmB compared with 39% (24 of 62) in those treated with d-AmB deoxycholate (P=0.02).[64] However, the patients in the L-AmB group were younger and most had received G-CSF and GM-CSF. In an Italian retrospective study of 59 patients with HM and proven or probable mucormycosis, the response rate was 23% (9 of 39) in patients who received d-AmB compared with 58% (7 of 12) in those who were treated with L-AmB.[30] L-AmB was given as primary therapy only to 4 patients, while to the other 8 it was administered as salvage treatment. In a recent study by Shoham *et al.*, the cases of 28 patients from five major medical centers who had been treated with L-AmB for invasive mucormycosis over a 7-year period (1998-2005) were analyzed.[31] The results of this study focused on those newly diagnosed patients with invasive mucormycosis who received L-AmB as primary therapy. Hematologic disorders were observed in 15 (54%) patients. Pulmonary disease was the primary site of infection in 50% of cases. The overall mortality was 61% (17 of 28 patients). This high mortality rate was reportedly related to the highly immunocompromised patient population. The importance of host response was also evident in the review by Roden *et al.* in which the mortality of patients with malignancies and HSCT was 66% while the mortality of patients with diabetes mellitus was 44%.

The other lipid formulation of AmB used in the treatment of mucormycosis is ABLC. Larkin and Montero described the efficacy and renal safety of ABLC in treating 64 immunocompromised patients with mucormycosis, on the basis of a search of the Collaborative Exchange of Antifungal Research (CLEAR) database.[37] The median daily ABLC dosage was 4.8 mg/kg (range 0.9–12.6 mg/kg) and the median duration of therapy was 16 days. The overall favorable clinical response to ABLC was 72% (46 of 64 patients) with a 64% success rate in patients with disseminated disease. In another study, 556 patients refractory to or intolerant of antifungal therapy were treated with ABLC; 71% of 24 patients with mucormycosis had a complete or partial response.[38] In a more recent retrospective analysis by Reed *et al.*[39] patients treated with ABLC had a significantly lower success rate at 30 days after hospital discharge than did those treated initially with AmB deoxycholate or L-AmB; however, the effect was driven by clinical failure experienced by patients with central nervous system involvement.

Data in the English literature regarding mucormycosis treatment with ABCD are limited. In a review of 21 patients with invasive mucormycosis treated with ABCD in 5 phase I and phase II studies, 12 of 20 (60%) responded.[40] The patients, all of whom had bone marrow or solid organ transplantation, hematologic malignancies, or diabetes, were given ABCD on the basis of pre-existing renal insufficiency, development of nephrotoxicity during d-AmB therapy, or fungal infection that failed to respond to d-AmB combined with surgical debridement.

ECIL recommendations

Based on the published data, it seems reasonable to recommend either LAmB or ABLC as first-line treatment for mucormycosis (BII), taking into account that the approach to mucormycosis should always be multi-modal, as already described. It is very important to start therapy early. Chamilos *et al.* showed that initiation of polyene therapy within five days after diagnosis of mucormycosis was associated with improvement in survival, compared with initiation of polyene therapy at six days or more after diagnosis (83% *vs.* 49% survival).[22] The optimal daily dose as well as the length of treatment have still to be defined. Starting dosages of 5-7.5 mg/kg/day for L-AMB and of 5 mg/kg/day for ABLC, respectively, are commonly used for adults and children.[35] It is not clear whether higher doses lead to a better outcome. In the study by Shoham *et al.*, daily L-AMB dosages ranged from 3-14 mg/kg and no pattern of improved response of mucormycosis in relation to dosage of the drug was noted.[31] In a formal prospective phase II study by Walsh *et al.*, in which the safety and pharmacokinetics of high doses of L-AmB were evaluated in various fungal infections, the maximum serum concentration was obtained with doses of 10 mg/kg/day and did not increase with higher doses (up to 15 mg/kg/day).[32] In a prospective, though non-comparative trial (AMBIZYGO), treatment of patients with mucormycosis with high doses of L-AmB (10 mg/kg/day) plus surgery resulted in 50% response rate at

Table 6. Open studies in the treatment of mucormycosis with lipid-based AmB.

