THE LANCET
Infectious Diseases

Supplementary appendix

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Supplement to: Hoenigl M, Salmanton-García J, Walsh TJ, et al. Global guideline for the diagnosis and management of rare mould infections: an initiative of the European Confederation of Medical Mycology in cooperation with the International Society for Human and Animal Mycology and the American Society for Microbiology. *Lancet Infect Dis* 2021; published online Feb 16. https://doi.org/10.1016/S1473-3099(20)30784-2.

Global guideline for the diagnosis and management of rare mold infections: An initiative of the ECMM in cooperation with ISHAM and ASM*

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* The American Society for Microbiology's contribution to this document was a review conducted by Stuart M. Levitz, MD, University of Massachusetts, Worcester, Audrey N. Schuetz, MD, Mayo Clinic, Rochester, MN, and Sean X. Zhang, MD, PhD, Johns Hopkins University, Baltimore, MD.

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Introduction and Methods

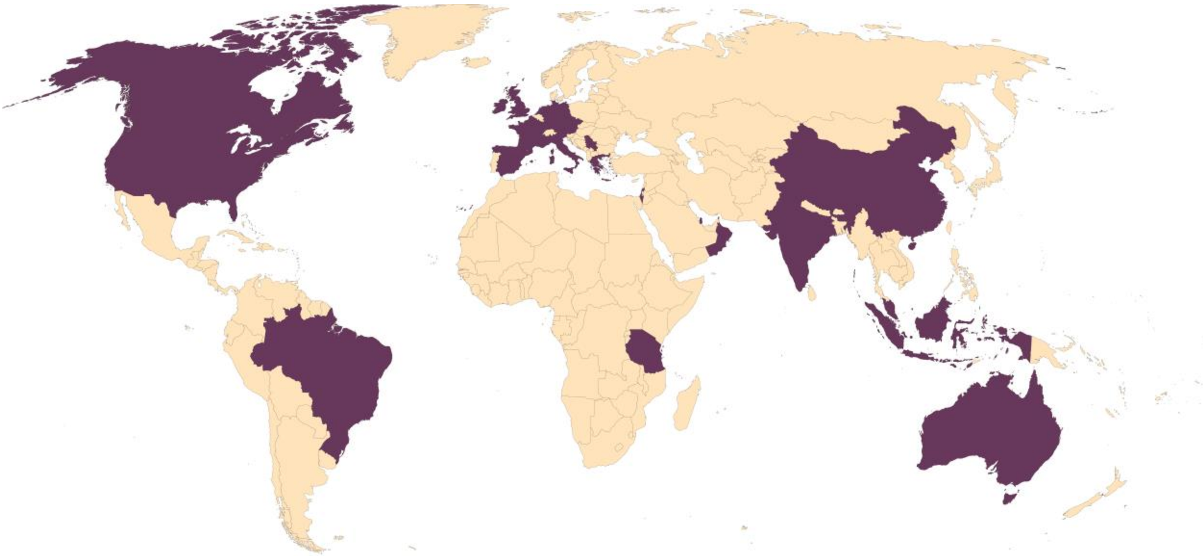
Invasive aspergillosis and mucormycosis have been the most commonly documented invasive mold infections over recent decades[1-4], but mycoses caused by rare molds are on the rise[5]. Mold-active antifungal prophylaxis in those at highest risk for invasive aspergillosis has proven effective in preventing invasive aspergillosis, and to a lesser extent also mucormycosis[6,7]. However, the selective pressure of antifungal prophylaxis also may be contributing to the emergence of less common invasive mold infections, caused by molds that are often intrinsically resitant against classes of antifungals, and include *Fusarium*, *Lomentospora* and *Scedosporium* species as well as even less common emerging molds such as *Rasamsonia*, *Scopulariopsis* and *Paecilomyces* spp., which have been described as opportunistic pathogens in patients with a variety of underlying diseases[8-10]. Intrinsic resistance of the fungal pathogens to many of the available antifungals limits successful therapeutic options. The prevalence of infection due to these fungal pathogens varies widely among geographic regions[11]. Readily available guidance on recognizing different disease patterns and the diagnostic and therapeutic options available, which differ across the regions of the world, is important to optimize patient management[12]. Current guidelines are limited to individual rare mold pathogens[13-15], focusing on specific groups of patients such as those with hematological malignancies[15], or are missing altogether for infections caused by many of the very rare, but emerging pathogenic molds.

Therefore, the European Confederation of Medical Mycology (ECMM)[16], together with the International Society for Human and Animal Mycology (ISHAM) and the American Society for Microbiology (ASM), issue this comprehensive guidance document as part of their "One World – One Guideline" initiative[12,17], to facilitate clinical decision-making, and simultaneously provide an overview of the areas of uncertainty for invasive mold infections caused by *Fusarium* spp., *Lomentospora* spp., *Scedosporium* spp., dematiaceous molds causing phaeohyphomycosis, *Rasamsonia* spp., *Scopulariopsis* spp., *Penicillium* spp., non-*marneffei Talaromyces* spp., *Paecilomyces* spp., *Purpureocillium* spp., and *Schizophyllum* spp. as well as other basidiomycetes. We aimed to address the limitations of previous recommendations by engaging physicians and scientists involved in all aspects of the management of rare mold infection, representing the fields of microbiology, mycology, pathology, radiology, infectious diseases, pharmacology, surgery, pediatrics, haematology, intensive care, and dermatology.

Panel: How the guideline group worked

In January 2018, experts were identified based on their publication activity in the field of rare mold infections in the previous five years, their involvement in patient management, and their distribution across the world regions as defined by the United Nations (**Figure 1**).

Figure 1. Worldwide distribution of the authors of the Rare Mold Guideline



Experts were invited in February 2018 to develop this guideline. From February to March 2018, videoconferences on the methodology were held, and a mandatory video tutorial added in March 2018. Supervision of the group was provided by the coordinators (MH, DS, OAC). Documents were shared among the authors on a password-protected OneDrive (Microsoft Corp, Redmond, WA, USA) repository, and were centrally managed and kept up-to-date with any new developments. Updates on PICO (population, intervention, comparison, and outcome) tables were written in red font; after spelling check and formatting, font color was changed to blue for evaluation by the group. Following discussion of contents and consensus, the font was changed to black. Once all tables were finalized, a writing group (MH, JSG, TJW, MN, CFN, JDJ, ML, MB, FC, TF, PK, TL, AK, JP, MR, SR, MS, JSt, BS, RS, ST, AW, PW, JY, DS, and OAC) contributed the first draft, which was circulated to all participants for approval in January 2020. Recommendations were consensus-based. If no consensus was found, the majority vote was used.

In April 2020, a four-week public consultation phase ensued. Comments received were evaluated, and used to modify the manuscript as appropriate, resulting in a final author review in July 2020. 54 scientific societies, including national societies from 38 countries and several international societies reviewed and endorsed the guidance document.

The following societeies have endorsed the guideline:

International

- International Immunocompromised Host Society (ICHS)
- The International Society for Human & Animal Mycology (ISHAM)

Africa

- Ghana Medical Mycology Group
- Federation of Infectious Diseases Societies of Southern Africa (FIDSSA)

Americas

- Medical Mycology Society of the Americas (MMSA)

Americas, Canada

- Association of Medical Microbiology and Infectious Disease (AMMI)

Americas, Latin America/Caribbean

- Asociación Argentina de Microbiología (AAM), Subcomisión de Micología Clínica
- Brazilian Association of Hematology, Hemotherapy and Cell Therapy (ABHH)
- Brazilian Society of Infectious Diseases
- Latin American Forum for Fungal Infections

Americas, United States

- American Society for Microbiology (ASM)

Asia

- Asia Fungal Working Group (AFWG)

Asia Central/Southern

- Medical Microbiology & Infectious Diseases Society of Pakistan (MMIDSP)
- Indian Society of Medical Mycologist (MSI)
- Iranian Society of Infectious Diseases and Tropical Medicine (ISIDTM)
- Iranian Society for Medical Mycology (ISMM)

Asia, Eastern/South-Eastern

- Indonesia Society for Medical Mycology
- Malaysian Society of Infectious Diseases and Chemotherapy (MSIDC)
- Infectious Diseases Society of Taiwan (IDST)
- Infectious Diseases Society of Thailand (IDAT) with Thai Medical Mycology Forum (TMMF)

Asia, Western

- Israeli Society for Infectious Diseases (ISID)
- Lebanese Society of Infectious Diseases and Clinical Microbiology (LSIDCM)
- Omani Society of Medical Microbiology and Infectious Diseases
- Infectious Diseases and Clinical Microbiology Speciality Society of Turkey (EKMUD)
- Turkish Society of Hospital Infection and Control (TSHIC)
- Turkish Febrile Neutropenia Society
- Turkish Society of Medical Mycology

Europe

- European Hematology Association (EHA)
- European Paediatric Mycology Network (EPMyN)

Europe, Eastern

- Czech Society for Medical Microbiology (SPLM)
- Hungarian Society of Infectious Diseases and Clinical Microbiology (MIFKMT)
- Romanian Society for Medical Mycology and Mycotoxicology (SRMMM)
- The Interregional Association for Clinical Microbiology and Antimicrobial Chemotherapy (IAC-MAC)
- Serbian Society of Medical Mycology (SSMM)
- Slovak Society of Chemotherapy
- Slovenian Society for Clinical Microbiology and Hospital Infections of SMC

Europe, Northern

- Nordic Society for Medical Mycology (NSMM)
- Finnish Society for Medical Mycology (FSMM)
- Irish Fungal Society (IFS)
- Swedish Society for Clinical Mycology (SSKM)
- British Infection Association (BIA)
- British Society for Medical Mycology (BSMM)

Europe, Southern

- Hellenic Society of Medical Mycology (HSMM)
- La Federazione Italiana di Micopatologia Umana ed Animale (FIMUA)
- Sorveglianza Epidemiologica Infezioni nelle Emopatie (SEIFEM)
- Società Italiana Terapia Antinfettiva (SITA)
- Associação Portuguesa de Micologia Médica (ASPOMM)
- Asociación Española de Micología (AEM), Sección de Micología Médica

Europe, Western

- Austrian Society for Medical Mycology (ÖGMM)
- Belgian Society for Human and Animal Mycology (BSHAM)
- French Society for Medical Mycology (SFMM)
- German Society for Hematology and Medical Oncology (AGIHO)
- German Speaking Mycological Society (DMykG)
- Paul-Ehrlich-Society for Chemotherapy (PEG)

Oceania

- ASID Australasian Society for Infectious Diseases

This guideline follows the structure and definitions of previous guidelines on invasive fungal infections which are in accordance with the Grading of Recommendations Assessment, Development and Evaluation (GRADE) and Appraisal of Guidelines for Research & Evaluation (AGREE) systems. The tables reflect the PICO approach, and the methodology including strength of recommendation (SoR), quality of evidence (QoE), and indexes (t, transferred evidence; h comparator group: historical controls; u, uncontrolled trials) as previously described[12,17].

1. Fusariosis

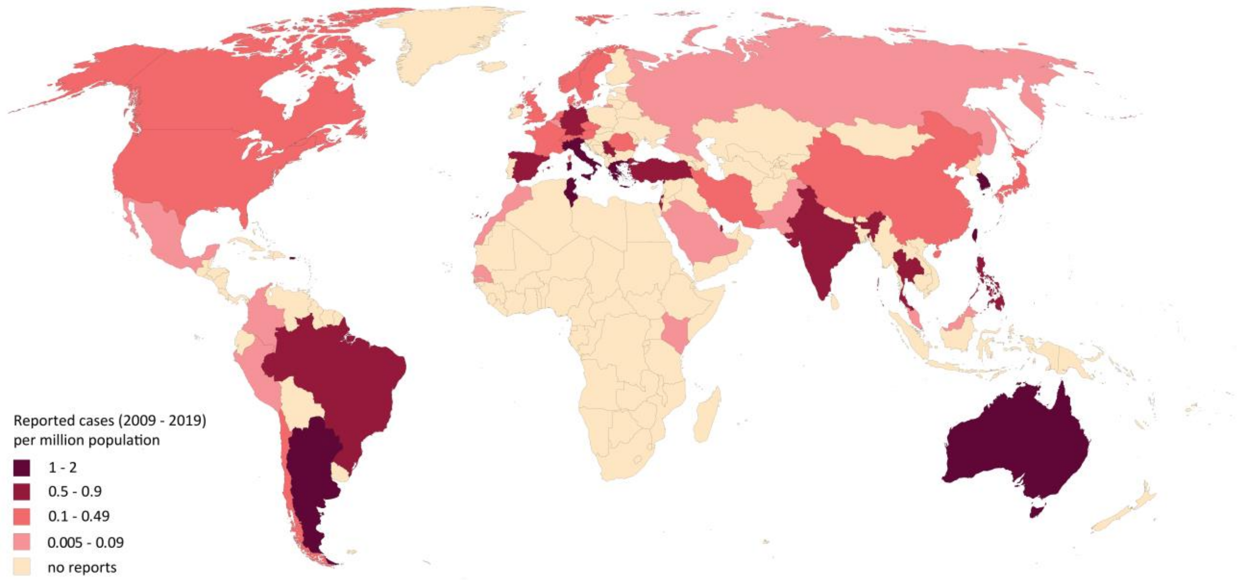
Epidemiology of fusariosis

Only a relatively small proportion of the more than 300 *Fusarium* species are opportunistic pathogens in humans[19]. *Fusarium solani* and *Fusarium oxysporum* spp. complexes are especially important, causing more than 50% and about 20% of severe fusariosis cases, respectively[18,20]. Other species causing infections are those from the *Fusarium fujikuroi* spp. complex [mainly *Fusarium verticillioides* (formerly *Fusarium moniliforme*), *F. fujikuroi*, *Fusarium subglutinans*, *Fusarium proliferatum*], and *Fusarium dimerum* spp. complex[20]. The main routes of infection are inhalation of airborne microconidia or direct inoculation through traumatic injury, including burns. In immunocompetent patients, fusariosis mostly results from direct contact with contaminated material and frequently presents as superficial infection, such as onychomycosis or fungal keratitis, that may become locally invasive[21-23]. Eye infections are mainly caused by species of the *F. solani* complex[24]. *Fusarium* spp. can adhere to plastic substrates such as catheters and soft contact lenses, predisposing those exposed to contaminated devices and material to associated infections[25]. Hospital water distribution systems may harbor *Fusarium* spp. and serve as a potential source of nosocomial transmission to hospitalized immunocompromised patients[26]. Outbreaks of *Fusarium*-related keratitis in contact lens wearers have been associated with contaminated lens cleaning solution[27]. In immunocompromised hosts, especially neutropenic patients with hematological malignancy or those undergoing hematopoietic stem cell transplantation (HSCT) or solid organ transplantation (SOT), fusariosis manifests as invasive infection mainly affecting skin and deep soft tissue, lungs and sinuses[20,28]. Infections in SOT patients tend to be locally invasive[29]. *Fusarium* spp. frequently disseminate, with positive blood cultures in as much as 70% of cases in immunocompromised patients[28]. This is in contrast to infections caused by many other molds, where blood cultures remain negative despite disseminated infection[30]. This distinctive clinical characteristic of positive blood cultures in disseminated fusariosis may be related to the ability of some *Fusarium* spp. to form *in vivo* adventitious conidia or aleurioconidia, which may then break away from invading hyphae and enter the blood stream[31]. Necrotic erythematous papular or nodular skin lesions are often evident in immunocompromised patients with systemic fusariosis and are a distinctive characteristic of these infections[30]. Dissemination to the central nervous system (CNS) and also hepatosplenic fusariosis have been described in isolated reports only[32-34]. Fusarial paronychial infection in neutropenic patients may result in a painful, erythematous infection of the great toe, which may serve as an important portal of entry for disseminated fusariosis[35].

