THE LANCET Infectious Diseases

Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Cornely OA, Alastruey-Izquierdo A, Arenz D, et al, for the Mucormycosis ECMM MSG Global Guideline Writing Grou. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. *Lancet Infect Dis* 2019; published online Nov 4. https://doi.org/10.1016/S1473-3099(19)30312-3.

Appendix to
Global guideline for the diagnosis and management of mucormycosis: An initiative of the ECMM in cooperation with the MSG ERC

Introduction

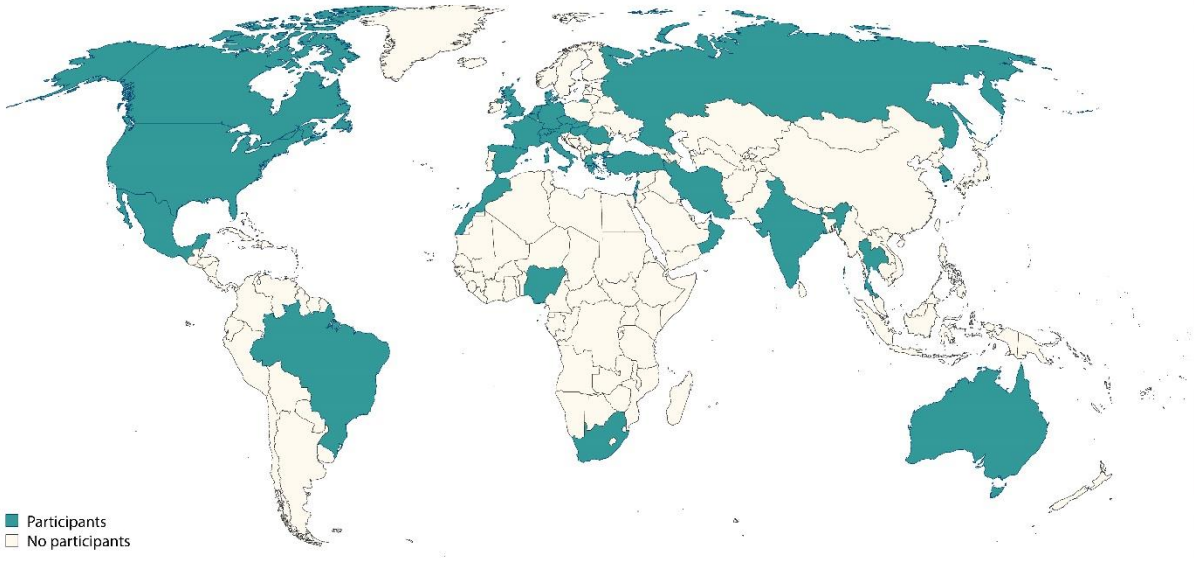
(continued)

Each of the over 150 recommendations tabulated can easily be traced back to the source references for maximum transparency. Any new relevant information, published after this document, can easily be placed in context, and any future updates can straightforwardly build on the tables. For consistency, the same methodology was used as in previous guidance documents.[1-3] As with any other guidance document, this guideline intends to assist management decisions. Whether specific recommendations are appropriate when managing individual patients needs to be carefully assessed by the treating physicians. Recommendations cannot and should not replace clinical judgment, and management of a patient with mucormycosis will always need to be individualised. Moreover, recommendations do not guarantee the availability of specific diagnostics or treatments, or reimbursement by healthcare systems. The recommendations do however reflect the current best available management for mucormycosis.

Guideline development

The general approach applied in the ECMM guideline programme has recently been described.[4] We invited experts to participate in this specific guideline in January 2018. Our selection of experts was determined by their publication activity in the field of mucormycosis, their personal involvement in patient management, and their distribution over the world regions defined by the United Nations https://unstats.un.org/unsd/methodology/m49/ (**Figure S 1**).

Figure S 1. Global distribution of authors of the mucormycosis guideline



Systematic approach

The guideline follows the structure and definitions of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Guidelines on Candidiasis and the ESCMID/ECMM guidelines on rare invasive fungal infections,[2,5] which are in accordance with the Grading of Recommendations Assessment, Development and Evaluation (GRADE) and Appraisal of Guidelines for Research & Evaluation (AGREE) systems.[6,7] Mucormycosis is a rare disease for which only one small randomised trial has been conducted,[8] and meta-analyses are therefore not applicable. The PICO (population, intervention, comparison, outcome) approach is applied, but in this set of guidelines, PICO is displayed within the tables. Both treatment strategies and diagnostic assays may alter patient course, and are thus regarded as interventions. The fixed sequence of seven columns in the tables is pre-defined, and increases transparency. First, a population is defined; then the intention or objective is stated, followed by the intervention. For that logical sequence, strength of recommendation (SoR) and quality of evidence (QoE) are provided, followed by the references on which the recommendation is based, and an index describing the source of level II evidence. In the last column, a comment may be added as appropriate. SoR and QoE are results of two independent evaluations, thus allowing a strong recommendation even in the absence of the highest quality evidence (**Table 1**). Empty tables covering prerequisite relevant topics were provided at the beginning of

the review exercise for population by the panel, and new tables were created by the authors to meet outstanding need as appropriate.

Authors and contributors

Authors fulfilled the criteria set forth by the International Committee of Medical Journal Editors (ICMJE). For the purposes of this guideline, further requirements reflecting sufficient author contribution were responsiveness throughout the guideline process, receipt of training on the guideline process, and disclosure of conflicts of interest. Contributors are individuals who do not meet all original ICMJE authorship criteria, but have contributed significantly to the guideline work.

Literature search terms

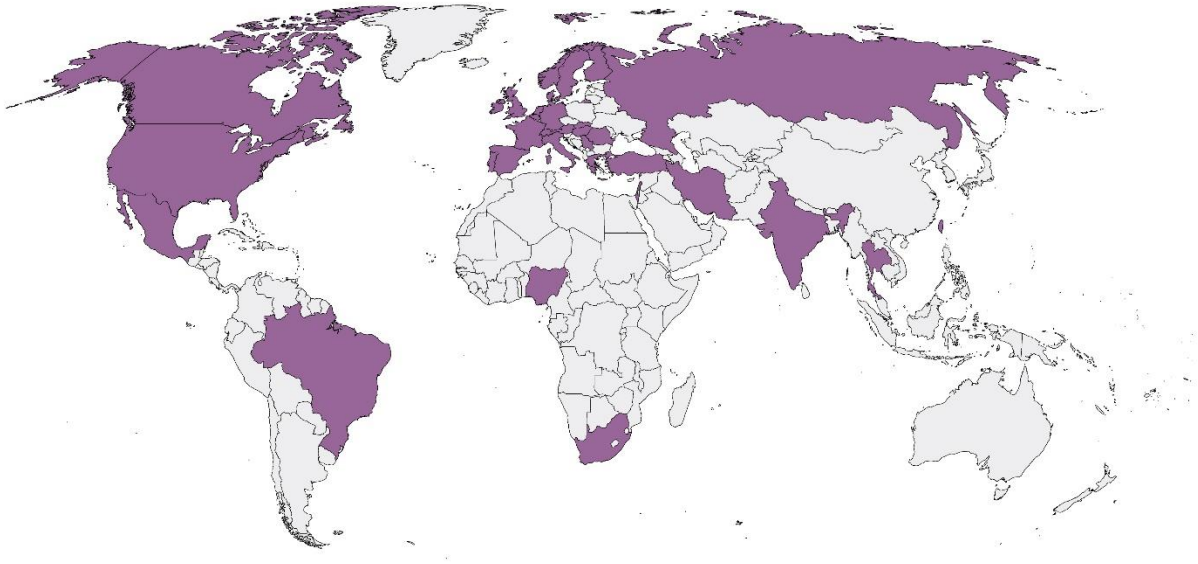
Authors used the following search strings: 'mucormycos* OR zygomycos*', 'ped mucormycos* AND child mucormycos* AND neonate', 'cavernous sinus syndrome or orbital apex syndrome* AND etiology*', 'epidemiology mucormycos* AND etiology*', 'mucormyc* AND susceptibility testing', 'conidiobolomyco*' rhinoconidiobolomyco*' basidiobolomyco*'. For the epidemiology section the following string was used 'mucormyc*[All Fields] OR zygomyc*[All Fields]) AND (case[Title/Abstract] OR cases[Title/Abstract] OR patient[Title/Abstract] OR patients[Title/Abstract] OR report[Title/Abstract]) AND ("2013/01/01"[PDat] : "2017/12/31"[PDat])'. With approximately 200 publications on mucormycosis cases per year, a five-year period was chosen to represent the current distribution of worldwide reports.

Work flow

Having members on the guideline group from 17 time zones is a challenge that we addressed by repeated video conferences on the methodology applied, as well as a video tutorial https://www.youtube.com/watch?v=1silWTWHwdg. Assistance to the group was provided by the coordinators (OAC, AC), who supervised and reminded contributors of timelines. Documents were shared among the authors on a password-protected OneDrive repository, and were updated several times per day. Updates on PICO tables were written in red font; after coordinators spell-checked and formatted, *e.g.* for consistent abbreviation use, the font colour was changed to blue for consideration by the group. Once the group discussed and agreed on contents, the font colour was changed to black. When all information on a slide had been agreed upon, the slide was flagged 'final'. Once all slides were final, a writing group (OAC, AAI, DA, SCAC, ED, BH, MH, HEJ, KL, REL, SCM, MMe, ZP, DS., DCS, RW, AC) volunteered to contribute the first draft, which was circulated to all authors and contributors.

At that time, any discrepancies in recommendations were resolved by majority vote. Additional aspects or publications missing in the manuscript could be contributed via a survey sent out to all authors. Once the authors and contributors agreed on a final draft, a 4-week public consultation phase followed. Comments received were evaluated, and either dismissed or led to changes in the manuscript. 51 societies from 33 countries worldwide have reviewed the manuscript and endorsed the guideline (**Figure S 2**).

Figure S 2. National societies endorsing the guideline



The following societies have endorsed this guideline:

International

- International Immunocompromised Host Society

Africa

- Medical Mycology Society of Nigeria
- Federation of Infectious Diseases Societies of Southern Africa

Americas, Latin America/Caribbean

- Brazilian Association of Hematology, Hemotherapy and Cell Therapy
- Mexican Academy of Dermatolgy
- Sociedad Mexicana de Dermatología (Mexico)

Americas, United States

- Mycoses Study Group Education and Research Consortium (USA)

Americas, Canada

- Association of Medical Microbiology and Infectious Disease (Canada)

Asia

- Asia Fungal Working Group

Asia, Central/Southern

- Clinical Infectious Disease Society (India)
- Society for Indian Human and Animal Mycology
- Iranian Society of Infectious Diseases and Tropical Medicine
- Iranian Society of Medical Mycology

Asia, Eastern/South-Eastern

- Infectious Diseases Society of Taiwan
- Infectious Diseases Society of Thailand with the Thai Medical Mycology Forum

Asia, Western

- Israel Society for Medical Mycology
- Israeli Society of Infectious Diseases
- Lebanese Society of Infectious Diseases and Clinical Microbiology
- Infectious Diseases and Clinical Microbiology Speciality Society of Turkey
- Society for Clinical Microbiologists of Turkey
- Turkish Febrile Neutropenia Society
- Turkish Society for Clinical Microbiology and Infectious Diseases

- Turkish Society of Hospital Infection and Control
- Turkish Society of Medical Mycology

Europe
- European Confederation of Medical Mycology
- European Pediatric Mycology Network

Europe, Eastern
- Hungarian Society for Infectology and Clinical Microbiology
- Romanian Society of Medical Mycology and Mycotoxicology
- Russian Interregional Association for Clinical Microbiology and Antimicrobial Chemotherapy
- Serbian Society of Medical Mycology
- Slovak Society of Chemotherapy
- Slovak Society of Infectious Diseases

Europe, Northern
- Irish Fungal Society
- Nordic Society for Medical Mycology
- British Infection Association
- British Society for Medical Mycology

Europe, Southern
- Hellenic Society of Medical Mycology (Greece)
- Federazione Italiana di Micopatologia Umana e Animale (Italy)
- Sorveglianza Epidemiologica Infezioni nelle Emopatie (Italy)
- Portuguese Association of Medical Mycology
- Asociación Española de Micología (Spain)
- Spanish Society of Medical Microbiology and Infectious Diseases

Europe, Western
- Österreichische Gesellschaft für Infektionskrankheiten und Tropenmedizin (Austria)
- Austrian Society for Medical Mycology
- Belgian Society of Human and Animal Mycology
- French Society for Medical Mycology
- Arbeitsgemeinschaft Infektionen in der Hämatologie und Onkologie (Germany)
- Deutsche Gesellschaft für Infektiologie (Germany)
- German Center for Infection Research
- German-Speaking Mycological Society
- Paul-Ehrlich-Society for Chemotherapy (Germany)
- Dutch Society for Medical Mycology
- Swiss Society of Microbiology

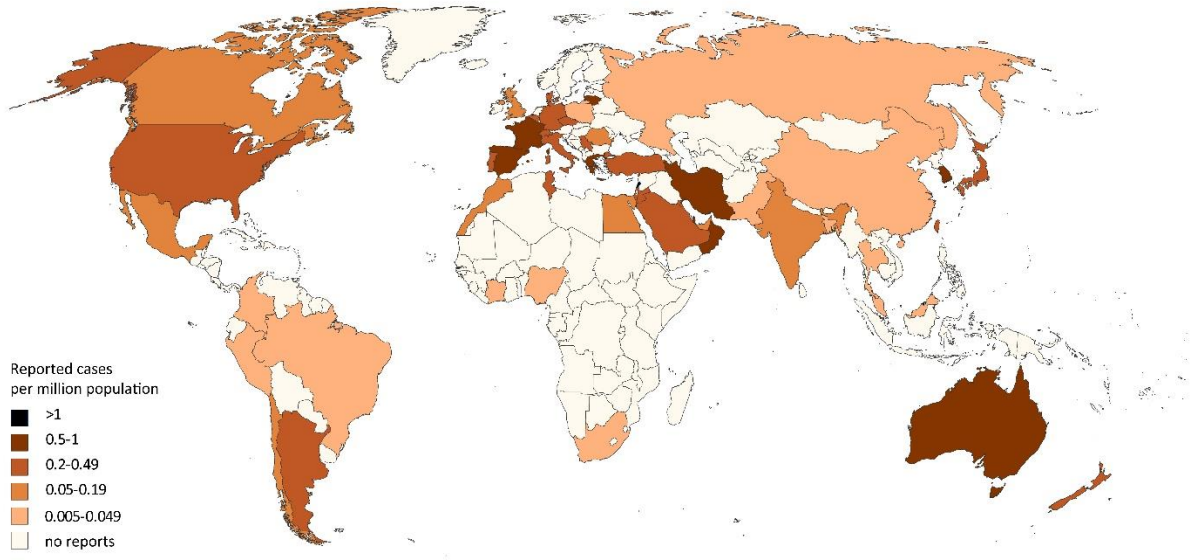
Epidemiology of mucormycosis

Patient populations

(continued)

Our approach characterises mucormycosis as a global disease and identifies areas from where the epidemiology of mucormycosis may be underreported. It should also be noted that given the difficulty in diagnosing mucormycosis the incidence of disease will likely be underestimated. **Figure S 3** represents cases per million population reported in the medical literature between 2013 and 2017.