Antifungal treatment	References	N. of patients with mucormycosis	Underlying conditions	Favorable outcome	Overall favorable ourcome (%)
ABCD	Oppenheim *et al.*[45]	41	n.r.	4	16/24 (67%)
	Herbrecht *et al.*[42]	40	7 HSCT, 5 HM	12	
ABLC	Walsh *et al.*[41]	38	n.r.	17	63/88 (71%)
	Larkin *et al.*[40]	37	17 HM, 8 HSCT	46	
L-AmB	Walsh *et al.*[43]	32	n.r.	4	65/95 (68%)
	Pagano *et al.*[38]	30	HM	7	
	Cordonnier *et al.*[46]	33	HM (including HSCT)	5	
	Cornely *et al.*[47]	34	HM	3	
	Sun *et al.*[36]	64	Solid organ transplant	10	
	Ruping *et al.*[16]	17	Mixed: HM and not	16	
	Lanternier *et al.*[44]	36	HM, diabetes and solid organ transplant	9	
	Shoham *et al.*[39]	31	Mixed: mostly HM	11	

HMs: hematologic malignancies; HSCT: hematopoietic stem cell transplantation; n.r.: not reported..

week 12.[36] Doses of 10 mg/kg/day are suggested for infections involving the CNS. For patients without CNS involvement, the suggested dosage is at least 5 mg/kg/day. The duration of antifungal treatment should be determined on an individual basis, but therapy usually continues for at least 6-8 weeks.

Azoles

Posaconazole

Posaconazole exhibits useful activity against the agents of mucormycosis. Compared with itraconazole and isavuconazole on a mg:mg basis, posaconazole has enhanced *in vitro* activity with reported 90% minimum inhibitory concentrations (MIC90) ranging from 1 to ≥ 4 μg/mL.[20,21,57,58,65,66] In the largest and most diverse collection of clinical isolates published so far, that included 217 clinical isolates of 11 species, 64-100% of the isolates were reported to be susceptible using an arbitrary breakpoint of 0.5 μg/mL or below. Comparatively higher MIC values were found for *Mucor circinelloides*[20] (Table 5). While fungicidal activity of posaconazole has been demonstrated against Rhizopus and Mucor spp., AmB was more rapidly fungicidal, with 95% killing noted at as early as 6 h and 99.9% killing at 24 h; for comparison, posaconazole showed less than 70% killing at 6 h and 99.9% killing at 48 h.[67] Similar observations have been made by *in vitro* studies using the XTT metabolic assay.[68]

A few animal studies have been conducted to explore the *in vivo* efficacy of posaconazole in screening models of disseminated mucormycosis that used survival and/or fungal tissue burden as end points.[69-73] Posaconazole prolonged the survival and reduced tissue burden in neutropenic mice with disseminated Mucor infection and was as effective as standard AmB at the highest dose level.[69] In non-immunocompromised mice, no beneficial effects were observed against *R. oryzae*, while partial activity was shown against *Lichtheimia corymbifera* and dose-dependent activity against *Rhizopus microsporus*.[70] In neutropenic mice, posaconazole started two days prior to inoculation was used against L. corymbifera and against one of the two isolates of *R. oryzae* in an inoculum-dependent manner. In both of these last two studies, AmB significantly prolonged the survival of mice infected with all isolates.[70,72] In a further neutropenic murine model of disseminated *R.oryzae* mucormycosis, posaconazole had modest, but significant effects on survival that were statistically inferior to AmB at 0.8 mg/kg/day.[73] Finally, in diabetic ketoacidotic or neutropenic mice with disseminated mucormycosis caused by *R. oryzae*, posaconazole monotherapy did not improve survival or reduce fungal burden as compared to placebo while L-AmB was effective.[71]