Incidence and prevalence of *Fusarium* infections vary depending on the underlying disease and geographical region. Comparable incidence of fusariosis in HSCT recipients has been identified in centers in Brazil and the United States, where ~6 cases per 1,000 transplants have been affected, ranging between 1.4 and 2 per 1,000 autologous HSCT recipients, and reaching 20 per 1,000 allogeneic HSCT recipients with HLA-mismatched related donors[36]. In Brazil, the 1-year cumulative incidence of fusariosis after allogeneic HSCT was 3.2%, and 0.6% after autologous HSCT[37]. In that study, fusariosis accounted for 29% of all invasive fungal infections. Similarly, an incidence of fusariosis of 14.8 cases per 1,000 patients with acute lymphoblastic leukemia and 13.1 cases per 1,000 patients with acute myeloblastic leukemia have been reported from another center in Brazil[38]. In contrast, a Spanish multicentre study analysing respiratory, blood and tissue samples, identified *Fusarium* spp. in 1.2% of all clinical isolates tested[39]. In a Spanish tertiary teaching hospital, median incidence of invasive fusariosis was 0.074 episodes per 10,000 admissions in hematological patients. The incidence increased during the study period[40]. Other centers in Europe, Asia and South America have also reported an increase in fusariosis cases in recent years[41-43]. A pediatric cancer center in Canada diagnosed five fusariosis cases over a period of 15 years[44].

Fusarium keratitis, often associated with contact lenses, is one of the most common fungal infections of the cornea[45]. In a Danish study, 20% of all cases of fungal keratitis were due to *Fusarium* spp. In this study, a mean incidence of 0.6 per million per year was estimated, ranging between 0 and 2 per million in 14 years (**Figure 2)**[46].

Figure 2. Worldwide distribution of fusariosis (reported cases between 2009 and 2019 per million population)

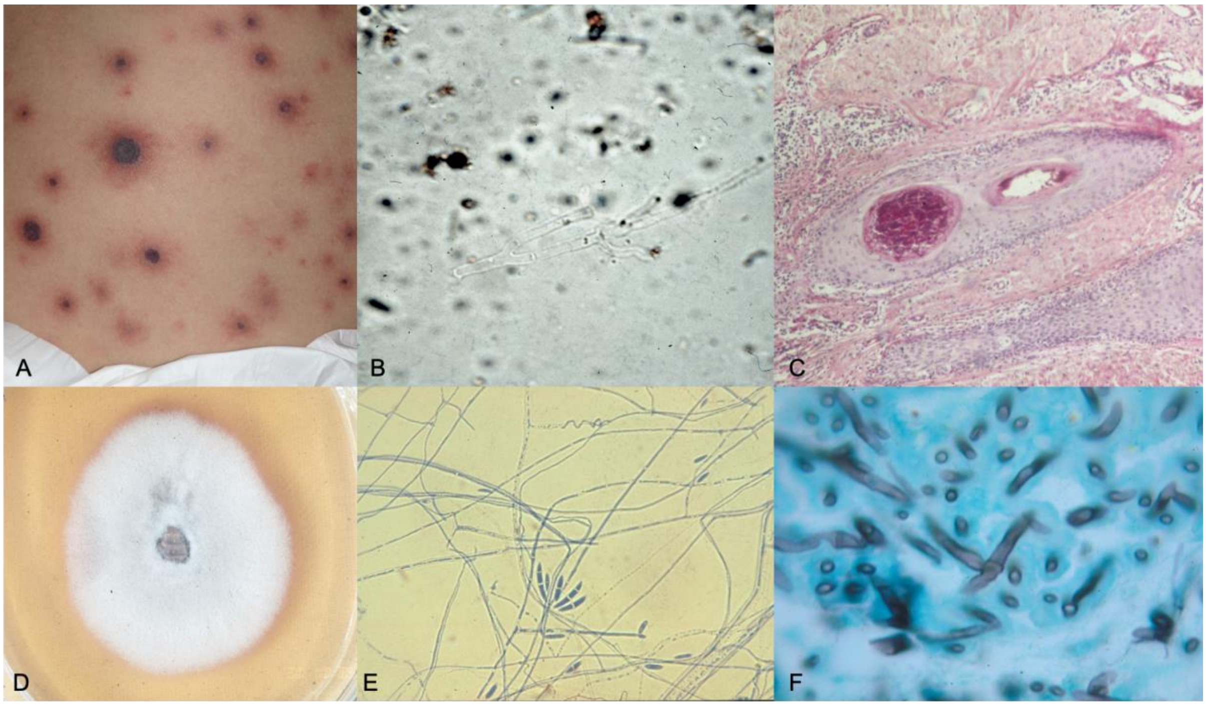


Cases of *Fusarium*-related infections reported in the medical literature were identified in a PubMed search on October 30, 2019 using the search string (*Fusarium* OR fusariosis) that yielded 1,850 publications. In total, 2,435 cases were selected from 48 countries, ~80% related to eye infections. Overall, the vast majority of cases were reported from India (n>1,200, more than 95% related to eye infections), China (n=189), Brazil (n=109), the United States of America (n=100), Philippines (n=94), Argentina (n=83), Italy (n=67), Germany (n=58), and Turkey (n=52) [13,18,21-24,32-34,37,38,41,42,46-320]. Outbreaks related to contaminated contact lens solution, tap water or other causes were not included. The number of cases reported between 2009 and 2019 is presented as cases per million population per country. The resident population per country was obtained from www.worldometers.info[321]. Of note, the maps in this guideline document are an underestimation of the true prevalence and only reflecting the reported prevalence. Reporting of cases and case series is highly depending on the ability diagnose those rare fungal infections and the ability to publish.

Fusarium spp. are also the cause of human and animal exposure to serious life-threatening toxins, especially in agricultural settings. For example ingestion of trichothecene toxins may result in fatal toxic alimentary aleukia which can cause pancytopenia, gastrointestinal distress, seizures, and death[322].

Diagnosis of fusariosis

Figure 3. Clinical, mycologic and histologic characteristics of invasive *Fusarium* infections (owned by co-author S. Taj-Aldeen)



Panel A. Cutaneous lesions resulting from fungemia in a hematopoietic allogeneic stem cell transplant pediatric patient. The lesions are painful and may depict different aspects according to the clinical progression. Skin lesions with ulcerated center, crusted necrotic center and surrounded by an erythematous halo ("target lesions") are suggestive and mostly observed on the trunk and extremities. **Panel B.** Immediate examination of potassium hydroxide digested cutaneous biopsies, may reveal hyaline septate 45° branching hyphae similar to other hyalohyphomycoses, although irregular branching patterns with up to 90° branching do occur. **Panel C.** Histopathology of skin lesions resulting from fungemia, shows vasculitis, the proliferation of capillary vessels with ectasiated lumen and intravascular thrombi containing fibrin and hyphae (PAS staining x 200). **Panel D.** Colony with cottony appearance surrounded by a tan pigment. **Panel E.** On slide culture, characteristically curved macroconidia ("banana-shaped") and thin hyaline hyphae are depicted (lactophenol cotton blue x400). **Panel F.** Histopathologic aspects are nonspecific and similar to other hyalohyphomycoses (Gomori's methenamine silver staining x 1000).

Diagnosis – Microscopy, culture and histopathology

Evidence – Blood cultures are positive in 40% of invasive cases[230], with faster detection of growth in fungal blood culture bottles compared to standard aerobic bottles[323]. This is true specifically for low inocula (10^2 and 10^3 CFU/ml) which are detected earlier in fungal media than in bacteriological media (10 hours earlier for *F. dimerum*, 14 hours for *F. solani*, and 35 hours for *F. verticillioides*)[230,323]. Although members of the genus *Fusarium* can be identified by the production of hyaline hyphae and pigmented, banana-shaped multicellular macroconidia with a foot cell at the base, species identification is difficult morphologically[324]. Microscopy has a very important role for diagnosing *Fusarium*-related infection particularly in many low and middle income countries, where culture may not be available and histopathology is only accessible in some tertiary facilities[45].

Direct examination of tissue, especially skin biopsy, allows for a rapid evaluation prior to culture results if the tissue sample can be examined in a timely fashion[230] **(Figure 3)**. In particular, to diagnose fungal keratitis, histopathologic examination and culture of corneal scrapings are employed[30]. In fresh tissue, hyphae are morphologically similar to those of *Aspergillus* spp., *e.g.* appearing as hyaline septate filaments that typically dichotomize in acute to 45° and sometimes even 90° angles. Adventitious sporulation may be present and the finding presence of reniform adventitious conidia is highly suggestive of fusariosis[324].

Hyphae are often difficult to visualize in tissue with routine haematoxylin-eosin (H&E) staining but can be easily identified with Grocott-Gomori's methenamine silver (GMS) or periodic acid-Schiff (PAS) staining. In the absence of microbial growth, distinguishing fusariosis from other hyalohyphomycosis may be difficult and requires the use of *in situ* hybridization of paraffin-embedded tissue specimens[325]. Matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) is also increasingly used for the identification of molds[326,327,328,329] **(Table 1)**.

Table 1. Microbiological, histopathological and imaging diagnostics of *Fusarium* spp. infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Microscopy, culture, MIC testing						
Any	To diagnose	Direct microscopy of skin biopsy	A	IIu	Nucci PLOSONE 2014[230]	Allows rapid presumptive diagnosis much earlier than culture
Any	To diagnose	Histopathology	A	II	Hayden DMP 2003[325]	Hyphae similar to *Aspergillus* spp., hyaline, septate filaments, dichotomizing in acute angles
Any	To diagnose	Blood culture	A	IIu	Nucci PLOSONE 2014[230]	Blood cultures positive in 40% of cases with disseminated infection
Any	To diagnose	Fungal blood culture bottles	B	IIIu	Hennequin EJCMID 2002[323]	Faster detection of growth compared with aerobic bottles
					Nucci CMR 2007[30]	Low inocula (10^2 and 10^3 CFU/ml) detected earlier in fungal medium
Any with keratitis	To diagnose	Culture	A	III	Nucci CMR 2007[30]	
Any	To determine MICs	Antifungal susceptibility testing, E-test	B	III	Dannaoui JFungi 2019[330]	
Any	To establish epidemiologic knowledge	MIC testing (CLSI or EUCAST)	A	IIu	Espinel-Ingroff AAC 2016[331]	
Any	To inform antifungal choice	MIC testing (CLSI or EUCAST)	C	IIu	Stempel OFID 2015[285]	N=9
					Lamoth JCM 2016[332]	N=12
Antigen-based assays						
Hematological malignancy	To diagnose	GM (Platelia, Bio-Rad) in serum	B	IIu	Nucci PLOSONE 2014[230]	83% sens., 67% spec., 73% serum GM positive before 1st clinical manifestation
Any	To diagnose	BDG (Fugitell, Assoc Cape Cod) in serum	C	IIa	Nucci Mycoses 2019[333]	For 2 tests >80 pg/ml, 90% sens. and 61% spec., high NPV, low PPV
Hematological malignancy with fever	To distinguish aspergillosis and fusariosis	GM (Platelia, Bio-Rad) in serum	D	II	Nucci CMI 2018[334]	GM positive 89% in IA, 73% in fusariosis
Any with GM-pos. fusariosis	To monitor response	Repeat GM (Platelia, Bio-Rad) in serum	A	II	Nucci PLOSONE 2014[230]	Persitently positive GM associated with worse worse outcome
Any	To rule out fusariosis	*Aspergillus*-specific LFD (OLM)	C	III	Thornton CVI 2008[335]	LFD highly specific, results negative in samples from patients with invasive fusariosis
					Hoenigl JCM 2014[336]	
Nucleic acid-based assays/MALDI-TOF MS						
Any	To diagnose	Multifungal DNA microarray	C	III	Boch Mycoses 2015[337]	
Any	To diagnose	Multiplex tandem PCR on blood cultures	C	III	Lau JCM 2008[338]	
Any	To diagnose	Panfungal semi-nested PCR	C	III	Landlinger JCM 2009[339]	ITS2 target + Luminex xMAP technology
Any	To diagnose	Panfungal semi-nested PCR	C	III	Landlinger EJCMID 2009[340]	ITS2 target + AFLP on different materials, N=60 species incl. N=4 *Fusarium* spp.
Any	To diagnose	Panfungal PCR + sequencing on fresh tissues and FFPE	C	III	Lau JCM 2007[341]	ITS1 target, 1/1 *Fusarium* spp. detected