Figure S 3. Worldwide distribution of mucormycosis (reported cases per million population)



In a literature search, 505 publications with at least one case of severe infection caused by fungi of the order Mucorales reported between 2013 and 2017 were selected from initially 955 search results. In case the study period began before 2013, reported cases were calculated proportionally. In addition, seven epidemiological studies reporting on mucormycosis cases diagnosed before December 31st, 2017 and published in 2018 were included.[9,10-520] Incidence estimations and non-severe infections were not included. High rates (> 1 per million) were calculated for Bahrain, Brunei , Lithuania, and Oman, although only three cases or fewer were reported in the past 5 years. Other countries with high rates may just reflect an actively publishing scientific community. The resident population per country was obtained from www.worldometers.info.[521]

Incidence and prevalence of mucormycosis

Incidence and prevalence rates of mucormycosis are difficult to determine for several reasons. Key limitations are the lack of standardised diagnostic strategies, centralised surveillance systems, as well as the limited awareness of this uncommon mould disease in many regions. One major problem regarding varying diagnostic strategies is the common approach of relying only on histopathological findings with lack of growth and identification of the fungus in culture. Apart from many missed or unreported diagnoses, a major obstacle for defining and comparing rates of mucormycosis globally is the lack of harmonised denominators (*e.g.* cases per 1,000 patient days, cases per specific patient population, cases per million population).[286,522-524]

In Italy, the incidence of mucormycosis was estimated at 0·35 cases per 1 million population per year.[525] An older surveillance study performed during 1992-1993 in the San Francisco Bay area that was comprising mostly HIV and non-haematological patients, estimated 1·7 cases per 1 million population per year.[526] In general, increasing incidence has been noted in several centres worldwide, especially in developing countries.[9,179,527-533] Increases have been associated with an ever-growing at-risk population, in particular in patients undergoing haematopoietic stem cell or solid organ transplantation in Europe and the United States and in patients with uncontrolled diabetes mellitus throughout the world.[365,528,533,534] An increase from 0·7 in 1997 to 1·2 per million population in 2006 was reported in a French surveillance study.[527] A similar increase from 2000 to 2009 has been noted in a single centre in Belgium.[531] A multicentre study in Iran showed a 2·5-fold increase in newly diagnosed cases in 2013 as compared to 2008.[529] A similar increase was noted in Lebanon from 2008 to 2017.[9] In Spain, cases of mucormycosis were reported to increase from 1·2 cases/100,000 hospital admissions in a single centre before 2007 to 3·3 cases since 2007.[179] A similar rise in numbers has been observed in a Swiss study.[535] The use of voriconazole for treatment of aspergillosis has also been linked to breakthrough mucormycosis in many centres.[522,536-538]

Varying rates of mucormycosis have been reported for specific at-risk populations, and for different institutions. A multicentre study in France reported a prevalence of one mucormycosis case per 1,000 acute lymphocytic leukaemia (ALL) patients.[76] In a single-centre study in France, 25 cases of pulmonary mucormycosis were diagnosed in 2,099 episodes of neutropenia in patients with acute leukaemia.[76,524] Incidence rates in transplant recipients was reported between 0·2 % and 3·5 % in American and European centres, whereas higher rates have been observed in Indian and Iranian centres.[539-543] For example, mucormycosis was reported in 12 per 1,000 kidney transplant recipients in an Indian centre but in only 1·1 per 1,000 kidney transplant recipients in a transplant centre

in Brazil.[540,544] Similar incidences of mucormycosis was reported in burn units in France, Greece, and the USA ranging from 4·9 to 6·3 per 1,000 admissions.[268,545,546]

Mucormycosis incidence rates compared to other mould infections

Mucormycosis has been known to be the second- or third-most common mould infection after aspergillosis in most countries. Mucormycetes account for up to 10% of moulds isolated from solid organ and HSCT recipients with invasive mould disease.[547-551] However, the frequency of Mucorales recovery from clinical specimen varies widely across geographic locations, as shown in one Iranian centre, where mucormycetes caused 52·3% of the mould infections in kidney transplant recipients.[552] In an Iranian multicentre study, an increasing rate compared to other mould infections has been noted from 9·7% in 2008 to 23·7% in 2014.[529]

Clinical manifestations of mucormycosis

Mucorales cause opportunistic infections in a heterogeneous population, mostly in patients with impaired immune status. This includes patients with uncontrolled diabetes mellitus, haematological malignancy, transplant recipients, patients with CARD9 deficiency, chronic granulomatous diseases, HIV, and neutropenic patients in particular.[199,250,524,550,553-560] Immunocompetent hosts can also develop mucormycosis, often via direct inoculation of organisms into disrupted skin or mucosa, for example following extensive burn, insect bite or traumatic injury.[561-567] Additionally, outbreaks of mucormycosis are associated with natural disasters, *e.g.* Joplin tornado of 2011 and Indian Ocean tsunami of 2004,[561,567,568] and combat related injuries.[562,569] Healthcare associated mucormycosis includes catheters, adhesive types and tongue depressors; few epidemics are also described.[570-578] Patients who inject illicit substances parenterally may develop isolated renal mucormycosis.[579] Rarely, patients with apparently normal immune system can also develop rhino-orbito-cerebral disease for unclear reasons.[580-583]
The clinical presentation of mucormycosis is highly variable, likely due to underlying host immune status,[9] wide variety of fungal species and strains causing infection, and sites of infection.[408,559,584] The spectrum ranges from cutaneous and soft tissue, to rhino-orbito-cerebral, sino-pulmonary, and gastrointestinal, to disseminated mucormycosis, whereas, blood stream infections are rarely proven, they likely have occurred in cases of disseminated disease.[131,408,522,557] Indeed any organ system can be affected, and cases involving the pleura, mediastinum, bones, and oral tissue before or after dental extraction are described.[379,585,586] Infections are characterised by rapid progression and angioinvasion, one hallmark of mucormycosis, resulting in extensive tissue necrosis and the invasion of adjacent organs and blood vessels.[203,587-589] However, the clinical presentation can be protean, and the rate of progression of infection can vary from extremely rapid (from symptoms to death in days) to more indolent (from symptoms to death in months).[132,185,298,499,590-592] Overall, sinusitis, pulmonary and cutaneous disease are the most frequent clinical presentations of mucormycosis.[408,534,559] The nature of the immune defect bears a close relationship to the site of infection.

Cutaneous and soft tissue mucormycosis

(continued)

Infection can progress into deeper tissue affecting muscles or bone.[593,594] Cutaneous mucormycosis may also develop in immunocompromised patients, where early recognition and treatment may prevent dissemination.[595,596] Chronic cutaneous mucormycosis has also been described.[597]

Rhino-orbito-cerebral mucormycosis

(continued)

This form of mucormycosis may sometimes be related to surgical intervention and thus occurs through direct inoculation.[513] Typical syndromes are cavernous sinus syndrome, acute orbital apex syndrome, and extraocular muscle dysfunction.

Gastrointestinal mucormycosis

(continued)

These infections are characterised by rapid progression with the risk of gastrointestinal perforation. Dissemination has been described to involve the liver, intestines, abdominal wall, kidney, and lung in immunocompetent patients.[598,599] Due to lack of clinical suspicion, diagnosis of gastrointestinal mucormycosis is often delayed or established post-mortem.[600-602] *Mucor indicus* has been found associated with gastrointestinal mucormycosis.[603] In children, especially premature neonates the combination of broad-spectrum antibiotics, formula feeding and abdominal mass, specifically in the presence of shock and metabolic acidosis may be suggestive of mucormycosis.[598,604] In adults with underlying risk factors for mucormycosis abdominal distension and fever accompanied by gastrointestinal bleeding may indicate mucormycosis.[598]

Renal and abdominal mucormycosis
(continued)
Apophysomyces elegans was identified as a cause of isolated renal mucormycosis in two cases.[605,606] The mechanism for infection of the kidney in immunocompromised hosts may arise from haematogenous dissemination from an infected vascular catheter.[607]

Mucormycosis of bones and joints
Mucormycosis of extracranial bones and joints most commonly occurs by direct inoculation in immunocompetent hosts, especially in patients subjected to prior trauma/accident or previous surgery, followed by haematogenous dissemination.[586] Infections of the cranial bones also may occur in the process of rhino-orbito-cerebral mucormycosis.[608]

Diagnosing mucormycosis
(continued)
Tests that may not be available, for example BDG, galactomannan, or certain nuclear amplification assays, are not strongly recommended at this time.

Imaging
Evidence – In some case series, a diagnosis of mucormycosis was more likely than aspergillosis if a neutropenic patient exhibited more than ten distinct nodular infiltrates on CT scan, pleural effusion, concomitant sinus disease, or vessel occlusion sign and negative serum and bronchoalveolar lavage (BAL) galactomannan assay results.[609-614] However, it should be emphasised that no radiographic finding alone has been shown to have adequate specificity or sensitivity to rule in or out pulmonary mucormycosis. Because a wide range of infectious and non-infectious diseases may present with these signs on CT, the diagnostic value of these findings depend on the pre-test probability.[610,615,616] Vessel occlusion detected by CT pulmonary angiography is a more sensitive and possibly more specific radiographic sign than other common CT findings of invasive mould disease in patients with haematological malignancies.[617]
In the sinus, the most common radiographic finding is sinusitis not distinguishable from bacterial infection. While mucosal thickening and partial or complete sinus opacification are frequent, bony erosion is a rare and very late finding.[618-621] Bone destruction is a late manifestation of possible orbital or cranial infection. Organisms may traverse the lamina papyracea to involve the orbit or invade the emissary veins of the ethmoid sinus to reach the cavernous sinus without bony destruction.[622] However, absence of sinus involvement by CT scan has a strong negative predictive value for rhino-orbito-cerebral diseases. MRI is substantially more sensitive than CT scan at detecting orbital and brain involvement; the most common finding of orbital disease is oedema in the orbital muscles.[618-621]
For other body sites, no imaging studies have been shown to be sensitive or specific for mucormycosis. Thus, once mucormycosis is suspected, treatment should be initiated empirically pending final confirmation, which typically requires biopsy and culture. In pulmonary mucormycosis, CT guided needle biopsy was found to be superior in diagnosing pulmonary mucormycosis over BAL.[623,624] However, since patients with haematological malignancies commonly have thrombocytopenia and coagulopathies, CT guided needle biopsy may be contraindicated and warrant the use of BAL as the initial diagnostic procedure.
Radiographic imaging has also been used to assess response to therapy. After adequate treatment, a decreasing extent of ground-glass opacities surrounding a reversed halo, central necrotic cavity or air-crescent sign may occur with recovery.[340,625] However, a confounding effect is seen during recovery of neutrophil counts in neutropenic patients. Such patients can have apparent radiographic worsening as the neutrophils recover. This radiographic effect has not been linked to worse clinical outcome. Indeed, in the only randomised controlled trial for mucormycosis ever conducted, the lack of radiographic changes during the first 30 days of therapy did not indicate negative clinical outcome up to 90 days.[626] Hence, clinicians should avoid making definitive therapeutic decisions based on short-term radiographic changes, particularly in the absence of changes in a patient's clinical condition.

Table S 1. Recommendations on imaging studies in mucormycosis

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Diabetic with facial pain, sinusitis, proptosis, ophthalmoplegia, amaurosis	To diagnose mucormycosis	Cranial CT	A	IIu	Centeno Radiology 1981[627] Gamba Radiology 1986[628] Reed CID 2008[620]	N=12 N=10 N=41 Destruction of bone is rare and late—most common finding is sinusitis indistinguishable from bacterial sinusitis.

Diabetic with facial pain, sinusitis, proptosis, ophthalmoplegia, amaurosis with bone destruction on CT	To determine extent of disease	Cranial MRI for orbit, CNS, cavernous sinus thrombosis	A	IIu	Mohindra Mycoses 2007[619] Koc IntJNeurosci 2007[618] Herrera Skull Base 2009[621] Reed CID 2008[620]	N=27 N=3 N=5 N=41 Higher sensitivity of MRI for orbit and CNS
Diabetic with facial pain, sinusitis, proptosis, ophthalmoplegia, amaurosis	To diagnose mucormycosis	Sinus endoscopy	A	III	Plowes Hernandez ActaOtoEsp 2015[375] Garcıa-Romero CaseRepID 2011[629]	N=5 N=2 Consider repeating endoscopy at individual intervals
Asia, specifically China and India: No underlying diseases, flank pain, fever, haematuria,with sterile urine & blood cultures	To diagnose and to determine extent of renal mucormycosis	CT or MRI	A	III	Chugh AmJKidDis 1993[630]	N=4
					Sharma BrJRadiol 2006[631]	N=1
					Marak MedMycol 2010[605]	N=2
					Dhaliwal Lancet 2015[632]	N=1, thin cortical enhancement ("cortical rim sign")
					Piccoli AmJKidDis 2014[633]	N=1, cortical rim sign
Asia, specifically China and India: No underlying diseases, flank pain, fever, haematuria,with sterile urine & blood cultures	To diagnose and determine	abdominal ultrasound	B	III	Sharma BrJRadiol 2006[634]	N=1
					Dhaliwal Lancet 2015[632]	N=1
Asia, specifically India: Diabetic adult on dialysis OR malnourished/pre-mature child with broad-spectrum antibiotic therapy and with abdominal mass, distension or bilious vomiting, with or without gastrointestinal bleeding	To diagnose mucormycosis	Endoscopic or CT-guided biopsy	A	IIr	Kaur Mycoses 2018[598]	N=176
Any	To prove mucormycosis	CT-guided biopsy	A	IIu	Lass-Flörl CID 2007[623] Rickerts CID 2007[624]	N=61 N=27 Higher sensitivity than BAL
Any	To determine disease response or progression	Weekly CT	A	IIu	Nam EurRadiol 2018[340] Choo DiagnIntervRadiol 2014[625]	N=20 N=5 In particular in unstable patients
Any	To detect dissemination	PET-CT	B	IIr	Douglas CurrOpID 2017[635]	
Haematologic malignancy	To detect dissemination	Staging images of head/sinuses, chest, abdomen	B	III	Pagano Haematol 2004[636] Chamilos CID 2005[609]	Mucormycosis may extend more rapidly than aspergillosis
Haematologic malignancy with pneumonia	To differentiate mucormycosis from invasive aspergillosis	CT / reversed halo	B	IIu	Wahba CID 2008[616] Marchiori Chest 2012[610] Legouge CID 2014[615] Jung CMI 2015[229] Nam EurRadiol 2018[340]	N=8 N=79 N=16 N=24 N=20 See **Figure 3**
Haematologic malignancy with pneumonia	To differentiate mucormycosis from invasive aspergillosis	CT / pleural effusion	C	IIh	Chamilos CID 2005[609]	N=16
					Marchiori Chest 2012[610]	N=18
Haematologic malignancy with pneumonia	To differentiate mucormycosis from invasive aspergillosis	CT / >10 nodular infiltrates	C	IIh	Chamilos CID 2005[609] Marchiori Chest 2012[610]	N=16 N=18
Haematologic malignancy with pneumonia	To differentiate mucormycosis from invasive aspergillosis	CT pulmonary angiography/ vessel occlusion	C	III	Henzler SciRep 2017[637] Stanzani CID 2015[617] Sonnet AJR 2005[611]	N=12 N=2 N=1 Negative serum and BAL galactomannan suggestive of mucormycosis

SoR, strength of recommendation; QoE, quality of evidence; N, number of subjects investigated; CT, computed tomography; MRI, magnetic resonance tomography; CNS, central nervous system; BAL, bronchoalveolar lavage; PET, positron emission tomography

Histopathology in mucormycosis

Table S 2. Recommendations on interpretation of tissue-based diagnosis of mucormycosis