Two separate, but overlapping series of patients receiving posaconazole within compassionate use protocols of the manufacturer have been published[52,53] (Table 7). The first is a summary of treatment of the first 24 patients (age 7-74 years) with active mucormycosis who were enrolled in 2 multi-center compassionate trials that evaluated oral posaconazole as salvage therapy for invasive fungal infections. Posaconazole was administered as an oral suspension at 200 mg QID or 400 mg BID for a median duration of 182 days (range 8-1004 days). Eleven (46%) of the infections were rhinocerebral, 9 were single site infections of different locations, and 4 patients had disseminated disease. Fifteen patients were post allogeneic HSCT or were treated for HM. Twenty-two of the patients (92%) had received prior therapy with AmB formulations and 18 (75%) had received adjunctive surgery. Rates of successful treatment (complete and partial response) were 79% in 19 subjects with mucormycosis refractory to standard therapy and 80% in 5 subjects with intolerance to standard therapy. Overall, 19 of 24 subjects (79%) survived the infection. Survival was associated with surgical resection of affected tissue, stabilization or improvement of the subjects' underlying illnesses, and absence of dissemination. Posaconazole was well tolerated and discontinued in only one subject due to a drug rash.[52] The second analysis was based on 91 patients (age 1-80 years) with mucormycosis (proven mucormycosis n=69 patients; probable mucormycosis n=22 patients), including 11 patients of the first analysis. Patients had infection that was refractory to prior antifungal treatment (n=81) or were intolerant of such treatment (n=10) and participated in the compassionate-use posaconazole (800 mg/day) program for 6-1005 days duration. Sixty-two percent of patients had single site infection; an HM was the most frequent underlying disease (53%), followed by insulin-dependent diabetes mellitus (33%). Similar to the first series, most patients (77 of 91, 85%) were pre-treated with AmB formulations, and most had undergone surgical debridement or resection (64 of 91, 70%). Complete or partial responses at 12 weeks after treatment initiation was 60% (55 of 91), and 21% (19 of 91) of patients had stable disease. Overall survival at one month post start of treatment was 62% (56 of 91).

Table 7. Clinical efficacy of posaconazole as second-line agent against mucormycosis.

Study	Study design	N. of patients [n]	Underlying diseases	Involvement of > 1 site [n/%]	AmB pre-treatment [n/%]	Adjunctive surgery [n/%]	Dose and duration of PCZ	CR/PR [n/%]	Survival [n/%]
Greenberg 2006[52]	Compassionate	24	HSCT, 11 SOT, 4 HM / BMF, 5 IDDM, 4	4 (17%)	22 (92%)	18 (75%)	200 QID / 400 BID for median of 182 days (r, 8-1004)	11 [46%] and 8 [33%] at no specified time point	19 (79%) at Day 90 post baseline
van Burik 2006[53]	Compassionate	91 (including 11 pts of study 1)	HM, 53 IDDM, 30 HSCT, 27 SOT, 10 *	35 (38%)	> 77 (85%)	64 (70%)	200 QID / 400 BID for range of 6-1005 days	14 [15%] and 46 [51%] at 12 weeks post baseline	65 (62%) at 1 mo post end-of-therapy

*HSCT: hematopoietic stem cell transplantation; SOT: solid organ transplantation; HM: hematologic malignancies; BMF: bone marrow failure; IDDM: insulin-dependent diabetes mellitus. PCZ: posaconazole; CR: complete response; PR: partial response. * more than one condition provided per patient.*

Treatment success in this analysis was independent of underlying condition, reason of enrolment, site, species, and performance of surgery.[53] Salvage therapy trials have strong limitations because of selection bias: a) patients are in better clinical condition since they survive the usual seven days of primary therapy; or b) are already responding, but because of immune reconstitution the clinical features worsen. This may explain the surprisingly high success rates.