Any	To diagnose	MLST, RT-PCR, LAMP	B	IIu	De Souza Mycopathol 2017[342]	
Neutropenic pediatric	To diagnose	Panfungal 28S qPCR	C	II	Landlinger Leukemia 2010[343]	96% sens., 77% spec.
Neutropenic patients	To diagnose	Multiplex PCR + DNA microarray hybridization	C	III	Spiess JCM 2007[344]	
Any	To detect and identify *Fusarium* spp.	Genus specific PCR	C	III	Hennequin JCM 1999[345]	includes *Acremonium* and *Cylindrocarpon*; 28S target, N=6 *Fusarium* spp.
					Hue JCM 1999[346]	
Hematology/oncology	To identify *Fusarium* spp.	DNA microarray, qPCR, LAMP and EF1α sequencing	A	III	De Souza Mycopathol 2017[342]	N=20 isolates
Any	To identify	TEF1α sequencing	A	IIu	Herkert FrontMicrobiol 2019[347]	N=43 isolates
Any	To identify	28S rDNA and TEF1α sequencing, 28S rDNA specific PCR	B	III	Gaviria-Rivera RSP 2018[348]	
Hematology/oncology	To identify	MLST with RPB2, TEF1α, ITS sequencing	B	III	Dalle Rosa JMM 2018[349]	N=1, new species *F. riograndense*
Keratitis	To identify	TEF1α sequencing	A	IIu	Boral Mycopathol 2018[24]	N=3
					Walther JCM 2017[21]	N=22
Eumycetoma	To identify	Culture + histopathology + TEF1α sequencing	B	III	Al-Hatmi Mycoses 2017[350]	N=2
Any	To identify species	Automated repetitive element sequence-based PCR	C	IIu	Healy JCM 2005[351]	N=26 isolates, web-based data analyses (Diversi-Lab)
Any	To identify species	ITS from primary clinical sample, EF1α from culture	A	IIu	Thomas JMM 2019[352]	N=33 isolates, retrospective
Any	To identify	MALDI-TOF MS	B	IIu	Triest JCM 2015[329]	
					Chalupova BiotechAdv 2014[328]	
Any	To detect and investigate an outbreak	Molecular genotyping	B	IIu	O'Donnell JCM 2004[353]	N=33 isolates, geographically widespread clonal lineage
Imaging studies						
Hematological malignancies	To differentiate fusariosis from aspergillosis	Chest CT	C	IIh	Nucci CMI 2018[334]	Similar images including macronodules with or without halo
Any	To differentiate fusariosis from other invasive mold diseases	Chest CT	C	III	Nucci SRCCM 2015[354]	Fusariosis suspected if hypodense sign w/o halo or vessel occlusion; search for sinusitis
					Sassi Mycoses 2016[265]	
					Marom AJR 2008[355]	

AFLP, amplified fragment-length polymorphism; BDG: Beta-D-glucan; CFU, colony-forming unit; CT, computed tomography; DNA, deoxyribonucleic acid; EF1α, elongation factor 1α; FFPE, formalin-fixed paraffin-embedded tissue; GM, galactomannan; IA, invasive aspergillosis; ITS, internal transcribed spacer; LAMP, loop-mediated isothermal amplification; LFD, Lateral Flow Device; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; MIC, minimal inhibitory concentration; MLST, multilocus sequence typing; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value; QoE, quality of evidence; qPCR, quantitative polymerase chain reaction; rDNA, ribosomal DNA; RPB2, ribonucleic acid polymerase II gene; RT-PCR, real time polymerase chain reaction; sen., sensitivity; spec., specificity; SoR, strength of recommendation; TEF1α, translation elongation factor 1α; w/o without.

Recommendations – Conventional methods are strongly recommended for the diagnosis of fusariosis and include direct microscopy, culture and histopathology. *Fusarium* spp. grow rapidly in most culture media without cycloheximide. The guideline group strongly recommends obtaining infected tissue or body fluids for histological or cytological evaluation and culture. Use of both culture and histopathology together should increase the yield of diagnostic testing.

Diagnosis – microbiology - serological testing

Evidence – Prior to establishing *Fusarium* spp. as the fungal cause of infection, other blood tests such as the *Aspergillus* galactomannan antigen test (GM; Platelia Aspergillus EIA BioRad) or (1→3)-β-D-glucan (BDG; Fungitell®, Associates of Cape Cod Diagnostics) assay, may be ordered. Knowledge of their performance in the setting of an active *Fusarium* infection is helpful. Serum GM antigen detection has 83% sensitivity and 67% specificity during *Fusarium* infection in the neutropenic immunocompromised host[230], with 73% of tests positive prior to the first clinical manifestation observed [230]. In a later study, *Aspergillus* serum GM antigen was positive in 89% of cases of invasive aspergillosis, while it was positive in 73% of cases of fusariosis[334]. In any patient with fusariosis with a positive *Aspergillus* serum GM antigen test, repeated testing over time (e.g., once weekly) may help with treatment stratification and outcome prediction, as continuously positive GM test results correlate with negative outcome[230]. When the BDG assay is used and two sequential tests are both >80 pg/ml, sensitivity is 90% and specificity 61% for *Fusarium* infections, since positive results may also indicate infections caused by *Aspergillus*, *Candida* and other fungal pathogens[333]. The *Aspergillus*-specific Lateral Flow Device (LFD) Test (OLM Diagnostics) is highly specific for aspergillosis, while results have been found negative in samples from patients with invasive fusariosis, which may therefore differentiate between aspergillosis and fusariosis[335,336].

Recommendations – Galactomannan (GM) testing is moderately recommended and BDG marginally recommended as part of the diagnostic evaluation for fusariosis. Monitoring serum GM during treatment is strongly recommended for those patients with positive serum GM results.

Diagnosis – Microbiology – Molecular testing

Evidence – Molecular testing is used for genotyping and species identification of clinical isolates, although this information is not usually useful in practice[347,351-353]. Investigators have used murine models to develop these molecular assays[356,357]. Molecular genetic methods include amplified fragment length polymorphisms (AFLP), loop mediated isothermal amplification (LAMP), multilocus sequence typing (MLST), and real-time polymerase chain reaction (RT-PCR). Accurate identification of *Fusarium* to the species level was often achieved by using *TEF1-α* sequencing, which allowed detection of various species including *F. oxysporum*, *F. solani*, *F. keratoplasticum*, *F. petroliphilum*, *F. napiforme*, *Fusarium falciforme*, *F. pseudensiforme*, *F. dimerum* and the new species *Fusarium riograndense*[21,24,342,350].

When *TEF1-α* was used as part of a multiplex PCR and DNA microarray hybridization panel used for species identification primarily in neutropenic cancer patients, *F. solani* and *F. oxysporum* could be reliably identified, but these tests are not validated for clinical implementation[337,344]. If *TEF1-α* was included in a panfungal semi-nested PCR (ITS2 target) and Luminex xMAP technology approach analysing various clinical specimens, *F. solani*, *F. oxysporum*, *F. verticillioides*, and *F. proliferatum* could be identified. These assays can be considered pan-*Fusarium*[339]. PCR has been used with a variety of specimen sources[338-340,343,344], including blood[338,343], spinal fluid, and tissue material[341,343]. Accurate identification of *Fusarium* spp. from invasive infections to species level is important not only from an epidemiological standpoint, but also for choosing the appropriate antifungal treatment. For example *F. solani* spp. complex show higher MICs to VCZ and AmB than *F. oxysporum* spp. complex. Within *F. solani* spp. complex, *F. keratoplasticum* had higher MICs than *F. falciforme* and *F. petroliphilum* in one study from Brazil[347].

Recommendations – Molecular-based diagnostic testing is not widely available; it is mainly based on in-house tests at centers where there is expertise in this area. If available, these tests are strongly recommended for species identification, which may have implications for antifungal susceptibility, and marginally recommended directly from clinical specimens.

Diagnosis – microbiology – susceptibility testing

Evidence – Susceptibility testing of isolates recovered in culture should be used for epidemiologic purposes in defining the range of minimal inhibitory concentrations (MIC) distributions for *Fusarium* spp.[331]. However, studies demonstrating that susceptibility testing results should be utilized to inform antifungal drug choice are lacking[285,332]. In one case series, there was a high rate of treatment failure (11/12) among patients with disseminated fusariosis who received VCZ as first-line treatment, where susceptibility testing showed a lack of *in vitro* activity of this drug (MIC ≥16 µg/mL)[332]. Conversely, in another study, among nine clinical isolates tested, there was no correlation between MIC and clinical outcome, and some cases responded well to VCZ treatment despite high MICs[285]. These discrepancies between MIC and outcome may be related to the critical role that host factors play in treatment of fusariosis in immunocompromised patients. Although there are no interpretative breakpoints for antifungal agents against *Fusarium* spp., compounds with MICs that are off-scale, such as >16 μg/ml, at the highest range of concentrations are unlikely to be active in patients. While susceptibility testing according to EUCAST or CLSI is primarily recommended, the E-test® (bioMérieux) is a good alternative method for testing of *Fusarium* spp., but unusually high MICs should be confirmed by the CLSI method[330].

Recommendations – Susceptibility testing of isolates is strongly recommended for epidemiologic purposes and marginally recommended for informing antifungal drug choice for fusariosis. Although there is little correlation between MIC and clinical success, knowing the causative species and its resistance pattern may help with some decisions such as combination therapy and duration of therapy.

Diagnosis – Imaging

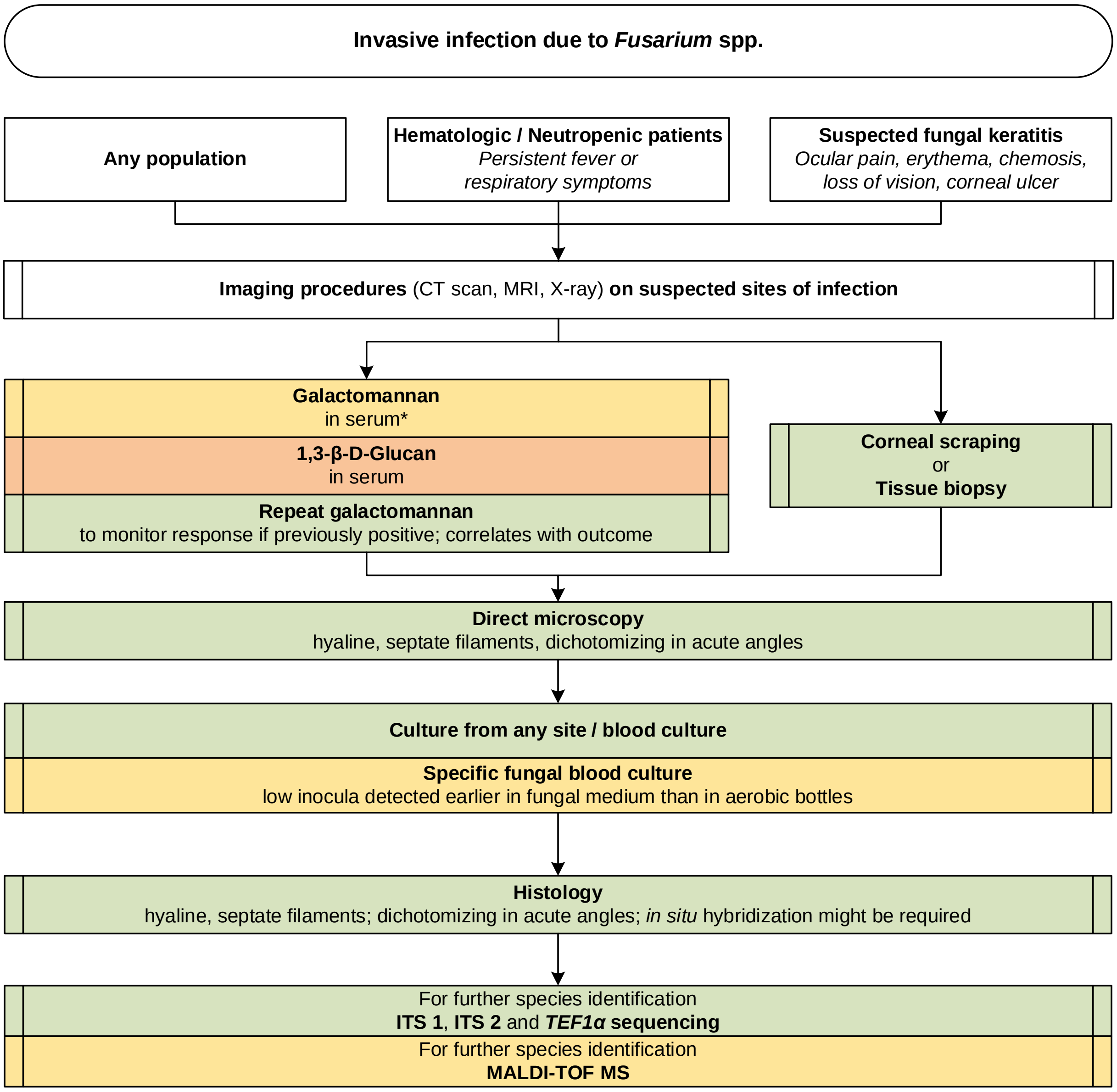
Evidence – Imaging studies may produce subtle findings that help differentiate fusariosis from aspergillosis[231,265,334,355]. Both fusariosis and aspergillosis present with macronodules on imaging. Cases of aspergillosis may have a halo sign, while cases of fusariosis are less likely to have a halo sign[231,334]. Invasive fusariosis should be suspected if chest computed tomography (CT) imaging demonstrates pulmonary infiltrates with a hypodense sign, but without the halo or the occluded-vessel signs. Suspicion is greater in the presence of hyperdense maxillary and ethmoid sinusitis.

Recommendations – CT imaging is marginally to moderately recommended for differentiating fusariosis from other invasive mold diseases including aspergillosis. Proceed with imaging for any suspected lung or sinus infection, since those body sites can have fluids or tissues to be examined by both culture and histopathology. As with other invasive fungal infections, imaging studies may assist in recognizing that there is a fungal infection, and may assist in the procurement of infected tissue or body fluids for further analysis when needed. No radiological findings reliably discriminate between different mold infections.