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To diagnose	Histopathology	A	llu	Chakrabarti PGJ 2009[638] Ben-Ami J Infect 2009[587] Rüping JAC 2010[639] Skiada CMI 2011[640] Frater APLM2001[641]	Hyphal diameter of invasive aspergillosis is typically 3-5 µm, whereas those of mucormycosis tend to be 6-16 µm or even up to 25 µm (**Figure 4A-C**).
Any	To diagnose	Immuno-histochemistry	B	llu	Jensen J Pathol 1997[642] Jung CID 2015[643] Sunagawa JpnJID 2013[454]	Monoclonal antibodies against *R. arrhizus* are commercially available (BioRad, code MCA2577, clone WSSA-RA-1) and have been proven useful for differentiating aspergillosis from mucormycosis. Sensitivity 100%, specificity 100% for mucormycosis. In 23 probable mucormycosis cases, 20 (87%) were positive.
Any	To diagnose	Molecular based tests on <u>fresh</u> tissue	B	IIu	Schwarz JCM 2006[644] Lass-Flörl CID 2007[623] Lau JCM 2007[645] Rickerts CID 2007[624] Kasai JCM 2008[646] Hrncirova JCM 2010[647] Bernal-Martinez CMI 2013[648] Buitrago JCM 2014[649] Alanio CMI 2015[650] Guinea J PlosOne 2017[179]	Not commercially available, limited standardisation, various DNA targets (ITS, 18S, 28S, CytB), various techniques (PCR +/- sequencing, semi-nested PCR, qPCR +/- HRM), Mucorales-specific or panfungal assays, fresh material preferred over paraffin embedded
					Springer JMM 2016[651]	Can be used on several clinical specimens incl. fresh and formalin-fixed paraffin-embedded.
					Zaman JMM 2017[652]	Identified Mucorales in 26 cases negative by culture.
						Fresh tissue is preferred over FFPE tissue.
Any	To diagnose	Molecular based tests on <u>FFPE</u> tissue	B	IIu	Hayden Diag Mol Path 2002[653] Nagao J Derm Sci 2005[654] Bialek J Clin Pathol 2005[655] Rickerts EJCMID 2006[656] Lau JCM 2007[645] Hata JCM 2008[657] Dannaoui JCM 2010[658] Hammond JCM 2011[659] Buitrago CMI 2013[660] Gade Med Mycol 2017[661] Guinea J PlosOne 2017[179] Ghadi Mycoses 2018[662]	Not commercially available, limited standardisation, wide heterogeneity of DNA targets and methods, variable sensitivity
					Bernal-Martinez CMI 2013[648]	Sensitivity 100%, specificity 100%
					Salehi JCM 2016[663]	Sensitivity 62%, specificity 100%, identification to genus and species level
					Springer JMM 2016[651,664]	Can be used on several clinical specimens incl. fresh and formalin-fixed paraffin-embedded.
					Drogari-Apiranthitou PRP 2016[665]	Sensitivity 79%, specificity 100%
						Fresh tissue is preferred over FFPE tissue.
Autopsy						
All	To diagnose	Histopathology and molecular testing on positive histopathology	A	llu	Ruangritchankul IJCE 2015[666] Ghadi Mycoses 2018[662]	Prevalence 0.5%, fungal DNA detected in all histopathologically positive samples.
Haematology	To diagnose	Histopathology	A	llu	Lewis Mycoses 2013[667]	Increasing incidence, 8-13% of all invasive fungal infections

All	To diagnose	Histopathology	A	llu	Shimodaira Mycoses 2012[668]	General hospital in Japan, over 50 yrs, prevalence ~0.1%, higher in haematology
All	To diagnose	Gross pathology	C	lltu	Chermetz Mycopathol 2016[95] Davuodi ECT 2015[669] Dhaliwal Lancet 2015[632]	Necrotic and haemorrhagic lesions in all organs incl. skin, haemorrhagic ulceration of skin and gastrointestinal surfaces with black necrotising margins, granulomatous reactions in chronic lesions.
SoR, strength of recommendation; QoE, quality of evidence; PCR, polymerase chain reaction; qPCR, quantitative PCR; RFLP, restriction fragment length polymorphism; FFPE, formalin-fixed-paraffin-embedded						

Antigen biomarkers

Evidence – Antigens to specifically detect Mucorales from clinical samples are not commercially available. In haematology patients or patients with compatible chest CT imaging results, galactomannan testing in blood and BAL has been used to decrease the likelihood of mucormycosis.[670-672] A high level of clinical suspicion is warranted since false positives and mixed infections can also occur.[673] Most Mucorales have low amounts of (1→3) ß-D-glucan (BDG), usually below the limit of detection of the assay, but certain *Rhizopus* spp. could yield positive results.[441,674-680] A recent publication has developed an ELISA and lateral-flow immunoassay (LFIA) that is able to detect several fungal pathogens including *Mucor* spp. and *Rhizopus arrhizus*.[681] This test could potentially be used as a new rapid diagnostic marker, although as currently developed, it is not able to distinguish Mucorales from Ascomycota.

Mucorales-specific T cells have been evaluated in haematological patients to diagnose and monitor treatment.[682-684] Although these tests are not commercially available, if further developed they might be useful as an alternative, non-invasive diagnostic marker for mucormycosis.

Detection of a serum disaccharide by mass spectrometry (MS) has been useful for the diagnosis of nine out of 10 patients with mucormycosis.[685] Although this method is unable to distinguish Mucorales from other fungal pathogens, it might be useful in the future if combined with other markers as well as clinical suspicion.

Recommendations — Specific serological markers to detect Mucorales are currently not available. Use of galactomannan to exclude mucormycosis is moderately recommended, although mixed infections do occur. Other assays such as ELISA, LFIA and MS still require validation and can be only marginally recommended currently. The group does not recommend the use of BDG for diagnosis of mucormycosis (**Table S 3**).

Culture and microscopy

Evidence – Culture of specimens is essential for diagnosis of mucormycosis, since it allows species identification and antifungal susceptibility testing. A positive culture from a sterile site confirms the diagnosis, while a positive culture from a non-sterile site must be combined with clinical and radiological evidence of disease to achieve a probable diagnosis.

Unfortunately, culture is falsely negative in up to half of cases of mucormycosis.[8,686] The organisms grow well *in vitro*, but homogenisation of the tissue may cause viability loss of the nonseptate, fragile hyphal forms of these fungi.[687] Thus, grinding of specimens should be avoided since this has been associated with lower recovery rates and slicing of tissue is recommended.[534,584,688-690]

As well, some strains grow better at 30°C than 37°C, and growth at two temperatures may also increase the yield of culture. Cultures of Mucorales are generally characterised by rapidly growing cotton candy like colonies. Identification to genus level can be reached if the isolate sporulate, but microscopic identification to species level requires a high level of mycological expertise. Of note, some species – such as *Apophysomyces elegans* or *Saksenaea vasiformis* – require specific media, for example, water agar with 0.1% yeast extract, potato dextrose agar or Czapek agar, to permit sufficient sporulation for microscopic identification. Subculture of the primary isolate and its incubation at different temperatures can help to differentiate genera. Specific morphological features such as presence of rhizoids, collumellae, shape and size of sporangia and sporangiospores can help in presumptive genus identification (**Table S 3**) .[691-695]

Direct microscopy specially using fluorescent brighteners together with dilacerating agents such as KOH can be used for a rapid presumptive diagnosis of mucormycosis, but identification to genus or species level is not possible at this stage. As in histopathological specimens, Mucorales are characterised by non-septate or pauci-septate, irregular, ribbon-like hyphae. As with histopathological investigations, artificial septa may occur due to hyphal folding or growth of one hyphae traversing another. An important diagnostic feature is the wide angle of non-dichotomous branching (≥45-90 degree) and greater hyphal diameter as compared to other filamentous fungi, ranging from 6 to 25 µm (and even larger) (**Figure 4D-F**).[534,584,623,641,696]

Table S 3. Recommendations on microscopy, culture and antigen biomarkers in mucormycosis

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To diagnose	Direct microscopy preferably using fluorescent brighteners (Calcofluor White, Blankophor)	A	IIu	Lass-Flörl CID 2007[623] McDermott OralSurgOralMedOralPathol OralRadiolEndod 2010[696] Roden CID 2005[534] Glazer Chest 2000[697] Lanternier CID 2012[584] Frater ArchPathLabMed 2001[641]	Allows rapid presumptive diagnosis, non-septate or pauci-septate, irregular, ribbon-like hyphae, branching angle 45-90°, hyphal diameter 6-25µm. No identification to genus and species level.
Any	To diagnose	Culture	A	IIr	Ribes CMR 2000[690] Roden CID 2005[534] Kontoyiannis AmJClinPathol 2007[689] Lanternier CID 2012[584] Kennedy CMI 2016[688]	Culture is essential since it allows species identification and susceptibility testing. Avoid grinding of clinical sample. Rapidly growing cotton-candy like colonies indicate Mucorales.
Any	To identify to genus level	Culture conditions including incubation at 30°C and 37°C	A	IIr	Padhye JCM 1988[694] Alvarez JCM 2010[691] Garcia-Hermoso MBio 2018[698] De Hoog CBS 2001[692] Walsh ASM Press 2018[695]	Microscopic identification can be performed if isolates sporulate in culture, but requires high level of expertise. Incubation at different temperatures helps to differentiate genera.
Haematology patients	To exclude mucormycosis	Galactomannan ODI in blood	B	IIu	Maertens CID 2005[671]	N=1/117 missed mucormycosis; if galactomannan ODI negative, consider false negative, mucormycosis, or alternative diagnosis; if galactomannan ODI positive, consider invasive aspergillosis, mixed infection or false positive result
Any with positive chest CT imaging	To exclude mucormycosis	Galactomannan ODI in BAL	B	III	Sinko TID 2008[672] Borras JCM 2010[670]	N=2 missed mucormycoses
Haematology patients	To diagnose	(1→3)-ß-D-glucan in blood	D	IIu	Odabasi MedMycol 2006[679] Ostrosky-Zeichner CID 2005[680] Chamilos PLOS One 2010[675] Ibrahim AAC 2005[677] Angebauld OFID 2016[674] Son PLOS One 2017[441] Liss Mycoses 2016[678] Egger JInfect 2018[676]	Some *Rhizopus* spp. contain 1,3-ß-D-glucan at a concentration above the threshold of detection
Haematology patients	To diagnose and monitor treatment	ELISpot or mucormycosis directed CD4+CD154+cells	C	IIu	Potenza PLOS One 2016[684] Potenza Blood 2011[683] Bacher AJRCCM 2015[682] Page IJMM 2018[699]	Not commercially available
Any	To diagnose	ELISA and LFIA	C	III	Burnham-Marusich mSphere 2018[681]	Potential new rapid diagnostic marker. Not discriminatory of Mucorales *vs.* Ascomycota

SoR, strength of recommendation; QoE, quality of evidence; N, number of subjects investigated; ODI, optical density index; BAL, bronchoalveolar lavage; ELISpot, enzyme-linked immuno spot; ELISA, enzyme-linked immunosorbent assay; LFIA, lateral-flow immunoassay

Susceptibility testing

Evidence – The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) have developed standardised methodologies for antifungal susceptibility testing of Mucorales.[700,701] These methods recommend 24 hours incubation time for this group. Agreement between these two methods has been found to be high (**Table S 4**), especially for triazoles, although EUCAST minimum inhibitory concentrations (MICs) tend to be higher than those of CLSI.[702-704] Commercial methods such as E-test® have been evaluated for Mucorales yielding, in some cases, conflicting results with standard methods, especially for amphotericin B and posaconazole.[705-707]

Epidemiological cut-off values have been determined for some species but clinical breakpoints have not been defined, by either the EUCAST or the CLSI, and as a result, classification of isolates as susceptible or resistant is not possible.[708] *In vitro* MICs show that amphotericin B is the most active compound against most species within the order, although some such as *Cunninghamella* spp. have higher MICs.[709-712] Posaconazole has two-fold (EUCAST) to three-fold (CLSI) fold lower geometric MICs than isavuconazole, while isavucaonzole reaches 3-4 times higher blood concentrations.[702,713] *Rhizopus* spp. usually have high MICs of posaconazole[714] whereas isavuconazole demonstrated reduced *in vitro* activity against *Mucor circinelloides.*[702] Other triazoles have MICs

that are above the clinically achievable drug concentrations.[702] Itraconazole showed variable MICs that have been reported to be strain-dependent. Voriconazole and echinocandins show high MICs against these fungi.[703,704,707-712,715-721]

High doses of amphotericin B were effective against experimental infections by Mucorales in mice.[722,723] Murine models of infections with *Rhizopus microsporus* and *R. arrhizus* have shown good correlation between posaconazole MICs and *in vivo* response.[724-726] Correlation between lower amphotericin B MICs and better outcomes has also been reported in patients with mucormycosis.[715,727] However, it should be emphasised that no clinical data are available to validate breakpoints for any antifungal drug against the Mucorales.

Antifungal combinations against these fungi have been studied *in vitro* in a few studies, and findings have shown synergistic combinations against some strains and species.[707,728,729] However, their correlation with the clinical outcome needs to be further analysed (**Table S 4**).

Table S 4. Recommendations on antifungal susceptibility testing in mucormycosis

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To establish epidemiologic knowledge	Susceptibility testing	A	IIu	Vitale JCM 2012[712]	N=66
					Almyroudis AAC 2007[711]	N=217
					Dannaoui JAC 2003[716]	N=36
					Sun AAC 2002[720]	N=37
					Torres-Narbona AAC 2007[721]	N=45
					Alastruey-Izquierdo AAC 2009[709]	N=77
					Chakrabarti JCM 2010[715]	N=18
					Chowdhary AAC 2015[704]	N=124
					Chowdhary Mycoses 2014[703]	N=80
					Espinel-Ingroff AAC 2015[708]	N=894
					Alastruey-Izquierdo CMI 2009[714]	Review
					Halliday IJAA 2016[717]	N=14
					Jain Mycopathologia 2015[718]	N=9
					Arikan Med Mycol 2008[707]	N=11
					Singh Mycoses 2005[719]	N=15
					Guinea PlosOne 2017[179]	N=19
Any	To guide treatment	Correlation of MIC/MFC with outcome	B	III	Rodriguez AAC 2009[724] Rodriguez AAC 2010[725]	Animal, posaconazole more effective in *R. microsporus* & *R. arrhizus* strains with MIC 0.25 µg/ml as compared to those with MIC 2 µg/ml.
					Spreghini JAC 2010[726]	N=1 strain *R. arrhizus*, high posaconazole MFC associated with failure
Any	To support combination treatment	Synergy testing	C	IIu	Biswas JAC 2013[728]	N=10, 3/10 synergy miltefosine/voriconazole, 5/10 synergy miltefosine/posaconazole
					Dannaoui AAC 2002[729]	N=35, 25% synergy amphotericin B/terbinafine, 44% synergy voriconazole/terbinafine
Any	To guide treatment	EUCAST / CLSI reference microdilution methods	C	IIu	CLSI M38-A2[730] EUCAST[700] Espinel-Ingroff AAC 2015[708]	Recommended incubation 24h, no data for clear correlation between MIC and clinical outcome
					Chowdhary Mycoses 2014[703]	87% agreement between Etest and CLSI amphotericin B MIC, EUCAST MIC higher than CLSI MIC
					Chowdhary AAC 2015[704]	essential agreement between isavuconazole 98%, posaconazole 87%, amphotericin B 66%
					Arendrup AAC 2015[702]	N=72, essential agreement 75-83% for isavuconazole
Any	To guide treatment	Correlation of CLSI MIC with outcome	C	IIu	Chakrabarti JCM 2010[715]	N=18, *A. elegans*, limited data, suggests *in vitro* and *in vivo* correlation
					Lamoth JCM 2016[727]	Some correlation of amphotericin B MIC
Any	To guide treatment	Commercial methods (E-test®)	C	II	Caramalho AAC 2015[705] Lamoth JCM 2015[706]	Conflicting results about correlation between E-test® and reference methods

SoR, strength of recommendation; QoE, quality of evidence; N, number of strains investigated; MIC, minimum inhibitory concentration; MFC, minimal fungicidal concentration; EUCAST, European Committee on Antimicrobial Susceptibility Testing; CLSI, Clinical and Laboratory Standards Institute

Molecular-based methods for direct detection

Evidence—Several studies have shown that molecular detection of Mucorales DNA in tissue samples has clinical utility in both fresh[179,623,624,644-652] and formalin-fixed paraffin-embedded tissue specimens.[179,645,648,653-661,663-665] In all studies, in-house techniques were used, as commercial test were not available until recently. Most of the described in-house techniques lack external validation. As various DNA targets (ITS, 18S, 28S, cytochrome B), and various techniques [PCR +/- sequencing, semi-nested PCR, qPCR +/- high resolution melting (HRM)] implemented in either Mucorales-specific or pan-fungal assays have been evaluated, there is a lack of standardisation. The International Society for Human and Animal Mycology (ISHAM) working group, Fungal PCR Initiative is currently working to address this issue. Overall, the test performance is improved with fresh specimens rather than formalin-fixed paraffin-embedded tissue samples.