Apart from a few small case series and anecdotal reports, the usefulness of posaconazole as second-line agent for mucormycosis is further supported by a retrospective outcome analysis of 70 consecutive patients with hematologic malignancy treated at the MD Anderson Cancer Center from 1989 to 2006. By multivariate analysis, salvage posaconazole-based therapy (P=0.01) and neutrophil recovery (P=0.009) were predictive of a favorable outcome.[22] Nevertheless, there are also emerging reports of breakthrough infections by agents of mucormycosis in patients receiving prophylactic posaconazole.[74-76] This means that even if a patient is on posaconazole prophylaxis, mucormycosis should be included in the differential diagnosis if signs of an invasive fungal infection are found.

Of note, no pharmacokinetic/pharmacodynamic investigations on optimization of treatment of invasive mucormycosis with posaconazole have been published. In a pivotal clinical second-line trial in patients with invasive aspergillosis, average plasma concentrations of approximately 0.5 μg/mL or over were associated with antifungal efficacy.[77] However, as noted elsewhere,[35] the MICs of *Aspergillus fumigatus* are consistently 0.5 μg/mL of below, which is in marked contrast to the MIC range of susceptible Mucorales.[20] The absence of a validated dosing target and the saturable absorption of posaconazole[78] justifies concerns about achieving adequate *in vivo* levels of oral posaconazole to treat mucormycosis. Furthermore, data from the 5 published investigations in murine models of mucormycosis demonstrate that posaconazole had consistently less efficacy to AmB and little efficacy against experimental *R. oryzae* infection.[69-73]

ECIL recommendations

Posaconazole monotherapy cannot be recommended as primary treatment of mucormycosis (CIII). However, the available clinical data from the compassionate use program suggest that posaconazole is an option for patients with mucormycosis who are refractory to or intolerant of AmB or who need prolonged continuation or maintenance therapy[52,53] (BII). Therapeutic drug monitoring is recommended where possible.

Important additional points in the assessment of posaconazole as a second-line option for invasive mucormycosis include the ongoing antifungal effect of prolonged and persistent polyene exposure in blood and tissue, and the key role of adjunctive surgery and control of predisposing conditions. For the immediate future, more information is needed on the interspecies differences in susceptibility, susceptibility testing and *in vitro/in vivo* correlations, site-specific pharmacodynamics, and the exposure-effect relationships of posaconazole against the Mucorales.

Other azoles

Fluconazole and voriconazole have no meaningful activity against agents of mucormycosis *in vitro* and in experimental models.[20] Clinical data have suggested that use of voriconazole for prophylaxis or empirical therapy may explain an increase in incidence of mucormycosis.[19,79,80] Whether voriconazole really impacts on incidence or just allows for longer survival and, therefore, exposure to other opportunistic pathogens of high-risk patients successfully treated for voriconazole-susceptible fungal infection remains a matter of debate.

Itraconazole has variable *in vitro* activity with differences between and within genera, best activity being reported in *Lichtheimia* spp.[20,81] In an experimental model, itraconazole reduced mortality of immunocompetent mice infected with Lichtheimia corymbifera and Apophysomyces elegans but not in animals infected with *Rhizopus microspores*.[82] Despite rare case reports,[83-86] data are insufficient to support its use as monotherapy for mucormycosis in clinical practice.

Isavuconazole is a broad spectrum triazole available as an oral and intravenous (iv) formulation currently in phase II clinical trial for candidemia and aspergillosis. Its spectrum includes Mucorales with MIC50 values of 1-4 μg/mL and MIC90 values of 4-16 μg/mL, as shown in a study on 345 isolates of five different genera.[66] In another more limited *in vitro* assessment, isavuconazole had MIC 90 values over 8 mg/mL against 36 strains of Mucorales while posaconazole had MIC 90 values of 1-4 μg/mL.[87] So far no clinical data are available for isavuconazole.