Diagnosis – Summary

Proceed with imaging for any suspected sinus, lung or liver involvement, since those body sites can have fluids or tissues to be examined by both culture and histopathology. Proceed with corneal scrapings of any corneal lesions. Proceed with biopsy of any skin lesions, particularly among neutropenic patients. While it is helpful to perform MIC testing of isolates recovered in culture, MIC results may not always correlate with clinical outcome. Additional molecular workup of organisms recovered from cultures will depend on the resources available at a particular center **(Figure 4)**.

Figure 4. Optimal diagnostic pathway for fusariosis, when all imaging and assay techniques are available



Legend:

strongly recommended
moderately recommended
marginally recommended
recommended against

CT, computed tomography; ITS, internal transcribed spacer; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectometry; MRI, magnetic resonance imaging; *TEF1α*, translational elongation factor 1α

* Serum galactomannan has been shown to have reduced sensitivity in the absence of neutropenia, and may therefore be less reliable as diagnostic in the non-neutropenic host

Treatment approaches for infections caused by rare molds – Standard Dosing recommendations for adults

Standard dosages of antifungals recommended in this guideline for treatment of rare mold infections in adults are outlined in **Table 2**.

Table 2. Standard dosing recommendations for adults with rare mold infections*

	Standard Dosage	Route of Administration	Therapeutic Drug Monitoring
Voriconazole(VCZ)[358]	2x 6 mg/kg d1, 2x 4 mg/kg from d2	iv; po	Yes, target trough level > 1.5-2 mg/l and <6mg/l
Posaconazole (PCZ)[7,359,360]	Suspension: 4x 200 mg or 2x 400 mg (lower exposure than 4x 200 mg) Delayed-release tablet/iv: 2x 300 mg on d1, 1x 300 mg from d2	iv; po (tablet preferable over suspension)	Yes (when used for treatment; target trough level > 0.7 mg/l)
Isavuconazole (ISA)[7,361]	3x 200 mg on d1+2; 1x 200 mg/d thereafter	iv; po	No
Itraconazole (ICZ)[362]	iv: 200-400 mg/d po: 100-400 mg/d SUBA-ICZ: 50-100 mg/d	iv; po; for po consider SUBA-ICZ	Yes
Liposomal amphotericin B (L-AmB)[363]	3-5 mg/kg qd	iv	No
Amphotericin B Lipid Complex (ABLC)[364]	3-5 mg/kg qd	iv	No
Amphotericin B colloidal dispersion (ABCD)[365]	6 mg/kg qd	iv	No
Amphotericin B deoxycholate (D-AmB)[363]	1 mg/kg qd	iv	No
Caspofungin (CASPO)[366]	70 mg on d1, 50 mg from d2 (if body weight ≤ 80 kg) or 1 × 70 mg per day from d2 (if body weight > 80 kg)	iv	No
Anidulafungin (ANID)[366]	200 mg on d1, 100 mg from d2	iv	No
Micafungin (MICA)[366]	100 mg/d	iv	No
Terbinafine (TRB)[367,368]	2x 250-500 mg/d	po	No
5-Flucytosine (5-FC)[369]	po: 50-150 mg/kg qd in divided doses every 6 hours iv: 70-150 mg/kg qd in divided doses every 6 hours	iv; po	Yes, target trough level 25-50 µg/ml, peak level 50-100 µg/ml

* For definitions of conditions that may require treatment stop/salvage treatment, including persistent, refractory, relapsed or breakthrough IFI, please refer to[370]
d, day(s); iv, intravenous; po, orally; qd, once a day; SUBA, super bioavailability

Treatment approaches to fusariosis

Treatment in adults

Primary prophylaxis

Evidence – While mold active prophylaxis that covers also *Fusarium* spp. has become standard of care in many high-risk settings, primary prophylaxis specifically for invasive fusariosis has been evaluated in one study only, in a subset of patients[302]. A previous study from the same group showed that high-risk hematological patients (acute leukemia or HSCT) with superficial skin lesions on the feet (interdigital intertrigo and / or onychomycosis) on hospital admission with positive culture for *Fusarium* spp. were at an increased risk of developing invasive fusariosis[22]. In this non-randomized trial, mold active prophylaxis (VCZ or PCZ) was given in 20 episodes at risk (neutropenia or graft versus host disease), while fluconazole or no prophylaxis was administered in 219 episodes[302]. Invasive fusariosis occurred in a similar proportion, namely 5.9% of the patients without and in 5% with anti-mold prophylaxis. However, in the subgroup of patients with superficial skin lesions with positive cultures for *Fusarium* spp., four out of five patients who had not received anti-mold prophylaxis developed invasive fusariosis vs. none out of six who received VCZ or PCZ (p=0.01). Cases of breakthrough invasive fusariosis have been reported in patients receiving primary mold-active prophylaxis with PCZ, VCZ or isavuconazole (ISA)[9,194,370-373] **(Table 3)**.

Recommendations – Primary prophylaxis with a mold-active triazole (VCZ or PCZ) is moderately recommended in high-risk hematological patients who present with superficial skin lesions with positive cultures for *Fusarium* spp. There are no data to support mold-active primary prophylaxis specifically to prevent fusariosis in other settings.

Secondary prophylaxis

Evidence – Secondary prophylaxis for invasive fusariosis was evaluated in a muticenter retrospective study of 40 patients who were successfully treated for invasive fusariosis and were exposed to subsequent periods of immunosuppression (neutropenia in 35 patients and graft versus host disease in five patients)[231]. Overall, 32 patients received secondary prophylaxis (VCZ in 24 patients, PCZ in two patients and a lipid formulation of amphotericin B (AmB) in six patients). Relapse of invasive fusariosis occurred in two of the eight patients (25%) who were not on secondary prophylaxis and in three out of 32 (9.4%) patients who received secondary prophylaxis (p=0.26). Considering only patients who had disseminated fusariosis, relapse occurred in both patients not on secondary prophylaxis and in three out of 26 (11.5%) patients who had received secondary prophylaxis (p=0.03) **(Table 3)**.

Recommendations – Secondary prophylaxis with a mold-active triazole or a lipid formulation of AmB is moderately recommended in patients with prior invasive fusariosis who will be exposed to subsequent periods of immunosuppression, especially if the previous disease was disseminated.

Diagnostic-driven treatment

Evidence – Patients with invasive fusariosis may frequently present with positive serum GM or BDG. In a multicenter study, 15 out of 18 patients with invasive fusariosis had at least one positive serum GM test. In one study, serum GM was positive at a median of 10 days before the first clinical manifestation of fusariosis in 73% of patients[230]. In another study, 12 out of 13 patients with invasive fusariosis had a positive BDG, in 11 the test was positive prior to the diagnosis of invasive fusariosis. However, the test lacked specificity and the positive predictive value for 2 consecutive positive BDG tests was 7% only[333]. Another study evaluated the strategy of using the area over the neutrophil curve (D-index) to stratify the risk for invasive mold disease in 29 high-risk neutropenic patients[374]. A cumulative index above 5,800 identified a group at higher risk, with a rate of invasive mold disease (including fusariosis) of 67%, 45.5% and 0% in high-, intermediate- and low-risk patients, respectively **(Table 3)**.

Recommendations – Serum GM, serum BDG and the cumulative D-index may be of help to establish a diagnostic-driven approach in high risk neutropenic patients. However, these tests lack specificity for the diagnosis of invasive fusariosis. Therefore, the guideline marginally supports the use of these tools for initiating specific treatment for invasive fusariosis.

First line treatment

Evidence – There are no randomized trials evaluating antifungal drugs for the treatment of invasive fusariosis. The largest series published to date is a multicenter retrospective study of 236 patients with invasive fusariosis diagnosed between 1985 and 2011 in 44 centers from 11 countries all over the world[28].

Among 206 patients who received treatment for invasive fusariosis, 110 received AmB deoxycholate (D-AmB), 38 were treated with VCZ, 34 with a lipid formulation of AmB (liposomal 20, lipid complex 8, colloidal dispersion 6; in a previous study lipid complex was less well tolerated than the liposomal formulation with more acute infusion-toxicity[375]), 21 received combination therapy (mainly VCZ plus AmB), and three received other therapies. The 90-day probability of survival was 27% for patients treated with D-AmB, 53% for patients receiving VCZ, and 48% for those receiving a lipid formulation of AmB.

Other studies reported lower numbers of patients receiving primary treatment with a single agent for invasive fusariosis with either VCZ (55 patients, response rates ranging from 44 to 100%, including localized disease)[18,201,285,376,377], AmB lipid complex (ABLC; 28 patients, 43% response rate)[378], liposomal AmB (L-AmB; 10 patients, response rates 0 to 100%)[9,371,379,380], and D-AmB (5 patients, 20% response rate)[379]. A few patients received treatment with either ISA, echinocandins, terbinafine (TRB) or PCZ[285,381-383].

Combination therapy with VCZ plus L-AmB or another agent was reported in the majority of studies, and is the preferred initial approach in many specialized centers because of high VCZ MICs, while other centers prefer monotherapy[18,28,159,201,285,377,382,384,385]. Response rates with combination therapy overall were similiar to monotherapy, and randomised controlled trials comparing monotherapy with combination therapy are lacking. In one study, combination therapy was used in 21 out of 236 patients (including VCZ plus L-AmB in 12 cases and VCZ plus D-AmB in 5 cases). Response rates did not significantly differ from monotherapy and receipt of combination therapy was not a significant predictor of 90-day survival[28]. However, as combination therapy may have been used in more critically ill patients, no conclusions can be drawn from this retrospective study between combination therapy and monotherapy.

Removal of indwelling central venous catheters has been associated with improvement in observational studies and thus should be considered in all cases of fungemia[386] **(Table 3)**.

Table 3. Prophylaxis and first line treatment of *Fusarium* spp. infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Prophylaxis						
HSCT with superficial skin fusariosis	To prevent invasive disease	Primary prophylaxis with PCZ or VCZ	B	IIu	Varon AAC 2016[302]	
					Bose JCM 2011[387]	N=2, breakthrough during PCZ
HSCT after disseminated fusariosis	To prevent recurrence	Secondary prophylaxis with PCZ, VCZ or L-AmB	B	IIu	Nucci Mycoses 2019[231]	
Fever-driven treatment						

Any	To cure	Initiate treatment upon fever	D	III	No reference found.	
Diagnosis-driven treatment						
Hematological malignancy	To increase survival rate	Apply D-Index for treatment initiation	C	IIu	Garnica BJID 2016[374]	
First-line treatment						
Any	To cure	L-AmB 3-9 mg/kg qd iv	A	IIu	Nucci CMI 2014[28]	N=20
					Jensen CMI 2004[380]	N=4
					Musa BJH 2000[379]	N=3
					Jenks IJAA 2018[384]	N=2
					Cudillo AH 2006[388]	N=1
					Lamoth JCM 2016[332]	
Any	To cure	VCZ iv, switch to po when stable,	A	IIu	Nucci CMI 2014[28]	N=38, 92% hematology, 90 d survival 60%
					Stanzani TCRM 2007[377]	N=19, 63% response
					Muhammed Medicine 2013[18]	N=8, 100% response
					Lortholary AAC 2010[201]	N=16, 44% response
					Perfect CID 2003[376]	N=11, 45% response
					Jenks IJAA 2018[384]	N=1, burn patient, success
Any	To cure	VCZ in combination with AmB lipid formulation	A	IIu	Lortholary AAC 2010[201]	N=13, diverse combinations
					Nucci CMI 2014[28]	N=21
					Muhammed Medicine 2013[18]	N=9
					Stanzani TCRM 2007[377]	N=3
					Horn Mycoses 2014[385]	N=19
					Stempel OFID 2015[285]	N=6
					Jenks IJAA 2018[384]	N=2, success
Any	To cure	ABLC	A	IIu	Nucci CMI 2014[28]	N=34, including ABLC (N=8), and ABCD (N=6); 90 d survival 53%
					Perfect CID 2005[378]	N=26 with ABLC 2-10mg/kg qd; 12 (46%) cured/improved, 3 (12%) stable response
Any	To cure	D-AmB	D	IIu	Nucci CMI 2014[28]	N=110, poorer response (90 d survival 28%, 95%CI 20-36%) compared with VCZ or AmB lipid formulation
					Musa BJH 2000[379]	N=5, response in 1/5 (20%)
Any	To cure	AmB lipid formulation + triazole/echinocandin/TRB	B	IIt	Campo JInf 2010[389]	N=44, 37/44 (84%) changed to combination; 12 wk mortality 66%
					Muhammed Medicine 2013[18]	N=2, L-AmB plus CASPO or MICA, 2/2 died
					Rothe AnnHematol 2004[390]	N=1, response to AmB plus TRB
Hematological malignancy	To cure	VCZ	A	IIt	Stempel OFID 2015[285]	N=15, 5 success
Hematological Malignancy	To cure	ISA	C	III	Cornely Mycoses 2018[381]	N=1, success
Hematological malignancy	To cure	Echinocandin	D	III	Stempel OFID 2015[285]	N=1
					Apostolidis CID 2003[383]	N=1
Hematological malignancy	To cure	TRB	C	III	Stempel OFID 2015[285]	N=1, success
Solid organ transplant	To cure	PCZ	C	III	Herbrecht JHLT 2004[382]	N=1, success
Hematological malignancy with endophthalmitis	To cure	VCZ for 10 d iv, switch to po +/- VCZ intravitreal +/- AmB intravitreal +/- vitrectomy	B	III	Simon JMM 2018[280]	N=1, success
					Bui JOPT 2016[82]	N=1, success
Hematological malignancy with endophthalmitis	To cure	L-AmB 5-9 mg/kg qd or other AmB formulations iv +/- AmB intravitreal 5 mg/0.1 ml qw +/- vitrectomy	C	III	Ocampo-Garza JEADV 2016[233]	N=1, success
					Rezai ArchOphthalmol 2005[391]	N=1, failure
					Malavade IDCP 2013[392]	N=1, failure
					Cudillo AnnHematol 2006[388]	N=1, success
Hematological malignancy with endophthalmitis	To cure	VCZ iv + L-AmB +/- VCZ intravitreal 100 mg/0.1 ml/wk, +/- AmB intravitreal	B	III	Kapp TID 2011[171]	N=1, failure
					Baysal CRH 2018[71]	N=1, success
					Rizzello Mycoses 2017[256]	N=1, success
					Perini Einstein 2013[244]	N=1, failure
					Malavade IDCP 2013[392]	N=1, success