Detection of Mucorales DNA in blood and other fluids has further been investigated as a non-invasive method of early diagnosis or pre-emptive therapy.[428,646,664,731-741] One of the first attempts to detect Mucorales DNA in serum was performed in a patient with pulmonary *C. bertholletiae* infection. The retrospective analysis of serum samples with pan-fungal PCR showed that DNA detection was positive two days before the appearance of pulmonary infiltrates.[734] Subsequently, two qPCR tests have been tested in a rabbit model of pulmonary mucormycosis, which demonstrated that DNA could be detected in serum of infected animals.[646] More recently, in a retrospective study, a combination of three separate qPCRs targeting the repeat target 18S rDNA allowing the detection of *Mucor*/*Rhizopus*, *Lichtheimia*, and *Rhizomucor*, was tested in 10 patients with mucormycosis. DNA detection was possible in nine out of 10 patients, 3 to 68 days before standard diagnosis.[738] In a nationwide retrospective study, the same set of qPCRs was tested in 44 patients with proven or probable mucormycosis. qPCR was positive in 81% of patients and preceded classical diagnosis by several days.[737] A new specific qPCR for *Cunninghamella* has also been developed.[732] One study also suggested that circulating Mucorales DNA could be detected several days before standard diagnosis in burns patients with wound mucormycosis and that early treatment of burns patients based on a positive qPCR positive in blood could be beneficial in terms of mortality.[735] It has been shown that DNA detection could be also of interest in other clinical samples such as cerebrospinal fluid (CSF),[428] and BAL.[736,739,741]

Recently, PCR amplification of the single target gene CotH, a Mucorales-specific gene, showed positive results when tested in serum, BAL, and urine; both in an experimental animal model and in patients with mucormycosis.[731] Overall, there is still a lack of standardisation of DNA detection and currently there are a limited number of commercial assays and these lack extensive clinical validation (**Table S 5**).

Table S 5. Recommendations on molecular-based methods for direct detection of Mucorales

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To diagnose	Molecular based tests on fresh tissue	B	IIu	Schwarz JCM 2006[644] Lass-Flörl CID 2007[623] Lau JCM 2007[645] Rickerts CID 2007[624] Kasai JCM 2008[646] Hrncirova JCM 2010[647] Bernal-Martinez CMI 2013[648] Buitrago JCM 2014[649] Alanio CMI 2015[650] Guinea J PlosOne 2017[179]	Not commercially available, limited standardisation, various DNA targets (ITS, 18S, 28S, CytB), various techniques (PCR +/- sequencing, semi-nested PCR, qPCR +/- HRM), Mucorales-specific or panfungal assays, fresh material preferred over paraffin embedded
					Springer JMM 2016[651]	Can be used on several clinical specimens incl. fresh and formalin-fixed paraffin-embedded.
					Zaman JMM 2017[652]	Identified Mucorales in 26 cases negative by culture.
						Fresh tissue is preferred over FFPE tissue.
Any	To diagnose	Molecular based tests on FFPE tissue	B	IIu	Hayden Diag Mol Path 2002[653] Nagao J Derm Sci 2005[654] Bialek J Clin Pathol 2005[655] Rickerts EJCMID 2006[656] Lau JCM 2007[645] Hata JCM 2008[657] Dannaoui JCM 2010[658] Hammond JCM 2011[659] Buitrago CMI 2013[660] Gade Med Mycol 2017[661] Guinea J PlosOne 2017[179] Ghadi Mycoses 2018[662]	Not commercially available, limited standardisation, wide heterogeneity of DNA targets and methods, variable sensitivity
					Bernal-Martinez CMI 2013[648]	Sensitivity 100%, specificity 100%

					Salehi JCM 2016[663]	Sensitivity 62%, specificity 100%, identification to genus and species level
					Springer JMM 2016[651,664]	Can be used on several clinical specimens incl. fresh and formalin-fixed paraffin-embedded.
					Drogari-Apiranthitou PRP 2016[665]	Sensitivity 79%, specificity 100%
						Fresh tissue is preferred over FFPE tissue.
Any	To diagnose	Molecular based tests on serum, plasma, or whole blood	B	IIu	Millon CID 2013[320]	N=10, 90% qPCR+ and earlier than culture/histology, good correlation with classical diagnosis
					Millon CMI 2016[742]	N=44, national study, 81% qPCR+
					Kasai JCM 2008[646]	Animal
					Kobayashi Respirology 2004[734]	N=1, PCR+ before pulmonary infiltrate
					Springer JMM 2016[664]	N=5, probable/proven mucormycosis, 5/5 PCR+
					Shigemura IJID 2014[428]	N=1, cerebral mucormycosis qPCR+ in serum and CSF
					Shigemura IJ Haem 2014[429]	N=1, disseminated mucormycosis
					Ino Int Med 2017[733]	N=4, whole blood testing
					Bellanger BMT 2018[732]	N=1, serum and BAL
Burn	To diagnose	Molecular based tests on serum, plasma, or whole blood	B	IIu	Legrand CID 2016[735]	N=77, circulating DNA may allow early detection of skin mucormycosis
Any	To diagnose	Molecular based tests on body fluids	B	IIu	Shigemura IJID 2014[428]	N=1, cerebral mucormycosis: CSF burden > serum burden
					Springer JCM 2018[741]	N=96, BALF, recommended extraction method provided
					Lengerova JCM 2014[736]	N=91, BALF, PCR/HRM +/- RQ-PCR: PCR/HRM has high NPV (haematology)
					Scherer JCM 2018[739]	N=24, BALF
Any	To diagnose	Molecular based	B	III	Baldin JCM 2018[731]	CotH amplification in urine samples from mice infected with different Mucorales (90% sensitivity and 100% specificity) CotH amplification in urine samples from 4 patients with mucormycosis Assay is discriminatory against aspergillus infected mice

SoR, strength of recommendation; QoE, quality of evidence; N, number of subjects investigated; ITS, internal transcribed spacer; CytB, cytochrome b; PCR, polymerase chain reaction; qPCR, quantitative PCR; HRM, high resolution melting; FFPE, formalin-fixed paraffin-embedded; CNS, central nervous system; BAL, bronchoalveolar lavage; BALF, BAL fluid; RQ-PCR, real-time quantitative PCR; NPV, negative predictive value

Genus and species identification

Evidence — (continued)

Identification of the species of Mucorales in culture by standard mycological methods such as morphology is notoriously difficult because the different species share similar morphological characteristics. This has been highlighted by molecular description of cryptic species that can hardly be distinguished morphologically.[691,710,743-745] Moreover, some species fail to sporulate on standard media, precluding a timely and easy morphological identification.[694] Comparison of morphological versus molecular identification showed better performance of molecular approaches.[522,746-748] A high level of concordance (>90%) between morphology and molecular identification may only be seen in reference laboratories.[746] The recent release of commercially developed Mucorales PCR assays are likely to be of limited use for species identification, as these are usually designed to detect the order Mucorales as a whole, or individual genera, but not down to a species level.

Several DNA targets have been evaluated for a reliable identification to the species level. The best informative target should have a large interspecies (between species) and a low intraspecies (within a given species) sequence variability. Moreover, a comprehensive and accurate database must be available. Several studies have shown that internal transcribed spacer (ITS) sequencing was a reliable and accurate method for identification to the species level.[644,654,703,749] Based on published results and expert opinions, both the ISHAM Working Group on Fungal Molecular Identification and the CLSI have recommended using ITS sequencing as a first-line method for species identification of Mucorales.[730,750] A reliable database, such as those developed by CBS

(http://www.westerdijkinstitute.nl/Collections/BioloMICSSequences.aspx) or ISHAM (http://its.mycologylab.org) should be used for sequence pairwise alignment. In one study, ITS2 PCR confirmed mucormycosis in 27 cases (54 %; CI 59·4–68·2). By comparison, Mucorales-specific PCR amplifying 18S was able to amplify DNA and the sequence enabled the identification of Mucorales species in all patients.[652] Other DNA targets have also been evaluated including 18S, 28S, cytochrome b, and FTR1,[657,751-754] and could be used as alternatives. However, for some of these targets there is less evidence of their usefulness. Whole genome sequencing has been used successfully to establish epidemiology in outbreak investigations, but overall there is limited evidence on genotyping methods.[698,755]

Alternative methods for rapid identification of filamentous fungi in clinical microbiology laboratories have been evaluated such as carbon assimilation profiles using the commercialised kits ID32C and API 50 CH (bioMérieux, Marcy l'Etoile, France),[756] and MALDI-TOF mass spectrometry.[757-768] Several studies showed a good identification by MALDI-TOF when an in-house database was used.[760,766] Commercial databases performed less well. In one study that tested 111 strains, only 49·5% of correct identification to the species level was achieved.[768] A multicentre study using another commercial database achieved 86% of correct identification to the species level.[765] Although MALDI-TOF identification of Mucorales seems promising, more data are needed to validate this technique and commercially available databases should be improved (**Table S 6**), molecular approaches remaining the gold standard.

For recommendations refer to **Table S 6**.

Table S 6. Recommendations on genus and species identification of Mucorales

Population	**Intention**	**Intervention**	**SoR**	**QoE**	**Reference**	Comment
Any	To establish epidemiologic knowledge	Molecular identification to species level by ITS sequencing	A	IIu	Schwarz JCM 2006[644]	ITS, good target
					CLSI MM-18A 2008[730]	Good discrimination of genera
					Balajee JCM 2009[750]	ITS sequencing as first-line identification technique
					Chakrabarti JCM 2003[769]	Good identification of *A. elegans* with ITS
					Chowdhary Mycoses 2014[703]	ITS, good target, N=80
					Nagao JDermSci 2005[654]	ITS, identification of 5 *Rhizopus* spp.
Any	To identify species	Molecular identification to species level vs morphology	A	IIu	Kontoyiannis JID 2005[522] Alvarez JCM 2009[746] Bonifaz Mycoses 2014[747] Yang AnnLabMed 2016[748]	N=27 N=190 N=22 N=12 Molecular identification superior over morphological identification
Any	To establish epidemiology in outbreak investigation	Whole genome sequencing	A	IIu	Etienne PlosOne 2012[755]	N=22, wound infections
					Garcia-Hermoso mBio 2018[698]	N=21, burns unit
Any	To establish epidemiologic knowledge	Molecular identification to species level with other DNA targets	C	IIu	Voigt JCM 1999[754]	28S PCR + sequencing
					Machouart JCM 2006[752]	18S PCR + RFLP
					Hall JCM 2004[751]	28S MicroSeq kit
					Hata JCM 2008[657]	Cytochrome b real-time PCR
					Nyilasi CMI 2008[753]	FTR1 sequencing
MALDI-TOF						
Any	To establish routine organism identification and epidemiologic knowledge	MALDI-TOF identification	B	IIu	De Carolis CMI 2012[759]	N=10 species
					Schrödl JCM 2012[766]	Range of MALDI-TOF available
					Dolatabadi JMM 2015[760]	*Rhizopus* and *Lichtheimia* studies
					Riat IJID 2015[764]	Limited evaluations for other Mucorales
					Ranque Mycoses 2014[763]	Limited data. In-house databases remain essential.
					Becker MedMycol 2014[757]	In-house library versus commercial library
					Schulthess JCM 2014[767]	Extraction preferred to direct plating
					McMullen JCM 2016[762]	
					Sanguinetti JCM 2017[770]	
					Chen FrontMicrob 2015[758]	3 strains, poor identification score
					Lau JCM 2013[761]	N=8 species
					Shao JCM 2018[768]	111 strains. 49·5% of identification with commercial database
Any	To diagnose	MALDI-TOF detection of pan-fungal disaccharide	C	IIu	Mery JCM 2016[685]	N=10 mucormycoses, Sensitivity 90%, may be useful if galactomannan negative with imaging positive
Any	To identify to species level	MALDI-TOF identification	B	IIu	Rychert JCM 2018[765]	N=118 strains, multicentre study Vitek MS v3.0 MALDI-TOF, confirmation by DNA sequencing, 86% correctly identified to species level

Any	To identify to species level	Carbon assimilation for species identification	C	IIu	Schwarz JCM 2006[644]	N=54, ID32C and API 50 CH kits allowed precise and accurate identification

SoR, strength of recommendation; QoE, quality of evidence; N, number of subjects investigated; ITS, internal transcribed spacer; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; MALDI-TOF, matrix assisted laser desorption ionisation time of flight; MS, mass spectrometry

Treatment approaches to mucormycosis

Surgical treatment for mucormycosis

Evidence – Various authors have reported higher cure and survival rates through surgical interventions.[94,534,584,586,638,640,771-781] It should be noted that many patients may be too sick to undergo surgery. Surgical treatment is important for local control of mucormycosis, but multiple sites of infection can be present in disseminated infection. Surgery can be separated into major groups: debridement of the skin and soft tissue, debridement of rhino-orbito-cerebral mucormycosis, orbital exenteration, lung resection, debridement of bone, and visceral resections in for example liver, spleen, peritoneal structures, or transplanted organs.