ECIL recommendations

Based on expert opinions and existing data, no other azoles, except posaconazole, are recommended in the treatment of mucormycosis.

Echinocandins

Caspofungin, anidulafungin and micafungin have no efficacy against agents of mucormycosis as single agents when tested by standard techniques *in vitro*.[20,88,89] However, *Rhizopus oryzae* expresses the target enzyme of echinocandins, 1,3-D-glucan synthase, and caspofungin has shown some efficacy in an animal model of infection but with an unexplained inverse-dose response relationship: low doses were more effective in reducing mortality than high doses.[90] This inverse dose-response relationship may be similar to the paradoxical effect previously described with caspofungin against *Candida albicans*.[91] No clinical data are available with echinocandin monotherapy in mucormycosis and occurrence of mucormycosis has been documented in HM currently receiving or recently exposed to caspofungin.[92] However, efficacy of combination therapy including an echinocandin has been reported.

Flucytosine

Flucytosine lacks activity against agents of mucormycosis.[20]

Terbinafine

Despite some *in vitro* activity, oral terbinafine failed to show efficacy in a murine model of mucormycosis, although absorption was demonstrated.[82] No clinical data are available for terbinafine monotherapy in mucormycosis.

Combination antifungal therapy

Most combination studies in mucormycosis include an AmB formulation and either an echinocandin or posaconazole. *In vitro* studies have consistently demonstrated absence of antagonism between posaconazole and AmB.[21,93] Using 30 clinical Mucorales, the combination of both agents was found to be significantly more synergistic (40%)

against hyphae (P<0.05) than against conidia (10%);[93] against 11 isolates of *Rhizopus oryzae*, there was no difference in between posaconazole and AmB.[21] Also, while lipid formulations appeared to enhance hyphal damage of human polymorphonuclear leukocytes against Mucorales *in vitro*, no such interaction was found for posaconazole in these experiments.[94]

The experiments on animal models have led to conflicting results. AmB lipid complex combined with caspofungin improved survival of diabetic ketoacidotic mice infected with *Rhizopus oryzae*.[95] L-AmB combined to anidulafungin or micafungin improved survival in mice infected intravenously with *Rhizopus oryzae* compared to placebo or monotherapy.[96] A paradoxical effect was observed with micafungin but not with anidulafungin. L-AmB combined to posaconazole has been assessed in mice infected with *Rhizopus oryzae*.[71] Combination therapy did not improve survival compared to L-AmB alone and posaconazole alone was not better than placebo is this model. In another study, the combination of low doses of AmB (0.3 mg/kg/day) with posaconazole (40 mg/kg/day) prolonged survival in a manner similar to those obtained with AmB given alone at 0.8 mg/kg/day.[73] The results of triple combination therapy (L-AmB, micafungin and deferasirox) in the treatment of murine mucormycosis showed that triple therapy was superior to all other treatment (i.e. placebo, mono or dual therapy) in prolonging 28-day survival of infected mice (n=18 per group) (40% survival for triple combination *vs.* 0-11% in all other treatment, P<0.05). Further, triple therapy resulted in 4.5 and 3 log10 reduction in brain and kidney fungal burden compared to placebo, respectively (P<0.0001).[97]

A retrospective single-center study in rhino-orbito-cerebral mucormycosis conducted in 37 evaluable patients compared monotherapy with d-AmB, ABLC or L-AmB (31 patients) to a combination of caspofungin and ABLC or L-AmB (n=6 patients).[39] Patients receiving a combination therapy had a significantly higher response rate and survival compared to patients receiving a monotherapy with a polyene. Interestingly, all these patients had only rhinocerebral localization of their disease and most of them had diabetes as predisposing factor. Also, importantly, all patients underwent surgery with a median number of 2 procedures (range 1-6). Although these results are impressive, their value is limited by: a) the low number of patients who received a combination treatment; and b) the restriction of the analysis to patients who were mostly diabetic with rhino-orbito-cerebral disease.