		5 mg/0.1 ml/wk +/- vitrectomy			Yoshida Mycopathol 2018[313]	N=1, success
					Bui JOPT 2016[82]	N=1, success
Immunocompetent patient with endophthalmitis	To cure	VCZ, AmB intravitreal, vitrectomy	C	III	Milligan AJOCR 2018[393]	N=1, failure
Liver transplantation with endophthalmitis	To cure	VCZ iv, intravitreal	C	III	Jørgensen JMCR 2014[164]	N=1, success
Postoperative endophthalmitis	To cure	VCZ intravitreal, AmB intravitreal, vitrectomy	C	III	Mithal ClinOphthalmol 2015[394]	N=1, success
Postoperative endophthalmitis	To cure	VCZ	C	II	Buchta Mycopathol2014[395]	N=18, response 18/18
Postoperative endophthalmitis	To cure	VCZ, AmB intravitreal, vitrectomy	C	III	Chander Mycopathol 2011[96]	N=1, success
Postoperative endophthalmitis	To cure	VCZ + VCZ intravitreal +/- topical + AmB intravitreal +/- topical, surgical intervention	B	II	Gungel Mycoses 2011[396]	N=9, response 4/9
					Cakir CER 2009[397]	N=8, success 6/8
Postoperative endophthalmitis	To cure	AmB 5 μg/0.1 ml intravitreal +/- vitrectomy	C	III	Alves da Costa Pertuiset CROM 2016[54]	N=1, failure, salvage treatment, vitrectomy
					Ferrer JCM 2005[398]	N=1, success
Exogenous endophthalmitis	To cure	VCZ iv, switch to po or VCZ po +/- VCZ intraocular	B	III	Troke Infection 2012[399]	N=8, response 5/8
					Comer ClinOphthalmol 2012[400]	N=2, failure 2/2
Exogenous endophthalmitis	To cure	PCZ po, topical	C	III	Sponsel BJO 2002[401]	N=1, success
Exogenous endophthalmitis	To cure	AmB + VCZ topical, po	C	III	Barrios Andrés RIM 2018[69]	N=3, success 2/3
Exogenous endophthalmitis	To cure	Ketoconazole 200 mg/d po + natamycin topical 5% + D-AmB topical 0.15%	D	III	Comer ClinOphthalmol 2012[400]	N=1, failure

Standard dose unless stated otherwise; ABLC, amphotericin B lipid complex; AmB, amphotericin B; bid, twice a day; CASPO, caspofungin, CI, confidence interval; CF, cystic fibrosis; d, day(s); D-AmB, amphotericin B deoxycholate; po, orally; HSCT, hematopoietic stem cell transplantation; ISA, isavuconazole; iv, intravenous; L-AmB, liposomal amphotericin B; PCZ, posaconazole; qd, once a day; QoE, quality of evidence; qw, once a week, SoR, strength of recommendation; TDM, therapeutic drug monitoring; tid, three times a day; TRB, terbinafine; VCZ, voriconazole; wk, week(s).

Recommendations – Data regarding primary therapy with a lipid formulation of AmB show similar numbers of patients treated and similar response rates with either L-AmB (doses from 3 to 9 mg/kg qd) or ABLC, which may be slightly worse tolerated than L-AmB. Likewise, data on primary therapy with VCZ show similar response rates compared with a lipid formulation of AmB. We therefore strongly recommend primary treatment for invasive fusariosis with either VCZ or a lipid formulation of AmB (L-AmB or other lipid formulatons of AmB). Given the broad dose range used and the small number of patients treated with the two lipid formulations of AmB, a formal recommendation for the dose of each agent cannot be made. For VCZ, we strongly recommend the standard dose intravenous treatment, with step-down to oral VCZ after disease control and the use of therapeutic drug monitoring (AIIu), with a target trough level of

1.5 mg/l – 6 mg/l, which has been shown to ensure efficacy and avoid toxicity in patients with invasive aspergillosis[358,402] (**Table 1**). D-AmB should not be used for treatment of invasive fusariosis when other active antifungal agents are available. For other agents a marginal recommendation is given.

Combination therapy is frequently used in the primary treatment of invasive fusariosis because of the severity of the disease, difficulties to achieve VCZ trough levels within the targeted range, and because MICs for azoles and polyenes are often high. Primary combination therapy with a potential early step down to monotherapy later – once MICs come back - is an approach we strongly recommend.

Endophthalmitis

Evidence – Ocular involvement in disseminated fusariosis occurs occasionally and may be associated with loss of vision[403]. Proposed treatment strategies have relied on the use of systemic and intravitreal anti-fungal agents, with or without vitrectomy. The literature on endophthalmitis in hematological patients is limited to case reports in which patients were treated with either systemic VCZ[82,280], AmB[233,371,391], or VCZ plus AmB[71,171,244,256,313], with or without intravitreal VCZ or AmB, with or without surgery.

Outside the setting of hematological patients, endophthalmitis caused by *Fusarium* spp. has been occasionally reported in other immunosupressed patients such as SOT recipients[164], in immunocompetent patients following ocular surgery[54,96,394-398], patients with keratitis (which is usually treated with natamycin 5% ophthalmic formulation)[69,105,399,401], or after dissemination from a primary skin lesion[393]. The majority of patients were treated with systemic VCZ plus intravitreal VCZ or AmB and surgery, with variable responses.

Recommendations – Considering that VCZ is an option for the treatment of invasive fusariosis and that it has good tissue distribution including the eyes, we favor the use of systemic VCZ (with or without AmB) with intravitreal VCZ or AmB (moderately recommended) over systemic AmB (marginally recommended). Surgical intervention (pars plana vitrectomy) should be discussed with an experienced ophthalmologist on a patient to patient basis.

Salvage therapy

Evidence – Because the outcome of invasive fusariosis is largely dependent on recovery of host defences[404], poor response to primary therapy does not necessarily mean that the antifungal drug is not active. Nevertheless, patients who fail to respond to treatment should receive salvage therapy. A caveat that must be acknowledged is that severely ill patients may die before a second treatment is offered and therefore response rates with salvage therapy may be inflated because of this selection bias[405]. Another consideration is that the drug chosen for salvage therapy depends on which agent was used for primary treatment. Considering these factors, since most patients with invasive fusariosis have received formulations of AmB in the past, the majority of data pertains to the use of a mold-active triazole as salvage therapy. This does not necessarily mean that a lipid formulation of AmB cannot be used as salvage therapy for a patient who received a triazole as primary therapy for invasive fusariosis.

The largest series of salvage therapy reported the outcome of 57 patients who received salvage therapy with VCZ[201]. The most frequent prior therapies were AmB (any formulation, 21 patients), an echinocandin (10 patients) and another triazole (10 patients). The overall response rate was 47%. The second largest series reported 21 patients who were refractory (n=17) to or intolerant (n=4) of primary therapy with another drug (lipid formulation of AmB in 20 patients). The overall response rate was 48%[406]. Another series reported 11 patients who received salvage VCZ, with a response rate of 45%[376]. Salvage therapy for fusariosis with ISA was given to four patients, with one positive response[381,407] **(Table 4)**.

Table 4. Antifungal salvage treatment for *Fusarium* spp. infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To cure	VCZ +/- other antifungal (CASPO, L-AmB, TRB, PCZ, or white blood cell transfusion)	B	IIu	Lortholary AAC 2010[201]	N= 57, success in 27
					Baden Transplantation 2003[408]	N=3, success in 3
Any	To cure	PCZ tablet or iv formulation preferred	B	IIu	Raad CID 2006[406]	N=21, success in 10
					Campo JInfect 2010[389]	N=2 hematology
Any	To cure	VCZ iv for ≥ 3 d, switch to po or VCZ	B	IIu	Perfect CID 2003[376]	N=11, success in 5
Any	To cure	ISA	C	III	Cornely Mycoses 2018[381]	N=4, success in 1
					Marty Mycoses 2018[407]	
Any with disseminated fusariosis	To cure	TRB 500-750mg qd + VCZ iv or L-AmB	B	III	Inano JIC 2013[159]	N=3, success in 3
					Rothe AnnHematol 2004[390]	
					Neuburger TID 2008[409]	

Hematological malignancy with skin infection and endophthalmitis	To cure	VCZ + AmB iv, intravitreal + vitrectomy	C	III	Malavade IDCP 2013[392]	N=1, outcome not reported
Exogenous endophthalmitis	To cure	VCZ +/- VCZ intraocular 1% +/- VCZ intravitreal 2.5 μg/0.1 ml +/- AmB intravitreal 5 μg/0.1 ml +/- vitrectomy	C	II	Troke Infection 2012[399]	N=16, response in 11
					Alves da Costa Pertuiset CROM 2016[54]	N=1, success
					Comer ClinOphthalmol 2012[400]	N=3, success
Exogenous endophthalmitis	To cure	PCZ	C	III	Tu AJO 2007[410]	N=2, response

Standard dose unless stated otherwise; AmB, amphotericin B; bid, twice a day; d, days; ISA, isavuconazole; iv, intravenous; L-AmB, liposomal amphotericin B; PCZ, posaconazole; po, orally; qd, once a day; QoE, quality of evidence; SoR, strength of recommendation; tid, three times a day; TRB, terbinafine; VCZ, voriconazole.

Recommendations – We moderately recommend VCZ as salvage therapy for patients with progressive disease who fail treatment with a lipid formulation of AmB. PCZ is a moderately recommended alternative, especially if the intravenous formulation and/or the modified release tablet formulation are available, which provide more reliable serum levels than the oral solution. Other moderately recommended options for salvage therapy include the combinations of TRB with VCZ or L-AmB, and combinations of VCZ with other antifungals. ISA as salvage therapy is a marginally recommended alternative, with stronger recommendations pending more data becoming available. Likewise, a lipid formulation of AmB is a reasonable alternative for a patient who fails primary treatment with a triazole, but only marginally recommended as strong supporting data are lacking.

Ancillary therapies

Evidence – Ancillary therapies for invasive fusariosis include surgical debridement of infected tissue, also following trauma[18,411,412], the use of colony-stimulating factors such as granulocyte colony-stimulating factor (G-CSF) and granulocyte-monocyte colony-stimulating factor (GM-CSF)[167,403], and the use of granulocyte transfusions[167,403].

In one study, surgical debridement for localized *Fusarium* infection in bone and joint resulted in control of infection in all six patients[411]. Similar results were observed in a cohort of immunocompetent patients with fusariosis confined to the skin[18].

The use of G-CSF or GM-CSF was evaluated in 17 patients with invasive fusariosis with hematological malignancies, with a response rate of 41%[403]. The contribution of colony-stimulating factors on the favourable outcome is difficult to evaluate.

The best evidence for the use of granulocyte transfusions in patients with invasive fusariosis comes from a study that analyzed 11 patients, with 10 showing unequivocal signs of clinical response[167]. It must be acknowledged that the use of colony-stimulating factors or granulocyte transfusions are measures undertaken to allow time for neutrophil recovery. If the patient remains neutropenic, the outcome is very poor[404] **(Table 5)**.

Table 5. Other treatment options for *Fusarium* spp. infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To cure	Resection of pulmonary infiltration/lobectomy and AmB	C	III	Lupinetti ATS 1990[412]	N=1, success
Hematological malignancies	To cure	Granulocyte transfusion	C	III	Boutati Blood 1997[403]	N=7, response 3/7
Neutropenic	To cure	G-CSF or GM-CSF	B	IIu	Boutati Blood 1997[403]	N=17, response 7/17
					Kadri Transfusion 2015[167]	N=11, granulocyte transfusion, response in 10; 90 d survival 73%
					Nucci Cancer 2003[404]	N=84
Any with fungemia	To cure	Removal of indwelling central venous catheters	B	IIut	Janum CDSR 2016[413]	
Any with skin fusariosis	To cure	Surgical debridement	A	IIu	Muhammed Medicine 2013[18]	N=11
Bone and joint infections	To cure	Surgical debridement and antifungal treatment	A	IIr	Koehler CRM 2016[414]	N=6, response 6/6

AmB, amphotericin B; d: day(s); G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; QoE, quality of evidence; SoR, strength of recommendation

Recommendations – We strongly recommend surgical debridement of infected tissue in cases of localized fusariosis of, for example, the skin following trauma, joints and bone. The use of G-CSF or GM-CSF should be considered if there is an expectancy of timely bone marrow recovery (moderately recommended).

Duration of treatment

Evidence – In the largest series of treatment for invasive fusariosis in severely immunocompromised patients, treatment was usually given until bone marrow recovery in neutropenic patients, or resolution of immunosuppression in non-neutropenic patients[28] **(Table 6)**.

Table 6. Treatment duration for *Fusarium* spp. infections

Population	Intention	Intervention	Sor	QoE	Reference	Comment
Hematological malignancies or HSCT	To cure	VCZ or AmB until resolution of neutropenia and of clinical manifestations of infection	A	III	Nucci CMI 2014[28]	
Exogenous endophthalmitis	To cure	VCZ for 4 wk to 4 mo	C	III	Buchta Mycopathol 2013[395]	N=20
					Comer ClinOphthalmol 2012[400]	N=2, success
					Alves da Costa Pertuiset CROM 2016[415]	N=1, success
Hematological malignancy with disseminated fusariosis	To cure	L-AmB +/- VCZ +/- TRB for > 2 mo	B	III	Neuburger TID 2008[409]	N=1, success
					Cudillo AnnHematol 2006[388]	N=1, success
Endogenous endophthalmitis	To cure	VCZ iv for 5 d, then 4 mo VCZ po	C	III	Milligan AJOCR 2016[416]	N=1, retinal detachment
Eye infections	To cure	VCZ for 6 wk to 7 mo	C	III	Troke Infection 2012[399]	N=24

AmB, amphotericin B; d, days; drug application as standard dose unless stated otherwise; HSCT, hematopoietic stem cell transplantation; iv, intravenous; L-AmB, liposomal amphotericin B; mo, month(s); po, orally; QoE, quality of evidence; SoR, strength of recommendation, TRB, terbinafine; VCZ, voriconazole; wk, week(s).

Recommendations – We strongly recommend continuing treatment for invasive fusariosis until recovery of host defences, and moderately recommend for disseminated disease a minimum treatment duration of 2 months.