Skin and soft tissue mucormycosis should be treated by radical surgical debridement with margins clear of infection, although it is currently unclear how to define such margins. Identifying margins of infected borders during the surgical procedure may be achieved in real time using fluorescent brightener on the resected tissue.[696] This approach limits unnecessary resection of non-infected tissue particularly in craniofacial areas. Currently, debridement extends until clean tissue is seen, but no intraoperative microscopic evaluation is done. Patients need to be closely followed after surgery to identify new necrosis, which must be managed by repeated debridement. Complete resection could cure mucormycosis and lead to long term survival.[747,773,775,776] In rhino-orbital infection, complete debridement, including endoscopic debridement or excision of infected tissues, increased survival rates in a cohort of solid organ transplant recipients.[782] Adapting the extent of surgery to the distribution of mucormycosis improves outcome and reduces unnecessary loss of healthy tissue.[781]

If lung resection is performed, patients may benefit from emergency surgery to prevent bleeding as well as from elective surgery, which has been shown to increase survival.[100,783] Liver resection with complete removal of mucormycosis ("R0 resection") is feasible and leads to prolonged survival. In addition, drainage of an abscess caused by mucormycosis followed by resection of an infected part of the liver is feasible. Resection of the peritoneal surface should be part of visceral surgical treatment. The rate of surgical complications after visceral resection appears to be acceptable.[474,772,775-777] For patients after solid organ transplantation recipients and graft mucormycosis, surgical debridement, removal of the transplanted organ and/or re-transplantation are options that increase survival probability. More than 80% of patients with osteoarticular mucormycosis undergo surgical intervention, including debridement, fixation, and grafting with an overall response rate of 76%.[586] Early surgical treatment is preferred over late surgical intervention, such that surgeons should be involved in the management team and in the decision plans at the time of diagnosis (**Table S 7**).[534,640,771,783]

In trauma patients, mucormycosis mostly manifests as a soft tissue infection, although subfascial muscular layers may also be involved. A wide variety of trauma mechanisms has been reported, ranging from traffic accidents, war theatre injuries and natural disaster, to injections and insect bites. Early, radical, repeated surgical debridement is indicated and can lead to a definitive cure (**Table S 7**).[562]

Table S 7. Recommendations on surgical treatment for mucormycosis

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To cure and to increase survival rates	Repeated surgery in addition to antifungal treatment	A	IIu	Tedder Ann Thor Surg 1994[778]	N=90
					Nithyanandam IJO 2003[781]	N=34
					Roden CID 2005[534]	N=470
					Greenberg AAC 2006[774]	N=19
					Zaoutis PIDJ 2007[780]	N=92, paediatric
					Chakrabarti PostgrMedJ 2009[638]	N=45
					Singh JID 2009[554]	N=50, SOT
					Sun AmJTranspl 2009[783]	N=11, SOT
					Sun Transplant 2010[782]	N=10, SOT
					Skiada CMI 2011[640]	N=99
					Lanternier CID 2012[584]	N=59
					Vironneau CMI 2014[779]	N=22, rhino-orbito-cerebral
					Taj-Aldeen MM 2017[586]	
Any	To cure	Intraoperative assessment of clean margins	C	III	McDermott OralSurgOralMed OralPathoOralRadiolEndod 2010[696]	N=1

HIV infected	To cure	Surgical debridement + amphotericin B formulation	A	IIr	Moreira JInfect 2016[560]	N=67
Skin	To cure	Surgical debridement + liposomal amphotericin B 5-7 mg/kg/d and/or posaconazole and/or caspofungin	B	IIr	Bonifaz CurrFunInfectRep 2015[784]	
Skin	To cure	Mohs microscopically controlled micrographic surgery as an alternative treatment method	C	III	Clark DermSurg 2003[773]	N=1, similar to the management of cutaneous carcinomas, Mohs surgery removes all the infected tissue in layers, giving adequate margins
Rhino-orbito-cerebral	To increase survival rates	Extensive surgical debridement and antifungal therapy	A	IIu	Bhansali PostgrMedJ 2004[785]	N=26
					Chakrabarti PostgrMedJ 2009[638]	N=14
					Vironneau CMI 2014[779]	N=22, 14/22 diabetic
Rhino-orbito-cerebral, SOT adults	To increase survival rates	Surgical debridement + amphotericin B, lipid formulation, dose not given	A	IIh	Sun Transplant 2010[782]	N=90, 52% died, 57% CNS, (74% died)
Lung, haematology patients	To cure	Emergency and elective lung resection	A	IIu	Chretien CMI 2016[100]	N=12, mostly aspergillosis, emergency surgery to prevent bleeding, elective surgery to cure before new chemotherapy or HSCT, 30 day mortality 6%, median survival after surgery 21 months
Lung, SOT adults	To cure	Surgical debridement + amphotericin B, lipid formulation or posaconazole, dose not given	A	IIh	Sun AmJTranspl 2009[783]	N=31, 90d mortality 45%
Intraabdominal	To cure	Abdominal resection (liver, spleen, omentectomy)	A	IIa	Li WorldJGastroenterol 2010[775] Su DMID 2012[777] Tuysuz Mycoses 2014[474] Busca Mycoses 2010[772] Schlebusch JCM 2005[776]	
Intraabdominal, post SOT	To cure	Debridement + AmB formulations	A	IIr	Almyroudis AmJTanspl 2006[771]	N=13
Intraabdominal & allograft, post liver SOT	To cure	Debridement + 2nd liver SOT	B	III	Gurevich TID 2012[786]	N=1
Liver, haematology patients	To cure	Drainage of liver abscess, then liver resection + liposomal amphotericin B	A	III	Su DMID 2012[777] Tuysuz Mycoses 2014[474] Busca Mycoses 2010[772]	Single cases, drainage of liver abscess followed resection, combined with Amphotericin B, liposomal
Healthcare-associated						
Any site, post-surgical	To cure	Debridement + antifungals	A	IIu	Roden CID 2005[534]	N=32, mostly soft tissue, 38% died
					Almyroudis AmJTanspl 2006[771]	N=70
					Tilak IndJDermVenereol 2009[564]	N=2, abdominal wall necrosis may be misdiagnosed as necrotising fasciitis
					Skiada CMI 2011[640]	N=18
					Nain IndJSurg 2015[338]	N=3, very aggressive disease
Wound infection, post kidney SOT	To cure	Debridement + vacuum sealing	B	III	Chen TransplantProc 2018[787]	N=4
Intramuscular injection	To cure	Surgical debridement and antifungal therapy	A	III	Chakrabarti JCM 2003[769]	N=2, recovery within 15-40 days
					Chander Infect Dis 2016[788]	N=4, severe skin and soft tissue necrosis
Intravenous access	To cure	Surgical debridement and antifungal therapy	A	III	Wollstein CanJPlastSurg 2010[789]	N=1, skin mucormycosis, iv access in the forearm
Injuries, accidents and disasters						
Insect/spider bite	To cure	Surgical debridement and antifungal treatment	A	III	Soman JIMSA 2010[790]	N=2, slower pace of illness, lack of suspicion of diagnosis
Motor vehicle accident	To cure	Repeat surgical debridement and antifungal therapy	A	IIu	Moran JHandSurg 2006[791]	N=3, soil contamination, average 10 debridements
					Ayala-Gaytan Mycoses 2009[792]	N=3, soil-contaminated skin lesions

					Ingram MedMycol 2014[793]	N=9, 78% of all trauma-related cases
					Nain IndJSurg 2015[338]	N=2, both died
Blunt trauma, at construction site	To cure	Repeat surgical debridement and antifungal therapy	A	III	Chakrabarti JCM 2003[769]	N=1, death within one day of therapy
Farm / gardening accidents	To cure	Surgical debridement and antifungal therapy	A	IIu	Moran JHandSurg 2006[791]	N=4, average 10 debridements
					Lanternier CID 2012[584]	N=11, trauma had lower mortality than haematology
Tornado	To cure	Surgical debridement and antifungal therapy	A	IIu	Neblett Fanfair NEJM 2012[561]	N=13
Volcanic cataclysm	To cure	Repeat surgical debridement and antifungal therapy	A	IIu	Patino WorldJSurg 1991[794]	N=8 among 38 patients with necrotising fasciitis
Combat or blast injury with recurrent necrosis	To cure	Surgery + antifungal treatment	A	IIur	Warkentien CID 2012[562] Rodriguez MilMed 2018[795]	N=16 28% mixed infection, risk factors for mucormycosis include dismounted blast injury, traumatic lower limb amputation, extensive perineal injury, mass transfusion
SoR, strength of recommendation; QoE, quality of evidence; N, number of subjects investigated; SOT, solid-organ transplantation; CNS, central nervous system; HSCT, haematopoietic stem cell transplantation						

Drug treatment for mucormycosis

Prophylaxis

Evidence – There is no evidence for primary prophylaxis directed solely towards mucormycosis. Usually prophylaxis is directed against a broad range of fungal infections, including candidiasis and aspergillosis. Breakthrough mucormycosis has been a rare event during prophylaxis with posaconazole oral suspension,[796-801] and exposure due to posaconazole delayed release tablets,[802,803] or intravenous infusions[804,805] may result in even lower invasive fungal infection rates (**Table S 8**).[806,807]

Secondary prophylaxis

Evidence – A frequent clinical question refers to the choice of secondary prophylaxis to prevent recurrence, specifically in immunosuppressed patients. In the absence of a consensus definition of secondary prophylaxis, we defined it as either continued treatment in a patient who had been diagnosed with mucormycosis and responded to treatment, or as restarted treatment in a patient with successful disease control now immunocompetent, but scheduled for a new period of immunosuppression, *e.g.* HSCT. The evidence base for treatment decisions, for example for transitioning to posaconazole,[808] or isavuconazole to facilitate outpatient treatment, is sparse (**Table S 8**).[809,810]

Table S 8. Recommendations on prophylaxis for mucormycosis

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Neutropenic or GvHD	To prevent	Posaconazole DR tablet 2x300 mg d1, 1x300 mg from d2	B	IIu	Duarte AAC 2014[802] Cornely JAC 2016[803] Chin AAC 2017[807]	Higher trough levels than oral suspension
Neutropenic or GvHD	To prevent	Posaconazole iv 2x300 mg d1, 1x300 mg from d2	B	III	Maertens AAC 2014[805] Cornely JAC 2017[804]	Intravenous administration recommended when oral dosing not feasible
Neutropenic or GvHD	To prevent	Posaconazole oral suspension 3x200 mg/d	C	IIu	Cornely NEJM 2007[796]	N=0/304 breakthrough
					Ullmann NEJM 2007[797]	N=0/301 breakthrough
					Pagano CID 2012[801]	N=0/260 breakthrough
					Cornely AAC 2012[806]	N=0/75 breakthrough
					Cho Mycoses 2015[799]	N=2/140 breakthrough
					Lamoth CID 2017[798]	N=2/279 breakthrough
					Lerolle CMI 2014[800]	N=2 over a 4 year period
Neutropenic	To prevent	Isavuconazole po/iv 3x200 mg d1-2, 1x200 mg/d from d3 or 1x200 mg/d from d1	C	IIu	Rausch CID 2018[811]	N=4/100 breakthrough
					Gebremariam AAC 2017[812]	Animal model, lower tissue burden of *R. delemar* with isavuconazole and with posaconazole, improved survival
ALL induction chemotherapy	To prevent	Amphotericin B, liposomal 5 mg/kg biw	D	I	Cornely JAC 2017[813]	Not better than placebo, but no proven breakthrough mould infection in either study arm
Neutropenic or GvHD	To prevent	Fluconazole, itraconazole, voriconazole, any dose	D	IIr	Lass-Flörl Drugs 2011[814]	Fluconazole and voriconazole not active, itraconazole may yield some activity, likely inferior to posaconazole

SOT adult, lung & heart	To prevent	Isavuconazole po/iv 3x200 mg d1-2, 1x200 mg/d from d3 or 1x200 mg/d from d1	C	IIu	Wu AAC 2018[815]	N=21, pharmacokinetic study
SOT, adult	To prevent	Isavuconazole po/iv 3x200 mg d1-2, 1x200 mg/d from d3 or 1x200 mg/d from d1	C	IIa	Rivosecchi ECCMID 2017[816]	N=187, no breakthrough mucormycosis
SOT adult, lung & heart	To prevent	Posaconazole iv 2x300 mg d1, 1x300 mg from d2	C	III	No reference found.	
SOT adult, lung & heart	To prevent	Posaconazole oral suspension 3x200 mg/d	C	III	Shields AAC 2011[817]	N=4
Immunosuppressed with prior diagnosis of mucormycosis	To prevent recurrence, secondary prophylaxis	Surgical resection and last drug effective in the same patient	A	III	Nosari BMT 2007[809]	N=3
					Hoover MedPedOnc 1997[810]	N=1
					Marty LancetID 2016[808]	N=12, transition from amphotericin B to oral posaconazole after 3-6 weeks if feasible
SoR, strength of recommendation; QoE, quality of evidence; N, number of subjects investigated; GvHD, graft-versus-host disease; DR, delayed release; ALL, acute lymphoblastic leukaemia; SOT, solid-organ transplantion						

Fever-driven treatment

Evidence – For the clinical situation of unexplained fever with negative imaging results, no reference supports initiation of mucormycosis-directed treatment. However, in high-risk patients with prolonged fever during neutropenia, empirical treatment with liposomal amphotericin B is an accepted strategy that is also active against mucormycosis.[818]

Diagnosis-driven treatment

Evidence – Short delays in treatment initiation substantially increase mortality rates in haematological malignancy patients.[819] With any new nodular opacities in haematology patients, breakthrough mucormycosis must be considered.[811]

Table S 9. Recommendations on treatment decision points in mucormycosis

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Fever-driven treatment						
Any with fever of unknown origin, imaging negative	To cure mucormycosis	Fever-driven treatment	D	III	No reference found.	
Any neutropenic with fever of unknown origin, unresponsive to antibiotics, galactomannan negative, imaging positive, but not typical	To cure mucormycosis	Fever-driven treatment	C	III	No reference found.	May be regarded as diagnosis-driven
Diagnosis-driven treatment						
Any immunocompromised with suspected mucormycosis	To increase survival	Immediate treatment initiation	A	IIu	Chamilos CID 2008[819]	N=70, treatment initiation ≥6 days after symptom onset increased 12-week mortality from 48·6% to 82·9%
Critically ill burn patients	To increase survival	Treatment based on qPCR serum screening	C	IIh	Legrand CID 2016[735]	N=75, period A: treatment after positive culture/biopsy, survival 1/5; period B: treatment after positive in house qPCR screening 2x/week, survival 2/3
Haematologic malignancy, radiologic suspicion, galactomannan negative	To direct appropriate therapy	Treatment based on qPCR blood screening	C	III	Ino Intern Med 2017[733]	N=4, all survived, in house qPCR
SoR, strength of recommendation; QoE, quality of evidence; N, number of subjects investigated; qPCR, quantitative polymerase chain reaction						

First-line antifungal combination therapy

For recommendations, refer to **Table S 10**.

Table S 10. Recommendations on first-line antifungal combination therapy for mucormycosis

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Combat or blast injury with recurrent necrosis	To cure	Liposomal amphotericin B + posaconazole or voriconazole + topical 0.025% Dakin's solution	C	IIur	Warkentien CID 2012[562] Rodriguez MilMed 2018[795]	N=16 28% mixed infection, risk factors for mucormycosis include dismounted blast injury, traumatic lower limb amputation, extensive perineal injury, mass transfusion
Any	To cure	Liposomal amphotericin B + caspofungin	C	IIu	Abidi Mycoses 2014[820]	N=101, no benefit for combination
					Kyvernitakis CMI 2016[821]	N=27, propensity score analysis, no benefit for combination
Any	To cure	Amphotericin B formulation + caspofungin	C	III	Reed CID 2008[620]	N=7 (6/7 diabetic), superior success and survival time compared to polyene monotherapy particularly for amphotericin B lipid complex and in patients with cerebral involvement
Haematologic malignancy	To cure	Liposomal amphotericin B + caspofungin	C	IIu	Klimko Mycoses 2014[250]	N=36, combination treatment (52%) was associated with favourable prognosis
Any	To cure	Liposomal amphotericin B + (anidulafungin or micafungin)	C	III	Ibrahim AAC 2008[822]	Animal study, improved survival rate with combination
Haematologic malignancy	To cure	Liposomal amphotericin B + posaconazole oral suspension	C	IIu	Kyvernitakis CMI 2016[821]	N=16, propensity score analysis, no benefit for combination
Any	To cure	Liposomal amphotericin B + (posaconazole DR tablet or iv)	C	IIu	Jenks IJAA 2018[823]	N=10, overall survival 4/6 in those with combination versus 0/4 in those with single agent therapy
Haematologic malignancy	To cure	Liposomal amphotericin B + caspofungin + posaconazole	C	IIu	Kyvernitakis CMI 2016[821]	N=106, propensity score analysis, no benefit for combination

SoR, strength of recommendation; QoE, quality of evidence; N, number of subjects investigated; DR, delayed release

Antifungal salvage treatment

For recommendations, refer to **Table S 11**.