ECIL recommendations

Although encouraging, these data are insufficient to support the recommendation for combination first-line therapy in mucormycosis (CIII). The use of a combination of a polyene and an echinocandin may, however, be an option in salvage therapy after failure of appropriate first-line therapy (BII).

Role of surgery in the treatment of mucormycosis

The characteristic angio-invasiveness of the agents of mucormycosis results in the formation of extensive thrombosis, tissue infarction and necrosis that may impair the penetration of antifungal agents to the site of infection. Timely debridement, if possible, of all devitalized tissue appears reasonable in order to reduce the mass of infecting molds and to prevent the extension of mucormycosis to adjacent structures. This is not always feasible in patients with HMs, who often have profound thrombocytopenia.

The most comprehensive review of mucormycosis so far, that included 929 cases published between 1885 and 2005, found higher survival rates for patients treated with antifungal therapy and surgery (328 of 470, 70%) compared with patients treated with d-AmB alone (51 of 90, 57%) or surgery alone (324 of 532, 61%).[5] Similarly, a review of 106 cases of solid organ transplant recipients with mucormycosis reported from 1970 to 2002 found a reduced mortality rate (34.3%) among patients receiving surgery in combination with antifungal treatment compared to those with antifungal therapy alone (62.5%). A favorable outcome was associated with limited disease accessible to surgical intervention and early surgery together with antifungal therapy.[98]

The role of surgery and its timely performance is also supported by contemporary prospective case series including 50 cases or over. In a matched case-controlled multicenter study on 50 consecutive solid organ transplant recipients with mucormycosis (48% pulmonary, 26% rhino-orbito-cerebral, and 22% with cutaneous-soft tissue disease) surgical resection was strongly associated with treatment success by multivariate analysis.[99] In a prospective multicenter Italian study on 60 cases of mucormycosis including 37 patients with HM (25% pulmonary, 22% rhino-orbito-cerebral, 20% with cutaneous-soft tissue, and 11% with disseminated disease) the mortality rate of patients receiving surgery in addition to antifungal therapy was lower (20%) compared to those given antifungals alone (28%). Interestingly, 28 of 30 (93%) surgical interventions were performed in patients with sino-orbito-cerebral and cutaneous disease.[44]

Rhino-orbito-cerebral disease

Prompt surgical debridement, repeated if necessary, is considered a crucial component of successful therapy. Surgery before disease progression to cerebral structures improves the chance for a successful outcome.[44] A single-center review of 27 patients with rhino-orbito-cerebral mucormycosis treated between 1997 and 2005 revealed a mortality rate of 22% among 23 patients treated with surgery and AmB.[45] All 4 patients who could not receive surgery died; importantly, the survival rate was higher (11 of 14 (79%) among patients presenting within two weeks following the start of symptoms compared with those with a delayed diagnosis (7 of 13, 54%). A single-center study which analyzed the impact of combination antifungal therapy for rhino-orbito-cerebral mucormycosis in 41 patients showed that all patients had at least one surgical intervention, illustrating that the standard approach includes surgical intervention when feasible.[40] A review of 34 cases of rhino-orbito- cerebral mucormycosis predominately in diabetics treated at a single center from 1992 to 2000 reported a 94% rate of treatment success in 18 patients with sino-nasal and limited sino-orbital disease treated with AmB and surgical debridement without orbital exenteration.[46] In contrast, combined antifungal and surgical treatment failed in 8 of 9 patients with extensive sino-orbital disease requiring orbital exenteration. None of the 7 patients with rhino-orbito-cerebral disease were offered surgery and all were considered treatment failures. Finally, in a recent review of the literature, which included 90 patients with rhino-orbito-cerebral mucormycosis and solid organ transplantation, sur-

gical debridement was shown to be independently associated with improved outcome.[47]