Treatment pathways for adults in different settings **(Figure 5, and Figure 6)**.

Figure 5. Optimal treatment pathway for fusariosis <u>in adults</u> when all treatment modalities and anti-fungal drugs are available

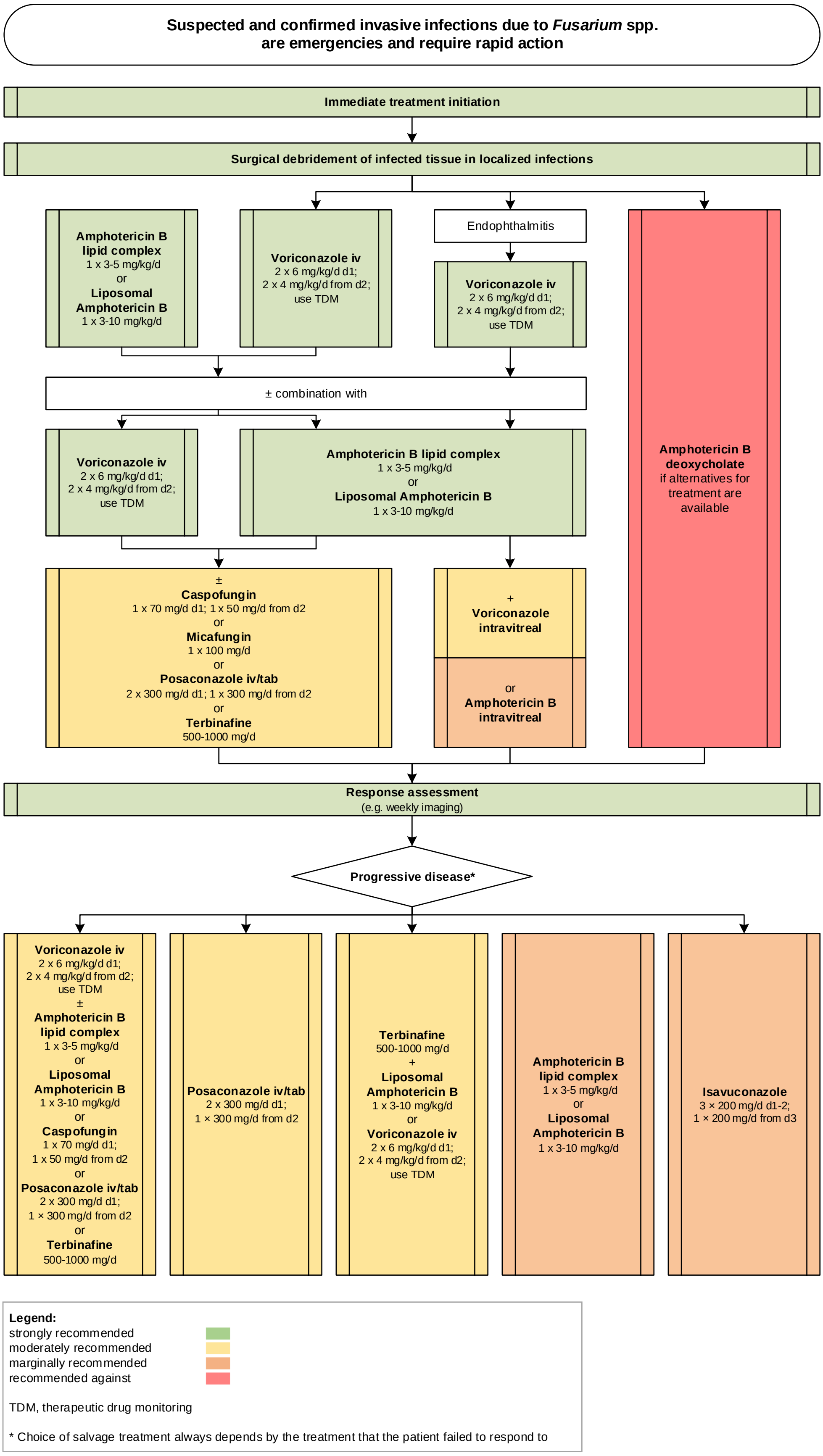
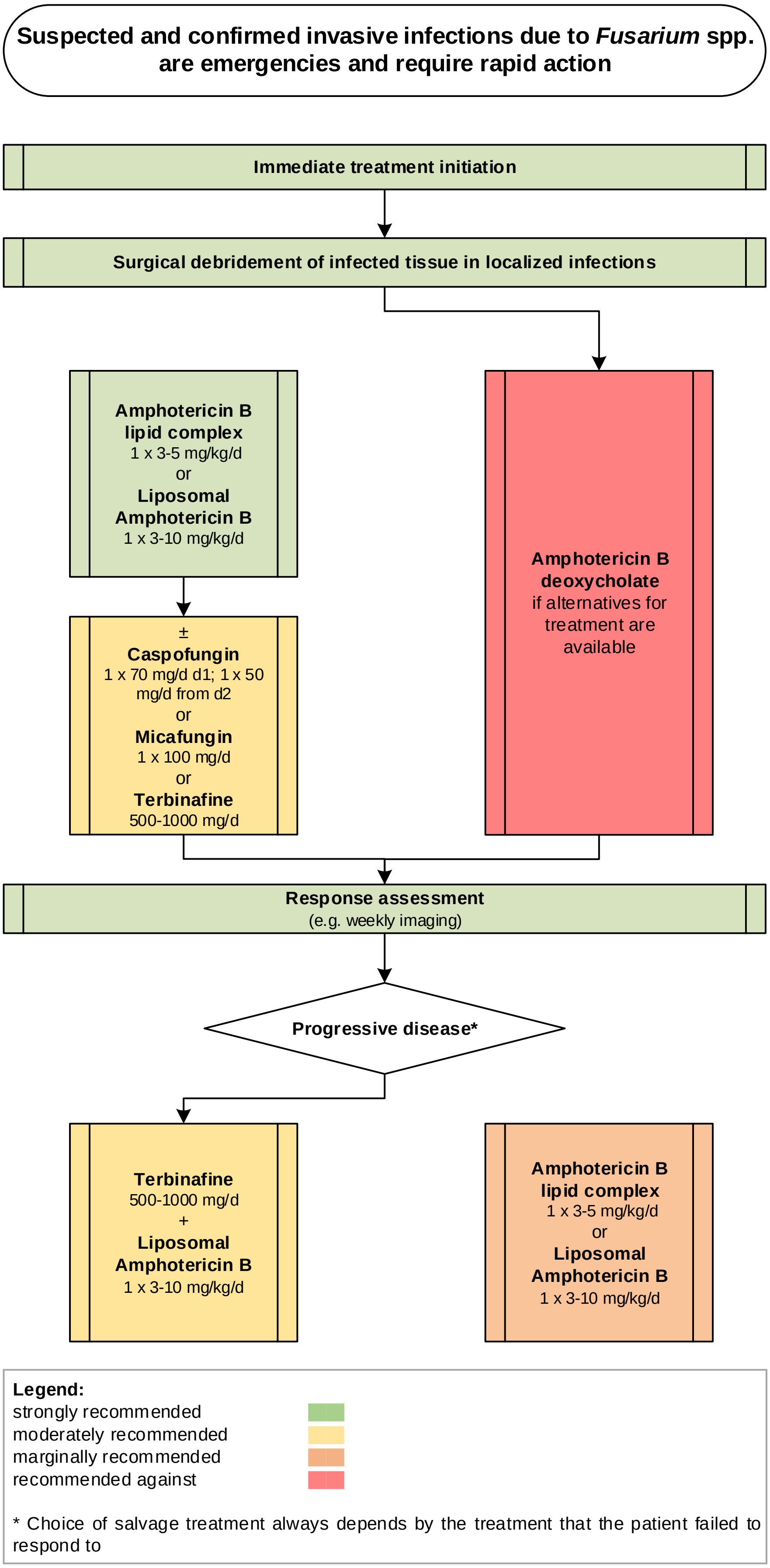


Figure 6. Optimal treatment pathway for fusariosis in adults when triazoles are not available



Specific considerations on treatment of fusariosis in children

Evidence – As in adults, *Fusarium* spp. can cause severe disseminated disease in children, which are associated with high mortality. To date, data in the pediatric setting are very limited and based on single cases or small case series, including in patients with burns[44,60,417,418].

Most immunocompromised children received either VCZ monotherapy or as part of combination therapy, which included any AmB formulation or an echinocandin. The limited data suggest that children receiving VCZ have better outcomes than those not receiving VCZ (cure rate 40/55 vs. 9/26). Similarly, in the seven case reports on salvage therapy, children receiving VCZ seemed to have a benefit **(Table 7)**. Surgical debridement can be an essential adjunctive treatment for localized infections.

Table 7. Therapy in children for *Fusarium* spp. infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
First-line antifungal therapy						
Hematological malignancy	To cure	AmB + 5-FC	C	III	Richardson RID 1988[419]	N=1, 7 yrs, failure
Hematological malignancy	To cure	L-AmB 1.5 mg/kg qd + ICZ 2.5 mg/kg qd po	C	III	Hsu PIDJ 1994[420]	N=1, 3 mo, failure
Hematological malignancy	To cure	D-AmB	D	III	Schwartz JPIDS 2015[263]	N=2, 9 yrs, 8 yrs, failure
					Litvinov CMI 2015[417]	N=2, 6yrs, 9 yrs, failure
					Albisetti Infection 2004[421]	N=1, 2 yrs, success
					Alvarez-Franco PedDermatol 1992[422]	N=1, 18 yrs, failure
					Ammari CID 1993[423]	N=1, 13 yrs, success
					Repiso PedDermatol 1996[424]	N=1, 7 yrs, success
Hematological malignancy	To cure	VCZ 4 mg/kg bid or 200 mg bid + D-AmB 1 mg/kg qd	C	III	Litvinov CMI 2015[417]	N=3, 10 mo-16 yrs, response 1/3
Hematological malignancy	To cure	VCZ + AmB lipid formulation	A	III	Hassler PIDJ 2017[418]	N=5, 0-13 yrs, success, granulocyte transfusions +/- G-CSF in 3/5
					Seban CNM 2017[425]	N=1, 12 yrs, localized infection, success
					Litvinov CMI 2015[417]	N=4, 8-16 yrs, response 1/4
					Arnoni Mycopathol 2018[60]	N=3, 6-11 yrs, response 2/3
					Uemura PIDJ 2018[298]	N=1, 10 yrs, failure, L-AmB 6 mg/kg
					Silva Mycopathol 2013[426]	N=1, 15 yrs, failure
					Schwartz JPIDS 2013[263]	N=1, 3 yrs, success
					Schwartz JPIDS 2013[263]	N=1, 15 yrs, failure
					Schwartz JPIDS 2013[263]	N=1, 1 yr, failure
Hematological malignancy	To cure	VCZ + echinocandin	C	III	Hassler PIDJ 2017[418]	N=3, 8-10 yrs, response 1/3
					Litvinov CMI 2015[427]	N=1, 17 yrs, success
Hematological malignancy	To cure	VCZ	A	III	Carlesse AMRIC 2017[428]	N=6, 1-8 yrs, success 6/6
					Hassler PIDJ 2017[418]	N=2, 3 yrs, 7 yrs, failure 2/2
					Vallerini JInfect 2017[429]	N=1, 12 yrs, success
					Arnoni Mycopathol 2018[60]	N=1, 9 yrs, success
					Sidhu IJPM 2013[278]	N=1, 12 yrs, survived
Hematological malignancy	To cure	L-AmB	C	III	Carlesse AMRIC 2017[428]	N=1, 9 mo, success
					Vagace BMC ID 2007[430]	N=1, 11 yrs, failure
					Tezcan JCM 2009[296]	N=1, 12 yrs, failure
					Cesaro Mycoses 2010[431]	N=1, 8 yrs, failure
					Hol BMT 2014[432]	N=1, 1 yr, success
					Morel PedDermatol 2013[433]	N=1, 13 yrs, failure
					Rodriguez BMT 2003[434]	N=1, 3 yrs, success

					Kivivuori EJP 2004[435]	N=2, 5 yrs, 8 yrs, failure 2/2
					Guzman-Cottrill PID 2004[436]	N=1, 3 mo, failure
Hematological malignancy with endophthalmitis	To cure	ABCD 5 mg/kg tid + VCZ intravitreal 100 μg/0.1 ml	C	III	Kah BMCO 2011[437]	N=1, 9 yrs, success
Chronic granulomatous disease	To cure	D-AmB + ketoconazole 150 mg qd	C	III	Bassiri-Jahromi MedMycol 2012[438]	N=1, 15 yrs, success
Chronic granulomatous disease	To cure	VCZ	C	III	Bassiri-Jahromi MedMycol 2012[438]	N=1, 12 yrs, success
Burn	To cure	Voriconazole + amphotericin B deoxycholate + surgical intervention	C	III	Rosanova BJID 2016[259]	N=15 (mean age: 2 yrs; range 1-9 yrs) success 14, failure 1
Burn	To cure	AmB	C	III	Schaal Burns 2015[267]	N=1, 2 yrs, success
Burn	To cure	VCZ	C	III	Muhammed Medicine 2013[18]	N=3, 4-17 yrs, success 3/3
Burn	To cure	L-AmB, VCZ	C	III	Muhammed Medicine 2013[18]	N=1, 8 yrs, success
Burn	To cure	L-AmB, MICA	C	III	Muhammed Medicine 2013[18]	N=1, 4 yrs, failure
Burn	To cure	L-AmB	C	III	Muhammed Medicine 2013[18]	N=1, 15 yrs, success
Antifungal salvage treatment						
Hematological malignancy	To cure	L-AmB 5-9 mg/kg qd + CASPO 70 mg/m^2 qd loading on d1, 50 mg/m^2 qd from d2	C	III	Uemura PIDJ 2018[298]	N=1, 10 yrs, ALL, L-AmB (9 mg/kg qd) + CASPO (70 mg/m^2 qd as loading dose followed by 50 mg/m^2 qd), *F. keratoplasticum* cultured, success
					Vagace BMCID 2007[430]	N=1, 11 yrs, ALL, L-AmB (5 mg/kg qd) + CASPO, treatment success
Hematological malignancy	To cure	VCZ + CASPO	C	III	Cesaro Mycoses 2010[431]	N=1, 8 yrs, ALL, success
Hematological malignancy	To cure	VCZ	C	III	Tezcan JCM 2009[296]	N=1, 12 yrs, HSCT, success
Hematological malignancy	To cure	L-AmB 10 mg/kg qd + VCZ start 4 d after L-AmB	C	III	Rodriguez BMT 2003[434]	N=1, 3 yrs, aplastic anemia, success
					Guzman-Cottrill PIDJ 2004[436]	N=1, 3 mo, L-AmB (10 mg/kg qd) + VCZ (6 mg/kg dose loading dose followed by 4 mg/kg dose) + granulocyte transfusion; AML, success
Hematological malignancy	To cure	AmB + 5-FC	C	III	Richardson RID1988[419]	N=1, 7 yrs, ALL, failure

Standard pediatric dose unless stated otherwise; 5-FC, 5-fluorocytosine; ABCD, amphotericin B colloidal dispersion; ALL, acute lymphocytic leukemia; AmB, amphotericin B; AML, acute myeloid leukemia; bid, twice a day; CASPO, caspofungin; D-AmB, amphotericin B deoxycholate; d, day(s); FCZ, fluconazole; G-CSF, granulocyte colony-stimulating factor; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; JMML, Juvenile myelomonocytic leukemia; L-AmB, liposomal amphotericin B; MICA, micafungin; mo, month(s); po, orally; qd, once a day; QoE, quality of evidence; SoR, strength of recommendation; tid, three times a day, TRB, terbinafine; VCZ, voriconazole; yrs, years.