Table S 11. Recommendations on antifungal salvage treatment for mucormycosis

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Refractory disease						
Refractoriness	To cure	Isavuconazole iv or po 3x200 mg d1-2, 1x200 mg from d3	A	IIh	Marty Lancet ID 2016[808]	N=11
Refractoriness	To cure	Posaconazole DR tablet or iv 2x300 mg d1, 1x300 mg from d2	A	IIt	Duarte AAC 2014[802] Maertens AAC 2014[805] Cornely JAC 2016[803] Cornely JAC 2017[804]	Higher serum concentrations than oral suspension, iv bridging when oral dosing not feasible
Refractoriness	To cure	Amphotericin B, liposomal 10 mg/kg	A	IIut	Cornely CID 2007[824]	N=4
					Lanternier JAC 2015[825]	N=44
Refractoriness	To cure	Amphotericin B, liposomal 5 mg/kg	B	III	Pagano Haematol 2004[636]	N=8, prior amphotericin B deoxycholate
Refractoriness	To cure	Amphotericin B, lipid complex 5 mg/kg	B	IIu	Walsh CID 1998[826]	N=16
					Larkin Inf Med 2003[827]	N=23
Refractoriness	To cure	Posaconazole oral suspension 4x200 mg/d or 2x400 mg/d	C	IIu	Greenberg AAC 2006[774]	N=19, 4 diabetes
					van Burik CID 2006[828]	N=81*, 30 diabetes
					Skiada CMI 2011[640]	N=61
					Vehreschild CRM 2012[829]	N=15[#]
Refractoriness	To cure	Combination of liposomal amphotericin OR lipid complex + posaconazole	C	IIu	Ibrahim AAC 2009[830]	Animal, not superior
					Pagano Haematol 2013[831]	N=29, posaconazole added, d84 response 16/29
Intolerance and toxicity						

Toxicity	To cure	Isavuconazole iv or po 3x200 mg d1-2, 1x200 mg from d3	A	IIh	Marty Lancet ID 2016[808]	N=5
					Marty Mycoses 2018[673]	N=8
					DiPippo Mycoses 2018[832]	N=23
Toxicity	To cure	Posaconazole DR tablet or iv 2x300 mg d1, 1x300 mg from d2	A	IIt	Duarte AAC 2014[802] Maertens AAC 2014[805] Cornely JAC 2016[803] Cornely JAC 2017[804]	Higher serum concentrations than oral suspension, iv bridging when oral dosing not feasible
Toxicity, renal	To cure	Amphotericin B, liposomal 5 mg/kg	B	III	Pagano Haematol 2004[636]	N=8, prior amphotericin B deoxycholate
Toxicity, renal	To cure	Amphotericin B, lipid complex 5 mg/kg	B	IIu	Larkin Inf Med 2003[827]	N=18, pre-existing nephropathy
Toxicity, renal	To cure	Amphotericin B colloidal dispersion 5 mg/kg	B	IIu	Herbrecht EJCMID 2001[833]	N=21
Toxicity	To cure	Posaconazole oral suspension 4x200 mg/d or 2x400 mg/d	C	IIu	Greenberg AAC 2006[774]	N=5
					van Burik CID 2006[828]	N=43*
					Vehreschild CRM 2012[829]	N=15[#]
SoR, strength of recommendation; QoE, quality of evidence; N, number of subjects investigated; DR, delayed release *33 patients had refractory disease and were intolerant; 11 individuals overlap between van Burik[828] and Greenberg[774] reports; [#]The reason for salvage treatment, *i.e.* refractoriness vs intolerance, was not reported in this study.						

Treatment duration for mucormycosis

Table S 12. Recommendations on treatment duration for mucormycosis

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To cure infection	Continue treatment until complete response on imaging and permanent reversal of immunosuppression	A	III	No reference found.	Treatment duration is being determined on a case-by-case basis and depends, *e.g.* on the extent of surgery, the organs involved, and ongoing immunosuppression
Any	To reach stable disease	1st line treatment by iv antifungal until stable disease	B	IIu	Lanternier JAC 2015[825]	N=34, median duration 21 days, followed by unspecified further treatement
Any	To reach stable disease	1st line treatment by intravenous antifungal until stable disease and PCR negativity	C	IIu	Millon CMI 2016[737]	N=44, no commercial test
Any	To facilitate oral treatment in stable disease	Isavuconazole po 3x200 mg d1-2, 1x200 mg from d3	A	IIh	Marty LancetID 2016[808]	N=37, median duration 84 days
Any	To facilitate oral treatment in stable disease	Posaconazole DR tablet 2x300 mg d1, 1x300 mg from d2	A	IIt	Duarte AAC 2014[802] Cornely JAC 2016[803]	Higher trough levels than oral suspension, prophylaxis study
Any	To facilitate oral treatment in stable disease	Posaconazole oral suspension 4x200 mg/d or 2x400 mg/d	C	IIu	van Burik CID 2006[828] Greenberg AAC 2006[774] Davoudi Mycopath 2014[112]	N=91 N=24 N=1 Across different studies mean duration approx. 6 months (range 6-1005 days), remains case-by-case decision
					Ma JGID 2015[298]	N=1, Late relapse in long-term survivor
					Kim Mycoses 2016[834]	N=5
CNS involvement	To facilitate oral treatment in stable disease	Posaconazole DR tablet 2x300 mg d1, 1x300 mg from d2	C	III	Andrey IJID 2017[835]	N=1, surgical resection
SoR, strength of recommendation; QoE, quality of evidence; N, number of subjects investigated; PCR, polymerase chain reaction; DR, delayed release						

Therapeutic drug monitoring (TDM)

Evidence – Antifungal agents, particularly triazole antifungals, may have unpredictable pharmacokinetics in patients with mucormycosis due to altered bioavailability, underlying organ dysfunction, or drug interactions that affect rates of drug metabolism and clearance. In some patients, this pharmacokinetic variability may contribute to treatment failure or drug toxicity.[836] TDM is the most direct approach for detecting potentially subtherapeutic drug exposures in patients and guiding dosage adjustments. TDM has also been recommended in patients receiving triazole antifungal agents for the treatment of other life-threatening invasive fungal disease.[614] Therefore, it is reasonable to assume TDM could have similar clinical utility when managing patients with mucormycosis.

The therapeutic range of isavuconazole and posaconazole for mucormycosis is unknown. Preclinical data from animal infection models have shown that triazole pharmacodynamics for *A. fumigatus* and *R. arrhizus* are similar when triazole serum exposures (area under the curve, AUC) are indexed to the MIC.[837] Simulations based on preclinical data predicted the highest probabilities of treatment response when posaconazole serum exposures exceeded 1-1·5 µg/ml for isolates with MICs up to 0.25 µg/ml, with proportionally higher serum exposures (>4 µg/ml) required for isolates with higher MICs (*e.g.* 2 µg/ml). However, these pharmacodynamic relationships have not been confirmed in patients with invasive mucormycosis. Serum levels of posaconazole >2 µg/ml may only be achievable in patients with non-licenced doses of posaconazole tablets or the IV formulation, and could predispose patients to increased risk of hepatotoxicity,[838] or pseudohyperaldosteronism (**Table S 13**).[839]

No TDM was undertaken in isavuconazole-treated patients in the VITAL study.[808] In the SECURE trial, TDM was performed in select clinical cases of patients with invasive aspergillosis (n=283 samples). Over 90% of patients achieved predicted target exposures and no relationship was observed between isavuconazole serum levels, treatment response, or liver toxicity.[713,840] Moreover, the more recent study of 283 serum samples from real-world use and the SECURE clinical trial demonstrates nearly identical values (>1 µg/ml in 90% of patients) with concentrations as high as 9 µg/ml.[841] Therefore, there is no current evidence to suggest that routine TDM for isavuconazole is necessary. However, TDM could be useful as part of a comprehensive clinical assessment for any patients with progressing mucormycosis on isavuconazole therapy, or after patients are switched from IV to oral therapy to confirm adequate drug exposures (**Table S 13**).

Recommendations – Routine TDM is strongly recommended for patients with mucormycosis receiving treatment with posaconazole. Serum trough posaconazole concentrations of 1 µg/ml or higher in patients with infecting isolates with elevated MICs are recommended to reduce the risk of treatment failure. There is no clinical evidence supporting a need for routine TDM with isavuconazole. However, documentation of serum drug concentrations could be useful in some clinical situations such as suspected treatment failure, drug interactions, suspected toxicity or intolerance, obesity, or after switching from IV to oral therapy in a patient with documented mucormycosis (**Table S 13**).

Table S 13. Recommendations on therapeutic drug monitoring in mucormycosis

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To reduce risk of treatment failure	Posaconazole oral suspension TDM for treatment target concentration >1 µg/ml	A	IIt	Dolton AAC 2012[836]	N=72, breakthrough during prophylaxis in those with significantly lower median concentrations, not specific for mucormycosis
SOT adults, lung/heart	To reduce risk of treatment failure	Posaconazole DR tablet or iv TDM for treatment target concentration >1 µg/ml	A	IIa	Haidar IDWeek 2017[842]	N=17
					Jeong JAC 2017[843]	N= 78, lung transplant
Any	To reduce risk of treatment failure in *Rhizopus arrhizus*	Posaconazole TDM for treatment target concentration >1·5 µg/ml	C	IIt	Lewis AAC 2014[837]	Maximum reduction in lung fungal burden observed with posaconazole doses achieving serum levels > 4 µg/ml for *R. arrhizus* isolate with MIC of 2.0 µg/ml
Any	To reduce risk of treatment failure in *Mucor* spp.	Posaconazole TDM for treatment target concentration >4 µg/ml	C	IIt	Lewis AAC 2014[837]	Maximum reduction in lung fungal burden observed with posaconazole doses achieving serum levels > 4 µg/ml for *R. arrhizus* isolate with MIC of 2 µg/ml
Any	To reduce risk of treatment failure	Isavuconazole TDM for treatment target concentration >1 µg/ml	C	III	Marty LancetID 2016[808]	No TDM undertaken in VITAL study
					Andes AAC 2018[841]	N=283 samples, 90% target reached
					Desai AAC 2017[840]	TDM in select clinical cases from the SECURE study
					Kaindl JAC 2018[713]	
Any	To reduce risk of treatment failure	Determine azole concentration in ascites, effusion, CSF	C	III	Felton CMR 2014[844]	Therapeutic range not established, single drug concentrations may not be interpretable

SoR, strength of recommendation; QoE, quality of evidence; N, number of subjects investigated; TDM, therapeutic drug monitoring; DR, delayed release; MIC, minimum inhibitory concentration; CSF, cerebrospinal fluid

Specific considerations on treatment of mucormycosis in children

Evidence – As in adults, diagnosis and treatment of mucormycosis in children remain challenging. Evidence derives from case series, case reports and is extrapolated from adults.[525,534,640,845-849] Predisposing factors, sites of infection and identified species are similar to adults.[534,640,780,846-850] Specifically for neonates the most commonly reported site of infection is the gastrointestinal tract (54%), which is associated with increased mortality rate (78%).[780,847,851,852] Mucormycosis is life threatening for immunocompromised children and premature neonates.[780,846,847,850,853,854] The overall mortality rate among all age groups ranges from 33% to 56%, and reaches 64% to 89% in neonates with disseminated disease.[846-848,850] In ages less than 12 months, disseminated infection and HSCT have been found to be independent prognostic factors (**Table S 14 and Table S 15**)[780,846].

As in previously published guidelines, the group considered four sources to grade therapeutic interventions: (i) evidence for efficacy from studies in adults; (ii) availability and quality of paediatric pharmacokinetic data and dosing recommendations; (iii) paediatric safety and supportive efficacy data; and (iv) regulatory approval for use in paediatric age groups (**Table S 14 and Table S 15**).[1]

Recommendations – Immediate initiation of effective antifungal therapy in combination with appropriate surgical debridement – and, if feasible, control of underlying predisposing conditions – is strongly recommended.[780,846,847,854-856] Liposomal amphotericin B and amphotericin B lipid complex are strongly supported as first-line treatment options among all age groups, with a preference for liposomal amphotericin B in CNS involvement.[298,639,640,826,827,848,854,857-864] Amphotericin B deoxycholate is an alternative choice in the neonatal population, if liposomal amphotericin B or amphotericin B lipid complex are not available based on the existing tolerability and safety data.[298,847,864] For both, paediatric and neonatal patients, a liposomal amphotericin B dose of 5-<10 mg/kg/d is strongly recommended.[639,640,858,859,865-867] Due to the lack of clinical efficacy data, a dose of 10 mg/kg in selected cases as in CNS involvement is recommended with marginal strength and based on extrapolated clinical evidence from studies in adults, paediatric studies of safety, tolerability, and pharmacokinetics, and data from animal models.[824,825,866] Salvage or continuation treatment options comprise isavuconazole and posaconazole for children ≥13 years and are supported with moderate strength.[774,808,828,868-874] Oral posaconazole is dose-adjusted based on age with a general preference given to the delayed release tablet formulation for children ≥13 years; therapeutic drug monitoring (TDM) is advised for the oral solution (**Table S 15**).

For salvage treatment, combination therapy of amphotericin B lipid formulation plus caspofungin, or amphotericin B lipid formulation plus posaconazole for children ≥2 years of age is recommended with marginal strength (**Table S 15**).[620,829,831,875-879] Indications of salvage therapy, duration of treatment and diagnosis are similar to those outlined for adults.

Table S 14. Recommendations on first-line treatment of mucormycosis in children

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Neonates	To cure	Amphotericin B formulation plus surgery	A	IIu	Roilides AmJPerinatol 2009[851]	N=59, neonates
Neonates	To cure	Amphotericin B, liposomal 5-<10 mg/kg/d	A	IIt		No neonatal PK available
					Juster-Reicher EJCMID 2003[858]	N=37, neonatal safety
					Kolve JAC 2009[859]	N=87, paediatric safety
					Rüping JAC 2010[639]	N=5, paediatric efficacy
					Shoham MedMycol 2010[860]	Adult efficacy
					Skiada CMI 2011[640]	Adult efficacy
Neonates	To cure	Amphotericin B, lipid complex 5 mg/kg/d	A	IIt	Würthwein AAC 2005[863]	N=30, neonatal PK and safety
					Wiley PIDJ 2005[862]	N=44, neonatal safety, <3 months
					Walsh PIDJ 1999[861]	N=111, paediatric safety
					Walsh CID 1998[826]	Adult efficacy
					Larkin InfMed 2003[827]	Adult efficacy
Neonates	To cure	Amphotericin B, deoxycholate 1-1·5 mg/kg/d	C	III	Ma JGlobID 2015[298]	n=12, <1 yr, intracranial mucormycosis
Paediatric	To cure	Amphotericin B formulation and surgery	A	IIu	Zaoutis PIDJ 2007[780]	N=157, paediatric, median 5 yrs
					Dehority JPHO 2009[854]	N=6, paediatric
					Roilides CMI 2009[847]	N=157, paediatric,median 5 yrs
					Pana BMCID 2016[846]	N=63, paediatric, 0-20 yrs
					Ardeshirpour Laryngosc 2013[856]	N=11, paediatric, 2-14 yrs

					Xhaard CMI 2012[855]	N=29, mixed paediatric and adult, 3-63 yrs
Paediatric	To cure	Amphotericin B, liposomal 5 - <10 mg/kg/d	A	IIt	Lestner AAC 2017[865] Seibel AAC 2017[866]	N=47, paediatric PK, 1-17 yrs
					Hong AAC 2006[867]	N=39, paediatric PK, 0.2-17 yrs
					Dehority JPHO 2009[854]	N=6, paediatric safety, median 11 yrs
					Kolve JAC 2009[859]	N=84, paediatric safety (median age 11 yrs)
					Rüping JAC 2010[639]	N=5, paediatric efficacy
					Shoham MedMycol 2010[860]	Adult efficacy
					Skiada CMI 2011[640]	Adult efficacy
					Wattier JPIDS 2015[848]	N=14, paediatric efficacy
					Phulpin-Weibel Mycoses 2013[876]	N=11, paediatric efficacy, 2-14 yrs
Paediatric	To cure	Amphotericin B, liposomal 10 mg/kg/d	C	IIt	Seibel AAC 2017[866]	N=12, paediatric PK and safety
					Lanternier JAC 2015[825]	Adult efficacy
Paediatric	To cure	Amphotericin B, lipid complex 5 mg/kg/d	A	IIt	Walsh AAC 2005[878]	N=6, paediatric PK, 21 d-16 yrs
					Walsh PIDJ 1999[861]	N=111, paediatric safety
					Wiley PIDJ 2005[862]	N=548, paediatric safety, 0-20 yrs
					Walsh CID 1998[826]	Adult efficacy
Paediatric	To cure	Amphotericin B, deoxycholate, any dose	C	III	Bonifaz Mycoses 2014[747]	N=22, paediatric, (6 m -18 y)
					Ma JGlobID 2015[298]	N =51, mixed paediatric and adult, 15 d-79 yrs, CNS involved
SoR, strength of recommendation; QoE, quality of evidence; N, number of subjects investigated; PK, pharmacokinetics; CNS, central nervous system						