Soft tissue infection

Soft tissue mucormycosis is rare in patients with HMs and is usually the result of nosocomial infection.[100-102] Adjunctive surgical excision of infected tissues is generally considered the standard treatment of cutaneous and surrounding tissue mucormycosis and has been found to improve outcome. Surgical debridement may have to be repeated and amputation in case of affected extremities may become necessary.[103,104] In a retrospective single-center analysis of mucormycosis in patients with mostly uncontrolled diabetes mellitus treated between 2000 and 2004, significantly higher survival rates were reported for patients treated with debridement surgery and AmB compared to antifungals alone (80% *vs.* 52%).[48] In addition to patients with rhino-orbito-cerebral disease, a survival benefit of a combined surgical intervention was also documented for 17 patients with cutaneous infection (91% *vs.* 80%). Both groups may have benefited from early definitive diagnosis due to rapid detection of the infection and the relative ease with which biopsies can be made. A review of cases with cutaneous infections due to *Lichtheimia corymbifera* following trauma documented a survival rate of 77% in 22 of 27 patients offered surgical intervention in addition to antifungals.[49]

Localized pulmonary lesion

Surgical resection of infected lung tissue may be associated with a survival benefit. In a case series on 30 patients with pulmonary mucormycosis from a single US center, patients who underwent surgery had a significant reduction in mortality (11%) compared with patients treated with antifungal drugs alone (68%).[50] A comprehensive review of cases of pulmonary mucormycosis published between 1971 and 1999 also indicated a reduced mortality rate of patients receiving combined medical-surgical treatment (27%) compared with those treated with antifungals alone (55%).[51]

Disseminated disease

In disseminated disease, surgery should be considered on a case-by-case basis using a multidisciplinary approach. A recent analysis of 70 consecutive patients with HM and mucormycosis treated at a single center included 11 cases with disseminated disease. Seven (64%) were treated with antifungals alone and all died. Three died despite surgery and the only survivor received surgery in addition to antifungal therapy.[22]

ECIL recommendations

In summary, recommendations regarding surgery in mucormycosis vary according to the site and extension of the disease. While there is good evidence to recommend surgery for rhino-orbito-cerebral and soft tissue diseases (AII), and moderate evidence for pulmonary mucormycosis (BIII), surgery should be considered on a case-by-case basis for disseminated disease (CIII). Repeated procedures may be necessary, but should now be investigated prospectively.

Other modalities used in the treatment of mucormycosis

Adjunctive treatment with deferasirox or deferiprone

Iron acquisition is central to the pathogenesis of the agents of mucormycosis. It is many years now since the first reports that deferoxamine, an iron chelator, acts as a siderophore for Mucorales and therefore supplies previously unavailable iron to the fungi and promotes their growth. In contrast, iron chelation by deferasirox or deferiprone that cannot be utilized as siderophores by the mold creates iron deprivation that reduces the fungal growth. These iron chelators appear to be a rational adjunct to antifungal treatment. However, the limited evidence currently available is insufficient to estimate the role of deferasirox or deferiprone as adjunctive treatment for mucormycosis in combination with surgery and antifungal treatment.[105]

In animal models of mucormycosis, deferasirox used in combination with lipid formulations of AmB improved outcomes.[96,106] In an open-label clinical study on deferasirox as adjunctive treatment for 8 patients (mostly diabetics with rhino-orbito-cerebral disease) with proven mucormycosis the drug was found to be safe and to improve clinical and radiological signs of disease.[107] Further anecdotal evidence of the beneficial effect of adjunctive deferasirox was published in a case report.[108] Failure, however, of deferasirox as adjunctive treatment in a severe case of mucormycosis has also been reported, therefore underlining the multifactorial nature of the disease.[109] A double-blinded, randomized, placebo-controlled phase II clinical trial of the safety and exploratory efficacy of adjunctive deferasirox therapy for patients with mucormycosis treated with L-AmB (the deferasirox-AmBisome therapy for mucormycosis (DEFEAT Mucor) study; NCT00419770) failed to demonstrate any benefit from combination therapy.[54] Furthermore, increased mortality was recorded in the patients receiving deferasirox. This, however, could have been due to the fact that more leukemic and neutropenic patients were included in the deferasirox arm. Further studies are needed in order to clarify the potential of deferasirox to add benefit to lipid polyene therapy for mucormycosis.[110]

ECIL recommendations

Routine use of adjunctive iron chelator therapy is not recommended (AI).