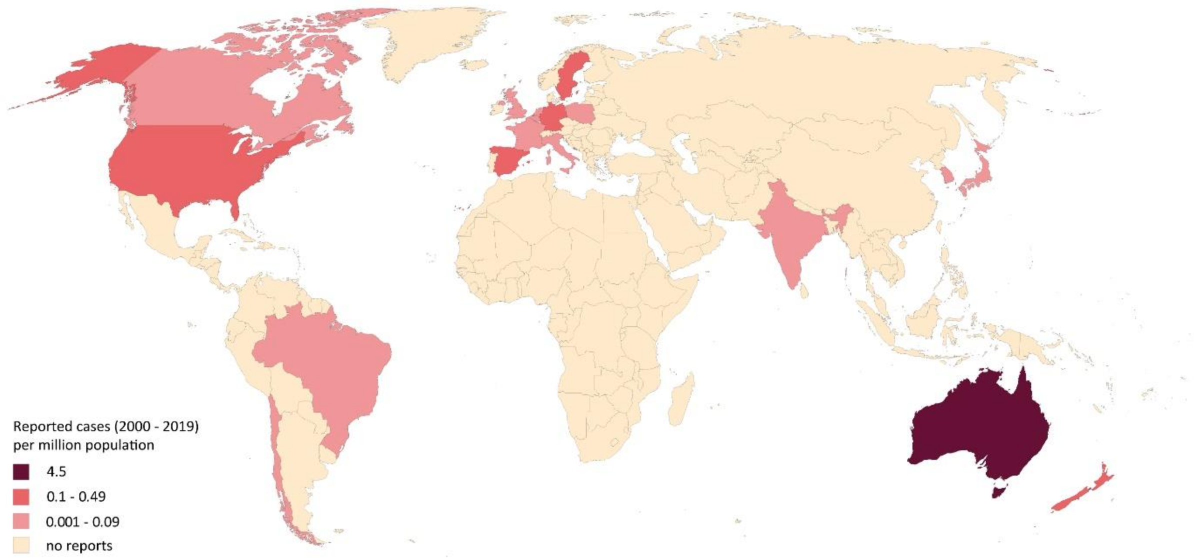
Recommendations – First-line treatment with VCZ monotherapy or combination therapy with VCZ and a lipid formulation of AmB is strongly recommended. Monotherapy with L-AmB is marginally supported; monotherapy with D-AmB is discouraged. Combination therapy with VCZ plus high-dose L-AmB or an echinocandin is recommended as salvage therapy with marginal strength. In line with recommendations in adults, surgical debridement is strongly recommended for localized infections.

2. Lomentosporiosis

Epidemiology of lomentosporiosis

Based on phylogenetic profiling, *Lomentospora prolificans* is now distinguished from *Scedosporium* spp.[439]. *L. prolificans* is ubiquitously found as a soil saprophyte predominately in arid climates of Australia, south-western US and Spain, reflected by the proportionally higher number of reported cases from these regions[9,440,441]. Prevalence and incidence data for lomentosporiosis are largely unknown. In a US study, *L. prolificans* accounted for 2% of mold infections and 6% of non-*Aspergillus* infections identified in liver and heart transplant recipients[442]. In France, four cases in hematological patients have been reported within 6 years[443]. Another study in the US reported 0.2 lomentosporiosis cases per 100,000 inpatient days in hematological patients (4 patients between 1989 and 2006)[444]. Immunocompromised patients treated for hematological malignancy and those undergoing HSCT or SOT are at highest risk for lomentosporiosis[11,445]. In more than 80% of hematological patients, *L. prolificans* causes disseminated disease, mostly with fungemia, which is associated with a particularly dire outcome[11,446]. Endocarditis and brain infections are commonly seen in disseminated disease. The risk of dissemination in HSCT and SOT patients depends on the type of transplantation and immunosuppressive regimen[447]. In one review, only 34 of 162 patients (21%) were noted to have no underlying disease[441]. Infections after direct inoculation via surgical wounds or after traumatic injuries may also disseminate to non-contiguous organs[11,440,444,445,448-456] (**Figure 7**).

Figure 7. Worldwide distribution of lomentosporiosis (reported cases between 2000 and 2019 per million population)



Cases of *Lomentospora*-related infections reported in the medical literature were identified in a PubMed search on November 15, 2019 using the search string "Scedospori* OR Pseudallescheri* OR Lomentospori*" that yielded 1,628 publications. In total, 233 cases were identified from 18 countries[11,162,173,440,443,444,446,456-540]. The vast majority of cases were reported from Australia (n=108), the United States (n=53), followed by Spain (n=20), Germany (n=15), and Japan (n=12). Australia reported ~8-times more lomentosporiosis cases per million population than the average number of all countries. The number of cases reported between 2000 and 2019 is presented as cases per million population per country. The resident population per country was obtained from www.worldometers.info[321].

Diagnosis of lomentosporiosis

Diagnosis – Microbiology – Conventional methods

Evidence – The definitive diagnosis of *L. prolificans* infection relies on isolation of the fungus from biopsies, sterile body fluids and blood cultures[13,495,516,541,542]. For respiratory tract samples of patients with cystic fibrosis (CF), a special selective medium (SceSel+) has shown improved rates of isolation as it inhibits the overgrowth by aspergilli[543-545]. Other selective fungal culture media that have been successfully used are the inhibitory mold agar (IMA), and brain heart infusion (BHI) agar[546]. If all three are not available, specimens can be cultured on sabouraud dextrose agar (SDA), or horse blood agar at 30°C or 37°C[468,469]. In contrast to *Scedosporium*, *L. prolificans* is not capable to grow in the presence of cycloheximide[445]. Species identification is achieved by identification by macroscopic and microscopic examination of the colonies. *L. prolificans* is usually characterized by the black color of its colonies, and its characteristic flask-shaped and annellated conidiogenous cells **(Table 8)**, but identification should be confirmed by subsequent ITS gene sequencing[445]. In direct microscopy *L. prolificans* may form pigmented hyphae in infected tissue sections, the organism is therefore classified as a cause of phaeohyphomycosis[13,541].

Recommendations – The guideline group strongly recommends obtaining infected tissue and body fluids for histological evaluation and culture. For respiratory tract samples from CF patients, the guideline group strongly supports the usage of SceScel+, IMA or BHI media.

Diagnosis – Microbiology – Serology

Evidence – Standardized commercial serological tests for the detection of L. *prolificans* are lacking[547]. Heat shock protein 70 and 90, enolase and immunomes (conidial and hyphal proteins/enzymes) reacting with human IgA have been identified in sera as candidate antigens for serodiagnostic tests[548-550] **(Table 8)**.

Recommendations – There is currently no commercial serological test, and in-house tests are only marginally recommended.

Diagnosis – Microbiology – Molecular-based

Evidence – Standardized commercial PCR assays are lacking for the diagnosis of *L. prolificans* infection. Various assay formats (oligoarray, multiplex PCR, PCR+reverse line blot hybridization, multiplex+microarray, pan-fungal PCR + ITS sequencing, and multiplex tandem PCR) have been published from different groups in the field mainly based on the ITS region[337,338,341,344,551-554] **(Table 8)**.

Recommendations – Based on case reports, the use of broad-range PCR with subsequent hybridization or microarray ID is marginally supported as no standardized commercial assay is available.

Diagnosis – Microbiology – Species identification

Evidence – Based on positive cultures, accurate species identification is mainly achieved by morphological identification or ITS sequencing[440,484,495,555-557]. On autopsy material broad-range PCR and subsequent hybridization or microarray identification is used[531,558]. MALDI-TOF MS also may identify *L. prolificans* from culture extract[327] **(Table 8)**.

Recommendations – The guideline group strongly supports obtaining pure cultures for species identification via morphological characteristics, MALDI-TOF MS or ITS sequencing. Also, establishing a diagnosis based on autopsy samples by culture and histopathology/ITS sequencing is strongly recommended.

Microbiology – susceptibility testing

Evidence – *L. prolificans* is a highly drug resistant fungus, sometimes even showing high MIC values for all antifungal agents tested [AmB, itraconazole (ICZ), VCZ, PCZ, TRB, caspofungin (CASPO), micafungin (MICA), and anidulafungin (ANID)], although occasionally lower MICs are observed against VCZ, sometimes PCZ and rarely for more antifungal classes[9,151,469,559,560]. Similar results were found using CLSI[561], EUCAST[559] and Sensititre® YeastOne® YO10 panel (Trek Diagnostic Systems Ltd.) methods[151,560] **(Table 8)**.

Recommendations – The guideline group strongly recommends susceptibility testing of *L. prolificans* to inform susceptibility patterns for epidemiological purposes and moderately for clinical decision making, despite the fact that clinical breakpoints are not available.

Diagnosis - Pathology

Evidence – Histological findings include mostly hyaline hyphae, although the cultures can be dark and hyphae may appear melanized in direct microscopy after KOH treatment, which may be in contrast to *Scedosporium* spp., *Aspergillus* spp., and *Fusarium* spp., which have hyaline hyphae[445]. Calcufluor white staining can provide better sensitivity than KOH. In general, however, *Lomentospora* hyphae may not differ markedly from those of *Scedosporium* spp. Other typical features of *L. prolificans* include irregular branching patterns and adventitious conidiation in tissue. In contrast, other common hyalohyphomycotic molds usually present with regular hyphal septation, and dichotomous branching **(Table 8)**.

Recommendations – The guideline group strongly recommends histopathological examination of biopsy tissue in cases of suspected infection.

Diagnosis – Imaging

Evidence – *L. prolificans* may cause CNS disease, usually during disseminated infection[495,510,536,562,563]. Case reports have outlined imaging procedures for brain, sinuses, lung, abdomen, heart, bones, and disseminated infections[411,446,479,495,510,529,536,562-568]. As with other invasive fungal infections, imaging is important to detect and localize *L. prolificans* infection and guide microbiological sampling of infected tissue and/or body fluids **(Table 8)**.

Table 8. Microbiological, histopathological and imaging diagnostics of *Lomentospora* spp. infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Microscopy, culture, MIC testing						
Any	To diagnose	Direct microscopy	A	III	Tortorano CMI 2014[13]	
					Cortez CMR 2008[541]	
Any	To diagnose and identify species	Culture from blood, species identification by morphological characteristics or ITS sequencing	A	III	Tortorano CMI 2014[13]	
					Cortez CMR 2008[541]	
					Lackner Mycoses 2011[542]	
					Kelly BMCID 2016[495]	N=1
					Penteado TID 2018[516]	
Any	To diagnose	Specimen culture, SDA +/- cycloheximide to differentiate from *Scedosporium*	A	II	Cobo MedMycol 2017[468]	N=7
CF	To diagnose	Culture of respiratory samples; Use SceSel+ selective medium or Inhibitory mold agar or brain heart infusion agar.	A	III	Horre Mycoses 2011[545]	
					Blyth JCM 2010[544]	SceSel medium better than Mycosel, Sabouraud agar, combination SceSel + Sabouraud best
					Hong JCM 2017[546]	Inhibitory mold agar or brain heart infusion agar.
					Sedlacek JCF 2015[543]	>11600 samples, SceSel+ better than standard media. 150 samples, *Scedosporium* recovered on SceSel+, Mycosel, and SDA from 91%, 50%, and 47%
Hematology	To diagnose	Culture of respiratory sample, blood, tissue onto SDA and horse blood agar (30° and 37°C)	A	IIu	Cooley EID 2007[469]	N=28
Any	To inform susceptibility patterns and treatment modalities	CLSI method	A	IIu	Jenks IJAA 2018[384]	6/7 isolates with MICs > 16 µg/mL against VCZ and/or PCZ