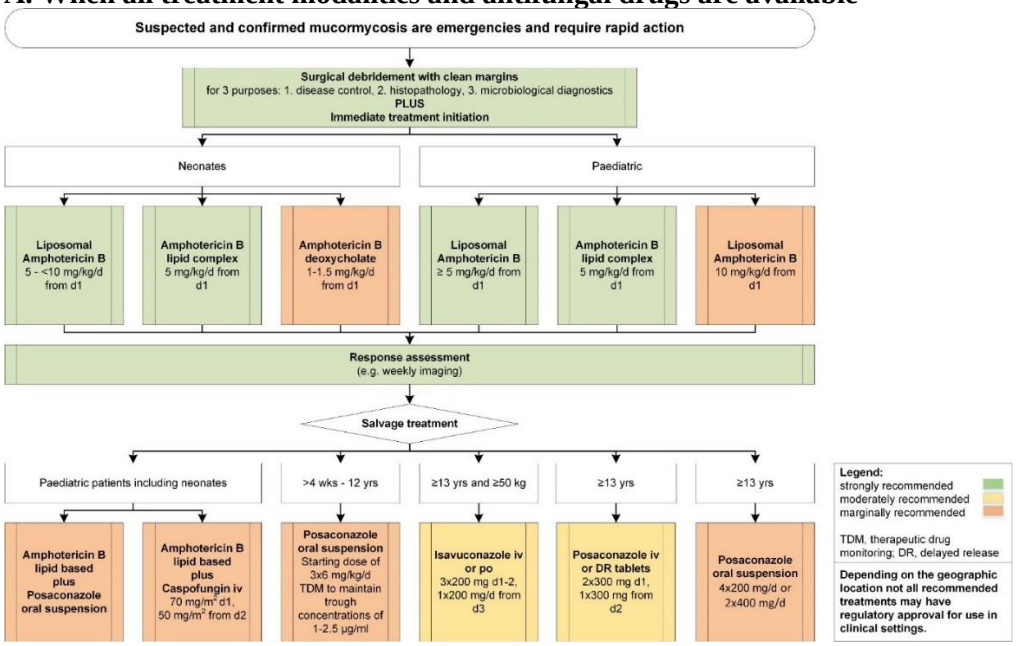
Table S 15. Recommendations on salvage treatment of mucormycosis in children

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Paediatric patients ≥13 yrs and weighing ≥40 kg	To cure	Isavuconazole iv or po 3x200 mg d1-2, 1x200 mg from d3	B	IIt	Marty LancetID 2016[808]	Adult;assumption of similar PK in post-pubertal adolescents and absence of dose-dependent safety concerns
Paediatric patients ≥13 yrs	To cure	Posaconazole oral suspension 4x200 mg/d or 2x400 mg/ d	B	IIu	Krishna AAC 2007[870]	N=12, paediatric PK and safety oral suspension, 12-18 yrs
					Welzen PIDJ 2011[873]	N=16, paediatric PK and safety, oral suspension, chronic granulomatous disease, 2-16 yrs
					Arrieta ICAAC 2013[874]	N=43, Paediatric PK and safety (oral suspension; 2-18 yrs)
					Lehrnbecher EJCMID 2010[871]	N=15, paediatric safety, oral suspension, 3·6-17·5 yrs
					Döring Med Mycol 2017[880]	N=27, paediatric safety, oral suspension, <17 yrs
					Greenberg AAC 2006[774]	Adult efficacy
					van Burik CID 2006[828]	Adult efficacy
					Walsh CID 2007[872]	TDM rationale in treatment
						No established paediatric dose; TDM for oral solution advised; target ≥1 µg/ml
Paediatric patients ≥13 yrs	To cure	Posaconazole DR tablet or iv 2x300 mg d1, 1x300 mg from d2	B	IIt	Groll, IDWEEK 2017[881]	N=57, paediatric PK and safety, iv and novel investigational DR suspension, 2-18 yrs
					Duarte AAC 2014[802]	Adult PK and safety
					Maertens AAC 2014[805]	Adult PK and safety
					Cornely JAC 2016[803]	Adult PK and safety
					Cornely JAC 2017[804]	Adult PK and safety
Paediatric patients beyond 4 weeks to 12 yrs	To cure	Posaconazole oral suspension No established dose; individualised dosing based on body weight: Starting dose of 3x6 mg/kg/d plus TDM to maintain trough concentrations of 1-2·5 µg/ml	C	IIu	Arrieta ICAAC 2013[874]	N=43, Paediatric PK and safety (oral suspension; 2-18 yrs)
					Döring BMCID 2012[868]	Paediatric safety (suspension; n=60; 0,7-11,5 yrs)
					Döring Med Mycol 2017[880]	N=27, paediatric safety, oral suspension, <17 yrs
					Welzen PIDJ 2011[873]	N=16, paediatric PK and safety, oral suspension, chronic granulomatous disease, 2-16 yrs
					Lehrnbecher EJCMID 2010[871]	N=15, paediatric safety, oral suspension, 3·6-17·5 yrs

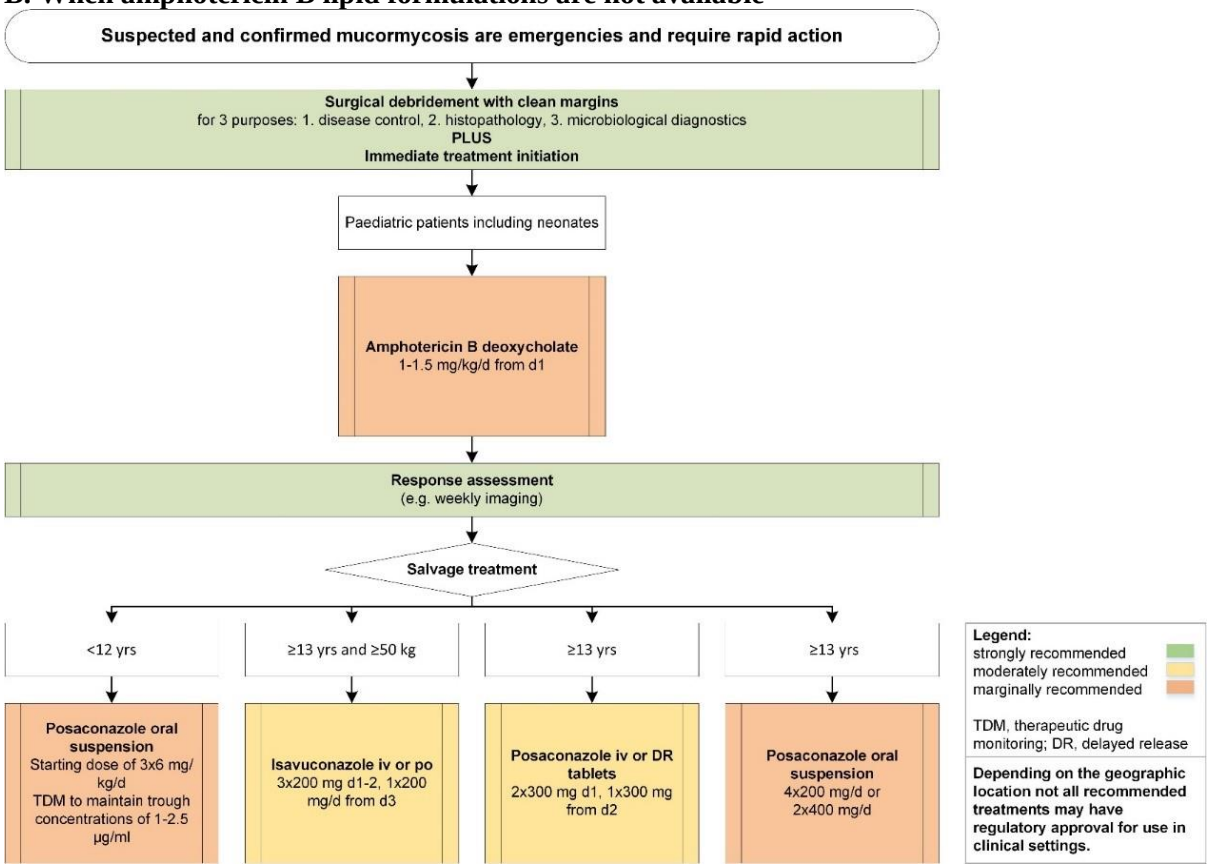
					Vanstraelen PIDJ 2016[882]	N=8, paediatric PK study, oral suspension, prophylaxis, 13 yrs or younger (mean 6·7 ± 2·8)
					Mesini EJH 2018[883]	N=1, paediatric PK, tablets
						No established paediatric dose; TDM for oral solution advised; target ≥1 µg/ml
Paediatric patients including neonates	To cure	Amphotericin B lipid formulations, plus posaconazole oral suspension	C	IIIt	Vehreschild CRM 2013[829]	Adult efficacy
					Pagano Haematologica 2013[831]	Adult efficacy
Paediatric patients including neonates	To cure	Amphotericin B, lipid formulations, plus caspofungin iv loading (1st day): 70 mg/m^2; maintenance: 50 mg/m^2 neonates: 25 mg/m^2	C	IIIt	Saez-Llorens AAC 2009[877]	Neonatal PK caspofungin (n=18)
					Walsh AAC 2005[878]	Paediatric PK caspofungin (n=39; 2- <18 yrs)
					Neely AAC 2009[875]	Paediatric PK caspofungin (n=9; 3-24 mo
					Zaoutis PIDJ 2009[879]	Paediatric safety caspofungin (n=171; 0- <18 y)
					Phulpin-Weibel Mycoses 2013[876]	N=5 paediatric efficacy
					Reed CID 2008[620]	Adult efficacy
SoR, strength of recommendation; QoE, quality of evidence; N, number of subjects investigated; PK, pharmacokinetics; DR, delayed release; TDM, therapeutic drug monitoring						

Figure S 4. Optimal treatment pathway for mucormycosis <u>in children</u>

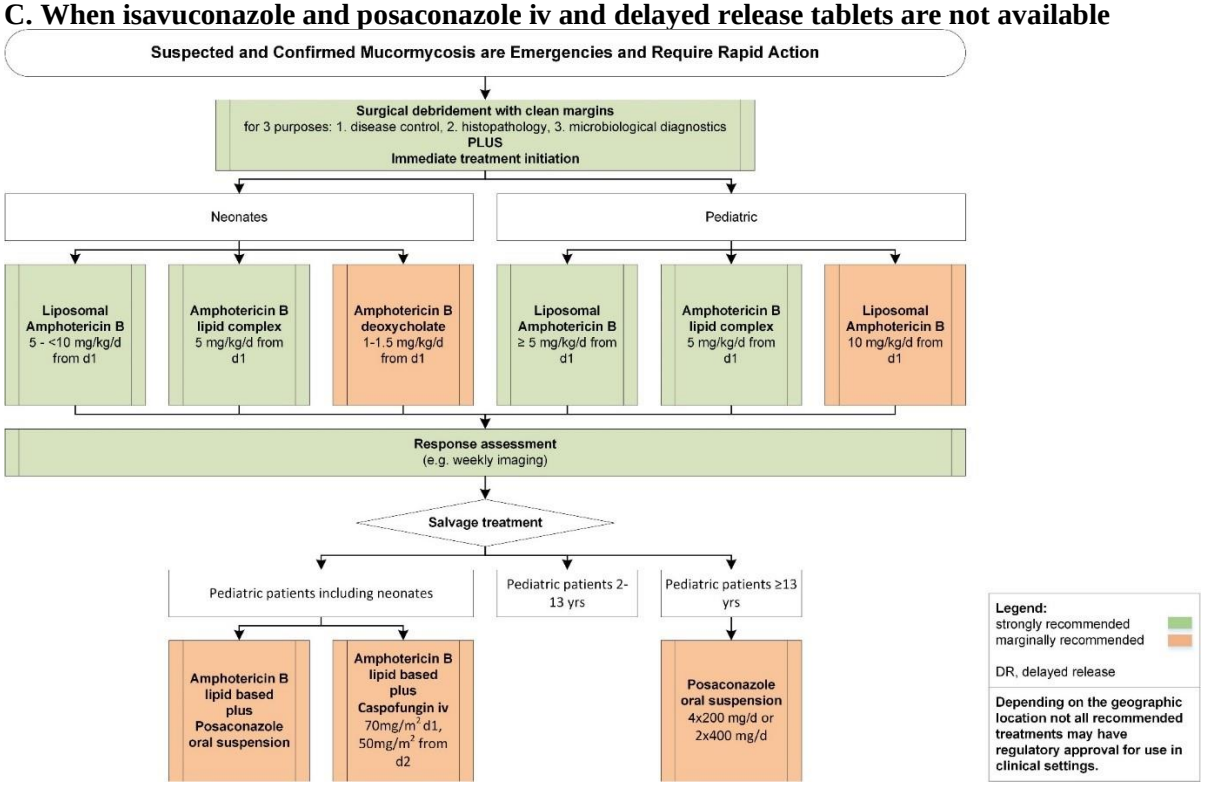
A. When all treatment modalities and antifungal drugs are available



B. When amphotericin B lipid formulations are not available



C. When isavuconazole and posaconazole iv and delayed release tablets are not available



Adjunctive treatments for mucormycosis

Iron homeostasis

Evidence – Iron is essential for the fungal metabolism.[884-886] In animal models administration of iron as well as of the drug deferoxamine, which is an iron chelator for humans but can be used as a siderophore delivering iron to fungi, actually worsened mortality rates.[887] Deferasirox is another iron chelator that cannot be used as a siderophore by Mucorales. Pre-clinical studies in mice found that deferasirox monotherapy in diabetic mice was as effective as liposomal amphotericin, and the combination of both drugs synergistically improved survival in mice.[884] Of note, in the mouse study, deferasirox was toxic in neutropenic mice, and in order to detect even minor efficacy, it had to be considerably dose reduced compared to the doses administered to diabetic mice. The drug was found safe in a single arm study on – mostly diabetic – patients.[888] A second, uncontrolled study successfully used deferasirox in diabetic patients.[889]

Subsequently, a double-blinded, randomised controlled study yielded an increased mortality rate in deferasirox recipients as compared to those treated with placebo (all patients treated with amphotericin) .[8] However, the study was small and enrolled mainly a haematologic malignancy population, rather than diabetic patients, for which the mouse study demonstrated greater efficacy.[8,888] Furthermore, due to its small size, there was an imbalance in randomisation, such that more patients in the iron chelation arm had leukaemia or stem cell transplant than in the control arm. Deferasirox is known to be potentially toxic to the bone marrow and kidneys, and hence there is biological plausibility around potential harm in neutropenic patients with mucormycosis, in contrast to diabetic patients for which there is biological rationale to reduce iron levels.[890,891] Indeed, in the randomised study, higher baseline serum iron and serum ferritin concentrations were associated with higher mortality.[8,626] Thus, future research in this space is warranted.

Deferiprone, mentioned for completeness, is the third iron chelator and is currently given in thalassaemia major.[892] Deferiprone was shown to protect diabetic mice from mucormycosis,[893] but has not yet been used to treat patients with mucormycosis. Enhanced iron delivery through iron chelators may be an untoward drug class effect,[894] although the conflicting results may be explained by differential risk profiles.[889] For now, this remains an area of uncertainty (**Table S 16**).