Adjunctive treatment with hyperbaric oxygen

Increased tissue concentration of oxygen may increase neutrophil antifungal activity and the putative oxidative killing mechanism induced by the polyenes. *In vitro*, the growth of Mucorales has been reported to be inhibited by high oxygen concentration.[111] There has been only limited clinical use of hyperbaric oxygen as adjunctive therapy, mostly in diabetic patients with rhino-orbito-cerebral disease. A retrospective single-center review suggested a survival benefit for 6 patients with rhino-cerebral mucormycosis treated with hyperbaric oxygen compared with a group of 7 patients treated with surgery and antifungals alone.[112] Similarly, another retrospective case series described 5 patients, all but one with rhino-orbito-cerebral disease, who received adjunctive hyperbaric oxygen and showed clinical improvement. The survival rate was 60% at three months.[113] A recent report summarizing the experience with hyperbaric oxygen as adjunct treatment gathered over the past 40 years concludes that there is not sufficient evidence to define the efficacy of this expensive intervention.[114]

ECIL recommendations

There are not enough data to support a recommendation

for routine use of hyperbaric oxygen as adjunctive treatment of mucormycosis (CIII).

Adjunctive cytokines

While the antifungal activity of polymorphonuclear leukocytes (PMLs) and macrophages against agents of mucormycosis and the mechanisms involved in this activity were clarified some time ago, there are few new data to help us better understand host defenses against these organisms and the role of cytokines.[115]

It is well known that PMLs and macrophages constitute an important defense mechanism against the agents of mucormycosis,[116] providing a rationale for the use of granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon-γ (IFN-γ) as adjunctive treatment beyond the setting of granulocytopenia. GCSF and GM-CSF have been shown to increase phagocytosis, oxidative burst and fungicidal activity of PMLs,[117-121] and IFN-γ to induce a T-helper cell type 1 (Th1) immunological response that favors resistance to invasive fungal infections and enhances PML's antifungal activities.[116,121,122] G-CSF and GMCSF are routinely given to neutropenic patients with invasive fungal diseases including mucormycosis. The use of γ-IFN in patients with GvHD, a group at high risk for mucormycosis, may augment the aGvH reaction in alloHSCT recipients so as to require augmented immunosuppression for control and thus lead to an even higher risk for invasive fungal infection. G-CSF and GMCSF have also been used in a limited number of cases of mucormycosis in non-neutropenic patients as adjunctive treatment with favorable outcomes.[16,123,124] While individual non-neutropenic patients with extensive or refractory disease may benefit from the use of adjunctive cytokine treatment, further studies are needed to assess the general utility of IFN-γ, G-CSF or GM-CSF as adjuncts to antifungal chemotherapy.

ECIL recommendations

The data suggest that growth factors should be used in patients with neutropenia and mucormycosis in order to reverse the underlying risk factor (BIII). Their use in non-neutropenic patients cannot be recommended at this point.

Conclusions

There are many unresolved issues concerning the epidemiology, diagnosis and treatment of mucormycosis. Although important advances have been made, there is still a need for better diagnostic tests in order to accurately identify patients with mucormycosis and initiate appropriate treatment as early as possible. Based on the existing data, ECIL has made these recommendations to aid clinicians. However, critical gaps in knowledge remain regarding management of these infections, including combination therapy, use of adjunctive treatments and evaluation of response.

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