Recommendations – Administration of iron or deferoxamine to patients with mucormycosis is discouraged, and a conservative approach to blood transfusions may be warranted given the risk of free iron release during transfusions. Adjunctive deferasirox use should be avoided in patients with haematological malignancy; its use in patients with diabetes as a predominant risk factor merits further exploration in clinical trials (**Table S 16**).

Augmentation of host response

Evidence – In haematology patients with mucormycosis and ongoing neutropenia, granulocyte colony stimulating factor (G-CSF) has been added to antifungal treatment in several small patient series (**Table S 16**).[525,534,557,636,895,896] In Belgium, a case has been described of a patient with extensive abdominal mucormycosis after trauma. Mucormycosis was unresponsive to conventional therapy and was treated successfully with a combination of the immunomodulator nivolumab and interferon-γ.[897]

Recommendations – The guideline group moderately supports G-CSF to augment host response against mucormycosis in patients with ongoing neutropenia (**Table S 16)**.

Reducing host vulnerability

Evidence – Hyperglycaemia and ketoacidosis facilitate host infection and mucormycosis.[898] Animal models of ketoacidosis express increased glucose-regulated protein (GRP-78), a receptor for *R. arrhizus* invasion.[899] Mucorales spore coat protein homologues (CotH) are fungal ligands to GRP-78,[900] and antibodies directed towards GRP-78 or CotH protect against experimental mucormycosis.[899,900] Correction of ketoacidosis alleviates mucormycosis *in vitro* and *in vivo* (**Table S 16**).[901]

Recommendations – The guideline group strongly supports controlling hyperglycaemia and ketoacidosis in patients with mucormycosis, specifically those with uncontrolled diabetes (**Table S 16**).

Hyperbaric oxygen exposure

Evidence – *In vitro* hyperbaric oxygen inhibits fungal growth.[902] Clinical reports on hyperbaric oxygen exposure in the context of mucormycosis are limited to cases or small series.[27,397,628,821,903-905] Hyperbaric oxygen exposure has mostly been reported from patients with correctable risk factors for mucormycosis.[534] These data are uncontrolled and retrospective, and the biological rationale for such therapy is unclear (**Table S 16**).

Recommendations – The guideline group supports a recommendation for hyperbaric oxygen exposure with moderate strength for diabetic patients (**Table S 16**).

Table S 16. Recommendations on adjunctive treatment for mucormycosis

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Iron homeostasis						
Other than haematology	To cure	Deferasirox 20 mg/kg/d, d1-14	C	IIu	Ibrahim JCI 2007[884]	Animal, deferasirox increased survival rates
					Spellberg AAC 2009[888]	N=8, 6 diabetes, 2 SOT
					Soman JAC 2012[889]	N=7, 5 diabetes, all 7 successful
Haematology	To cure	Deferasirox 20 mg/kg/d, d1-14	D	II	Spellberg JAC 2012[8]	N=20, open randomised controlled, excess mortality with deferasirox
Any	To increase survival rate	Deferoxamine	D	IIt	Van Cutsem KidInternat 1989[887] Boelart JCI 1993[885]	Animal, deferoxamine increased mortality
Any	To increase survival rate	Administration of iron	D	IIu	Van Cutsem KidInternat 1989[887]	Animal, iron increased mortality
					Boelaert JCI 1993[885]	
					Ibrahim JCI 2007[884]	
					Spellberg MedMycol 2012[626]	Higher baseline serum iron and ferritin associated with mortality
Augmentation of host response						
Haematology, ongoing neutropenia	To augment host response	G-CSF, dose not reported	B	IIu	Pagano BJH 1997[557]	N=8
					Kontoyiannis CID 2000[896]	N=14
					Pagano Haematol 2004[636]	N=18
					Roden CID 2005[534]	N=18
					Kara IntJClinPract 2007[895]	N=5
					Pagano JChemoth 2009[525]	N=8
Haematologic malignancy ongoing neutropenia	To augment host response	Granulocyte transfusion, dose not reported	C	IIur	Kontoyiannis CID 2000[896]	N=8
					Roden CID 2005[534]	N=7
Haematologic malignancy ongoing neutropenia	To augment host response	Granulocyte transfusion + IFNy1b	C	III	Safdar Cancer 2006[906]	N=4
Diabetes	To augment host response	GM-CSF 250-425 µg/d	C	III	Garcia-Diaz CID 2001[907]	N=3
Any	To cure	Adoptive immunotherapy, T cells generated in response to *R. arrhizus* antigens	C	III	Schmidt JID 2012[908]	*in vitro*
Any	To cure	Nivolumab + interferon-γ	C	III	Grimaldi Lancet ID 2017[897]	N=1
Reducing host vulnerability						
Diabetes	To improve response to treatment and to cure	Control of hyperglycaemia and ketoacidosis	A	III	Ibrahim MolMicro 2010[886]	Animal, anti-fungal iron permease
					Rammaert Diabetes Metab 2012[898]	Review
					Gebremariam JCI 2014[900]	Animal, anti-GRP-78
					Gebremariam JCI 2016[901]	Animal, bicarbonate
Glucocorticosteroid recipients	To cure	Rapidly taper glucocorticosteroid dose to discontinue, if feasible, or reduce dose to minimum required	A	IIr	Lionakis Lancet 2003[909]	
Hyperbaric oxygen exposure						
Diabetes	To cure	Exposure to 100% hyperbaric oxygen	B	IIr	Gamba Radiology 1986[628]	N=5
					Ferguson RevInfectDis 1988[904]	N=5, 4/5 recovered
					Garcia-Covarrubias RevInvClin 2004[905]	N=5, 1 diabetes
					John CMI 2005[903]	N=28, 17 diabetes (6% died)
Haematology	To cure	Exposure to 100% hyperbaric oxygen	C	IIu	John CMI 2005[903]	N=28, 5 haematologic
					Roden CID 2005[534]	N=44 (50% died), mixed
					Ribeiro Mycopathol 2013[397]	N=1
					Almannai PHO 2013[27]	N=1, paediatric
					Kyvernitakis CMI 2016[821]	N=11

SoR, strength of recommendation; QoE, quality of evidence; N, number of subjects investigated; SOT, solid organ transplantation; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRP, gastrin releasing peptide

Intensive care and critically ill patients with mucormycosis

Evidence – A paucity of data exists regarding mucormycosis in the intensive care unit (ICU). Patients may develop mucormycosis in the ICU, or be admitted to the ICU for further management.[910-913] Mucormycosis as a complication of critical care was initially reported four decades ago, when three previously non-immunocompromised patients developed mucormycosis following corticosteroid therapy.[914] A few case reports and small series in the ICU setting have subsequently been reported.[910,913,915] Unpublished data from a single centre found that 37% of mucormycosis cases were in patients being treated in the ICU.[916] Many patients at risk for mucormycosis are frequently managed in the ICU. These include patients with haematological malignancy, solid organ transplant recipients, patients with other immunodeficiencies, trauma patients, those with diabetes mellitus, and a multitude of patients with wound dressings. An outbreak of gastric mucormycosis in ICU patients was described in association with the use of contaminated wooden tongue depressors in critically ill patients.[574]

Management of mucormycosis in the ICU involves a four-pronged approach in combination – correction of underlying conditions where feasible, source control, appropriate antifungal therapy, and relevant supportive care. Correction of underlying conditions includes the management of ketoacidosis and correction of hyperglycaemic states in diabetic patients, modulation and weaning of corticosteroids and immunosuppressives, and reducing the duration of neutropenia in haematology patients. Source control involves the removal of infected tissue, drainage of septic collections, and removal of any infected devices. Source control and surgical intervention are key elements in the ICU management of mucormycosis, particularly for rhinocerebral, complicated gastrointestinal, skin and soft tissue forms.[771,778,917-920] Other presentations should be evaluated on an individual basis. Repeated surgical intervention may be necessary to achieve suitable source control. Appropriate and specific antifungal therapy is discussed in detail in the context of the guideline.

Relevant supportive care of the critically ill patient with mucormycosis is an integral component of the quartet of management of such patients, and entails the same principles as those advocated for all patients with sepsis and septic shock. Important aspects include need for airway protection and mechanical ventilation, attention to haemodynamics, glycaemic control, venous thromboembolism prophylaxis, nutritional support, transfusion policy, and renal replacement therapy where necessary.[917,921] Several of these aspects are reviewed in detail in recent evidence-based publications addressing sepsis.[917,921] In general, haemodynamic support involves judicious fluid administration, and in the setting of septic shock, the introduction of vasopressor support to achieve an initial target mean arterial blood pressure of 65 mmHg.[917,922] The use of corticosteroids, although controversial, may be considered in the setting of septic shock (hydrocortisone 50 mg 6-hourly intravenously for 5-7 days, or up to the weaning of vasopressor therapy, followed by tapering of the dose as guided by clinical response) .[921,923] Airway protection with mechanical ventilation should be instituted where necessary, in patients unable to protect their airway, or where airway clearance is problematic, with the aim of maintaining normoxia.[924,925] Glucose levels should be maintained at <180 mg/dL (10 mmol/L), and nutritional support commenced once the patient is haemodynamically stable, preferably via the enteral route.[921,923] A restrictive haemoglobin target of 7 g/dL is appropriate for non-bleeding patients without active myocardial ischaemia.[926,927] Pharmacological venous thromboembolism prophylaxis should be provided to all patients where no contraindications exist. Low molecular weight heparins are preferred to unfractionated heparin. Mechanical modes of prophylaxis such as intermittent pneumatic compression devices should be used when pharmacological prophylaxis is contraindicated. Combination mechanical and pharmacological prophylaxis should be used whenever possible.[921,923]

Recommendations – In critically ill patients in the ICU, a combined four-pronged management approach is strongly recommended. This includes correction of the underlying conditions where feasible, source control, appropriate antifungal therapy, and relevant supportive care.

Health economics

Evidence – Only very few studies have analysed the economic burden of mucormycosis. Based on hospital charges and on ICD-9 coding in the USA, mucormycosis was associated with an average hospital stay of 17 days, and with one out of three patients requiring re-admission. The costs per stay were estimated at USD 112,419.[530] In paediatric patients with fungal sinusitis, the duration of hospitalisation and associated costs were estimated to be 3 to 5 times, and 7 to 13 times higher, respectively.[928] In patients with haematological malignancies in the United Kingdom, first-line treatment with isavuconazole was shown to reduce costs compared to standard treatment with liposomal amphotericin B followed by posaconazole.[929] This was primarily driven by lower costs for drug acquisition and hospitalisation.

Recommendations – Due to variation of drug acquisition costs and duration of hospitalisation, future studies are needed to validate findings in other geographical settings and patient groups.

Future directions

Unmet needs

Unmet needs in mucormycosis differ between regions and institutions. Rapid diagnostics to identify with good specificity patients with mucormycosis in the early stages of infection are critical. Newer molecular-based approaches, including the detection of Mucorales DNA in the blood of patients,[320,742] and Mucorales-specific *CotH* gene in clinical samples,[930] have yielded promising results but require large-scale clinical validation. Similarly, markers of host response that recognise fungus-specific T cells or their products (*e.g.* cytokines are worthy of further exploration).[682,684]

As a medical emergency, a multidisciplinary approach with early consultation with specialists from the treating team, radiologists, infectious diseases specialists, microbiologists, pathologists, and surgical colleagues is of paramount importance in the proper management of mucormycosis. This is often challenging, as clinical manifestations vary, and present to physicians with disparate expertise. Access to multidisciplinary care may be limited by logistic difficulties, and in some countries, by geographical isolation.

The absence of ready access to current or new antifungal drugs also limits proper care and affects outcome. In resource-limited countries, liposomal amphotericin B and posaconazole availability can be restricted due to high costs, and pharmaceutical companies are constrained by their own terms of references. In high-income countries, the delay from discovery of a new agent with promising *in vitro* and *in vivo* animal results to the stage of clinical trials and then to registration and community availability spans years. Human ethics review boards, government and regulatory bodies need to work with clinicians to accelerate their access.

The importance of continuing to develop novel antifungal agents that are characterised by good efficacy, pharmacokinetic and pharmacodynamic characteristics, and safety cannot be overemphasised. Ideally, these new drugs should have a novel mode of action, be fungicidal and orally available, have a long half-life, and penetrate well into difficult body sites, such as the brain.

Constraints in optimising management

Successful treatment of mucormycosis remains challenging due to several reasons. Diagnosis of deep-seated mucormycosis is often delayed due to the unspecific clinical features of the infection and a lack of rapid, user-friendly, biomarker tests. In daily clinical practice, diagnosis of mucormycosis is often based on conventional techniques such as microscopy and culture with poor sensitivity. In addition, mucormycosis may be masked in patients with *Aspergillus* co-infections. Evaluation of a consecutive case series showed that delaying amphotericin B therapy is an independent predictor of mortality.[819] Lipid formulations of amphotericin B are the cornerstone for the treatment of mucormycosis. However, due to cost issues amphotericin B deoxycholate remains the only affordable treatment option in many low and medium income countries, despite its toxicity. Moreover, amphotericin B is unavailable in 27% of countries, resulting in an unserved population of nearly 500 million.[931] A study conducted in a West-Indian infectious diseases clinic revealed that as many as 35% of patients with mucormycosis left against medical advice because of various reasons including hospitalisation costs and drug toxicity.[365] Liposomal amphotericin B has a much more favourable safety profile than amphotericin B deoxycholate, but toxicity rates increase with increasing dosages. Nephrotoxicity and hypokalaemia rates as high as 30% were reported in patients treated with 10 mg/kg liposomal amphotericin B.[824]

Surgical debridement is associated with better survival, and is often feasible in cases of skin and soft tissue involvement but much more difficult in severely immunocompromised and/or critically ill patients suffering from deep-organ mucormycosis such as pulmonary or cerebral mucormycosis.

Priority research questions

To address unmet needs in the management of mucormycosis, the immediate research priorities can be grouped into three broad categories: microbiology laboratory tools, antifungal therapeutics, and clinical management.

In the microbiology lab, the development and validation of sensitive and specific diagnostic tests for mucormycosis that can be applied to serum, BAL and tissue is the most pressing research priority. Studies in this area should not only include standardisation and validation of existing techniques, for example PCR, but also pursue novel diagnostic targets. The success of cell-wall glucan-based assays for other medically relevant fungi suggests that identification of cell-wall polysaccharides unique to mucormycetes should be pursued. Developing antibodies for tissue immunohistochemistry that can distinguish hyphae of mucormycetes from those of other filamentous hyphae will not only aid in the diagnosis of mucormycosis, but also help identify cases of mixed mould infection. In addition to these diagnostic studies, the development of clinically relevant antifungal susceptibility testing, including the identification and clinical validation of breakpoints is required.

Although the introduction of azoles with activity against mucormycetes has offered alternatives to amphotericin B products, direct comparisons of these agents with each other and with amphotericin B lipid formulations are lacking. Understanding the relative efficacy of these agents in specific populations will be critical in refining treatment algorithms for mucormycosis. The identification and development of therapies with novel mechanisms of action against these organisms are also clearly required. In addition to conventional small-molecule approaches,

innovative solutions such as passive immunotherapy and chimeric antigen receptor T-cell therapy may hold promise and should be explored.
Additionally, studies to define optimal care pathways that incorporate existing and emerging tools and therapies should not be neglected. Defining the duration of antifungal therapy and markers of treatment response are needed to guide appropriate antifungal stewardship and optimal clinical care. Further research is required to define the role of antifungal prophylaxis, diagnostic/imaging-driven and clinically driven approaches for specific patient populations. Finally, clinical studies evaluating the role of TDM in guiding azole antifungal therapy in specific clinical populations will be invaluable in managing these challenging infections.

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