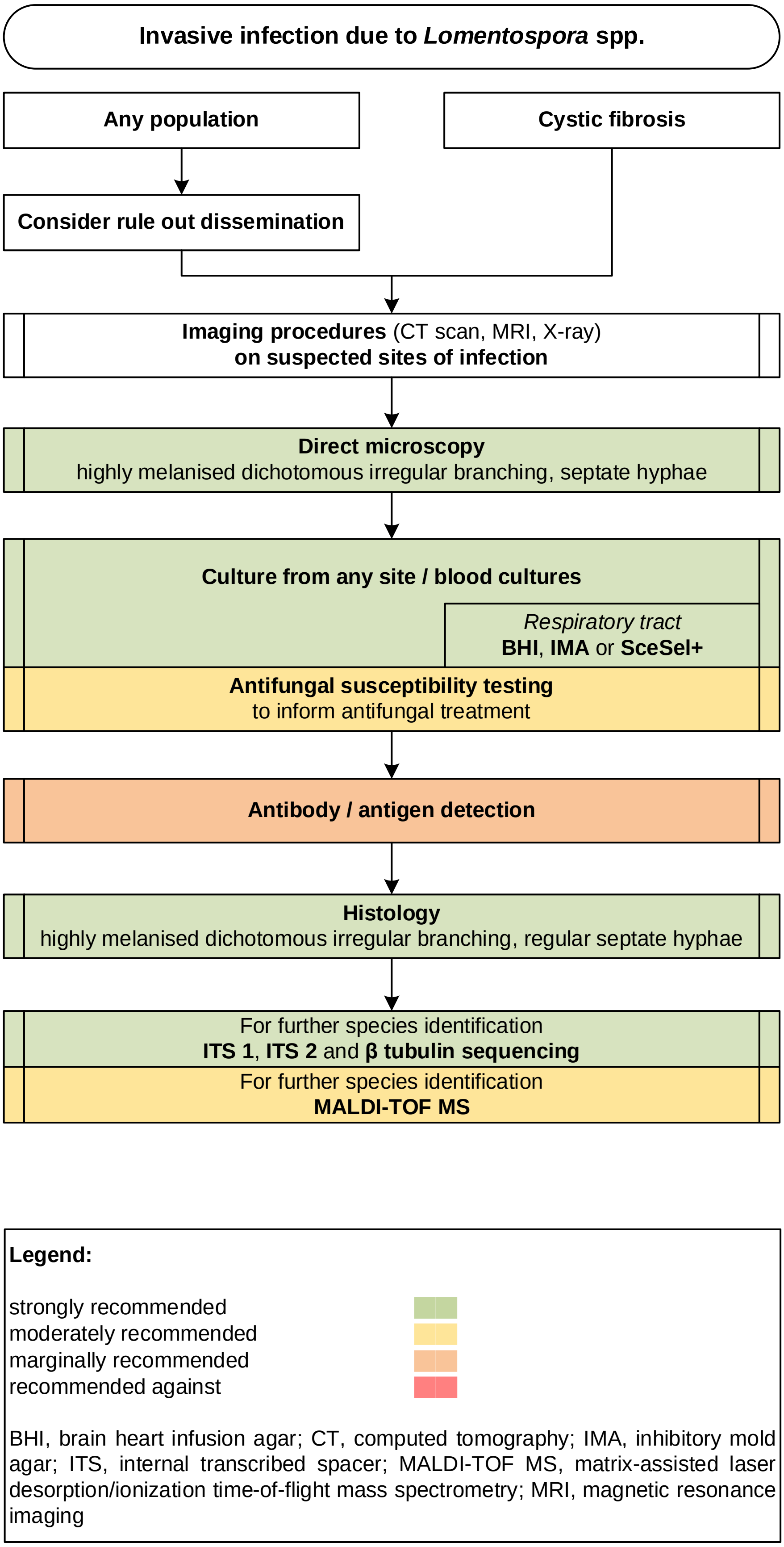
Any	To inform susceptibility patterns and treatment modalities	EUCAST microdilution method	A	IIu	Alastruey-Izquierdo AAC 2018[559]	*L. prolificans* is panresistant
Any	To inform susceptibility patterns	Sensititre® YeastOne® YO10 panels (Trek Diagnostic Systems Ltd.) to test susceptibility	A	III	Halliday IJAA 2016[151]	N=4 isolates, MIC ≥4 mg/L for AmB and echinocandins; ≥8 mg/L for FCZ, ICZ and PCZ; and ≥1 mg/L for VCZ
Any	To guide antifungal treatment	Susceptibility testing with microdilution EUCAST or CLSI method	B	III	Alastruey-Izquierdo AAC 2018[559]	No clinical breakpoints available
Serology assays						
CF or immunocompromise	To diagnose	Antibody detection (Heat shock proteins 70/90, and enolase)	C	III	Pellon JPR 2016[548] Buldain Vaccines 2019[550]	
Oncology	To diagnose and identify	Blood cultures, potato dextrose agar	A	III	Kelly BMCID 2016[495]	
Any	To diagnose and identify	Culture and histopathology	A	III	Holmes MMCR 2013[484]	N=1
					Guadalajara JCN 2018[569]	N=1
All	To identify candidate antigens	Antibody and antigen detection	C	IIII	Pellon FungalBiol 2014[549]	
Nucleic-acid based assays						
Hematology/oncology/ ICU/trauma	To diagnose	Multifungal DNA microarray	C	III	Boch Mycoses 2015[337]	
Any	To diagnose	Panfungal PCR (ITS1 target) + sequencing on fresh tissues or FFPE	C	III	Lau JCM 2007[341]	
Neutropenic patients	To diagnose	Multiplex PCR + DNA microarray hybridization	C	III	Spiess JCM 2007[344]	
Any	To diagnose	Multiplex tandem PCR on blood cultures	C	III	Lau JCM 2008[338]	
CF	To diagnose from sputum	Oligoarray, ITS2	C	III	Bouchara JCM 2009[551]	20 isolates, sens. 100% and spec. 99.2%
CF	To diagnose from sputum	Multiplex PCR, ITS	C	III	Harun JCM 2011[552]	Sens 70%, spec. 99%
CF	To diagnose from sputum	Reverse line blot hybridization after group-specific PCR	C	III	Lu Mycoses 2011[554]	*L. prolificans* in 2/52 CF patients
Any with meningitis	To diagnose and identify	Culture, broad-range PCR, not specified further	C	III	Tamaki TID 2016[531]	
Any	To identify	Culture and ITS sequencing	A	III	Heath CMI 2009[440]	N=49 isolates
					Ziesing MedMycol 2016[555]	*L. prolificans*
					Wangchinda MMCR 2018[556]	
					Penteado TID 2018[516]	N=1
					Elizondo-Zertuche Mycopathol 2017[557]	N=11
Any	To identify	MALDI-TOF MS	B	III	Sitterle CMI 2014[570]	
CF	To identify	ITS sequencing/ microarray	C	III	Schwarz PlOSOne 2017[558]	N=2
Tissue-based diagnosis						
Any	To diagnose	Histopathology of biopsies using *e.g.* KOH treatment or Calcufluor white stain	A	III	Ramirez-Garcia MedMycol 2018[445] Kimura PatholInt 2010 [571]	*L. prolificans* may be melanised, unlike many other hyphomycetes. *Lomentospora* and *Scedosporium* may branch irregularly. Intravascular and intratissue conidiation
Imaging studies						
Any with brain lesions / abscesses	To assess clinical manifestations and imaging characteristics	CT scan of the brain	B	III	Berenguer Medicine 1997[562]	N=3
Any with brain lesions / abscesses	To assess clinical manifestations and imaging characteristics	MRI of the brain	A	III	Kelly BMCID 2016[495]	N=1
Any with sinusitis	To assess clinical manifestations and imaging characteristics	CT scan of the sinuses	A	III	Ochi IJH 2015[510]	N=1
Any with pneumonia	To assess clinical manifestations and	Chest radiography	C	III	Berenguer Medicine 1997[562]	N=12
					Maertens AnnHematol 2000[446]	N=1

	imaging characteristics				Salesa SJID 1993[566]	N=1
					Uno JIC 2014[536]	N=1
					Pickles JInfect 1996[567]	N=1
					Idigoras CID 2001[568]	N=6, disseminated
					Bouza CID 1996[565]	N=1
Any with pneumonia	To assess clinical manifestations and imaging characteristics	CT scan of the lungs	A	III	Berenguer Medicine 1997[562]	N=2
					Maertens AnnHematol 2000[446]	N=1
					Uno JIC 2014[536]	N=1
					Ochi IJH 2015[510]	N=1
					Bouza CID 1996[565]	N=1
Any with abdominal / lymph node infection	To assess clinical manifestations and imaging characteristics	CT scan of the abdomen	A	III	Ochi IJH 2015[510]	N=1
Any with bone infection	To assess clinical manifestations and imaging characteristics	Bone radiography	C	III	Gosbell Mycoses 2003[479]	N=1
					Taj-Aldeen Medicine 2015[564]	N=23
					Pickles JInfect 1996[567]	N=1
Any with bone infection	To assess clinical manifestations and imaging characteristics	CT scan of spine / bones	A	III	Gosbell Mycoses 2003[479]	N=1
					Koehler CRM 2016[411]	
Any with bone infection	To assess clinical manifestations and imaging characteristics	MRI of spine / bones	A	III	Gosbell Mycoses 2003[479]	N=1
					Taj-Aldeen Medicine 2015[564]	N=23
					Koehler CRM 2016[411]	
					Steinbach JCM 2003[529]	N=1
Any with dissemination	To assess clinical manifestations and imaging characteristics	PET/CT	B	III	Kelly BMCID 2016[495]	N=1
Any with endocarditis	To assess clinical manifestations and imaging characteristics	Echocardiogram (preferably transesophageal)	A	III	Uno JIC 2014[536]	N=1
					Kelly BMCID 2016[495]	N=1
					Wakabayashi IntMed 2016[538]	N=1

AmB, amphotericin B; CF, cystic fibrosis; CLSI, Clinical and Laboratory Standards Institute; CT, computed tomography; DNA, deoxyribonucleic acid; EUCAST, European Committee for Antimicrobial Susceptibility Testing; FCZ, fluconazole; FFPE, formalin-fixed paraffin-embedded; HSCT, hematopoietic stem cell transplantation; Hsp, heat shock proteins; ICZ, itraconazole; IgA, immunoglobin A; ITS, internal transcribed spacer; KOH, potassium hydroxide; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; MIC, minimal inhibitory concentration; MRI, magnetic resonance imaging; PET, positron emission tomography; PCR, polymerase chain reaction; PCZ, posaconazole; QoE, quality of evidence; SceSel+, *Scedosporium*-selective medium; SDA, Sabouraud dextrose agar; SoR, strength of recommendation; VCZ, voriconazole.

Recommendations – For the detection and localization of lomentosporiosis and imaging-guided sampling of biopsies and body fluids, the guideline group strongly recommends magnetic resonance imaging (MRI) and CT scan for bones, MRI scan for the brain, transesophageal echocardiogram for the heart, and CT scan of the sinuses, lungs, and abdomen, based on suspected site of infection. The guideline group moderately supports the usage of positron emission tomography (PET) scan for disseminated lomentosporiosis and CT of the brain. Conventional radiography of the bones and the chest is marginally supported **(Figure 8).**

Figure 8. Optimal diagnostic pathway for lomentosporiosis, when all imaging and assay techniques are available



Treatment approaches to lomentosporiosis

Treatment in adults

First-line antifungal monotherapy

Evidence – *L. prolificans* appears to be intrinsically resistant to most antifungals[572,573], with VCZ showing the best *in vitro* activity against this fungus[573]. In several case series, the use of VCZ monotherapy led to the successful treatment of invasive lomentosporiosis in patients with various organ involvement patterns[367,447,468,574], with successful outcomes varying by case series between 25% to 66%. In two case series, outcome with AmB monotherapy was inferior to VCZ monotherapy, with survival in 2/13 patients receiving AmB monotherapy in one case series[447] and 0/4 patients successfully treated in another case series[367]. ISA monotherapy was effective in one case report in a patient with interstitial pulmonary disease[407] and miltefosine was effective in a patient with disseminated lomentosporiosis and an azole drug-drug interaction[535] **(Table 9)**.

Table 9. First-line antifungal therapy for *Lomentospora spp.* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To cure	VCZ iv + TRB +/- other antifungals	A	IIu	Jenks CMI 2020[575]	N=40, VCZ + TRB combination (+/- other antifungals) 10/16 (63%) success, other treatments 7/24 (29%) success
					Seidel CRM 2019[11]	N=56, mortality with VCZ 52.6% vs. therapy w/o VCZ 68.8%, mortality with VCZ mono 50% or in combination 55.3%
					Jenks IJAA 2018[384]	N=6, VCZ + TRB 3/3 survived vs. VCZ or L-AmB 0/3 survived
					Wangchinda MMCR 2018[556]	N=1
Any	To cure	VCZ + either L-AmB OR MICA	B	III	Jenks IJAA 2018[384]	N=2, 1/2 survived
					Jenks CID 2020[575]	N=8, response 2/8 (25%)
					Seidel CRM 2019[11]	N=17 7/17 (41%) survived
					Tamaki TID 2016[531]	N=1, failure
Any	To cure	VCZ iv bid	B	IIu	Cobo MedMycol 2017[468]	N=5, success 2/4
					Troke AAC 2008[574]	N=36, success 16/36 (44%)
					Hussain CID 2005[447]	N=18, VCZ 2/3 survived vs. AmB 2/13 survived
					Jenks CID 2020[575]	N=7, success 3/7; breakthrough lomentosporiosis in N=6 with VCZ prophylaxis
Any	To cure	L-AmB	D	III	Jenks CID 2020[575]	N=15, L-AmB mono 0/4 success vs. L-AmB combination 3/11 success
Interstitial pulmonary disease	To cure	ISA	C	III	Marty Mycoses 2018[407]	N=1, success
Hematological malignancy patients	To cure	VCZ + TRB	A	III	Cooley EID 2007[469]	N=7, VCZ plus TRB 2/4 survived vs. ICZ plus TRB or AmB 0/3 survived
CF with lung infection	To cure	VCZ + either MICA iv, TRB po OR AmB inhaled	C	III	Schwarz JCF 2018[456]	N=3, achieved improvement but no eradication

Standard dose unless stated otherwise; AmB, amphotericin B; bid, twice a day; d, day(s); FEV1, forced expiratory volume in one second; HSCT, hematopoietic stem cell transplantation; ICZ, itraconazole; ISA, isavuconazole; iv, intravenous; L-AmB, liposomal amphotericin B; MICA, micafungin; po, orally; qd, once a day; QoE, quality of evidence; TDM, therapeutic drug monitoring; tid, three times a day; SoR, strength of recommendation; SOT, solid organ transplants; TRB, Terbinafine; VCZ, voriconazole.

Recommendations – While combination antifungal therapy is the preferred option (see next paragraph), the guideline group moderately supports first-line treatment with VCZ monotherapy specifically in those who are more immunocompetent and have localized infection. Given superior outcomes seen with other antifungal treatment strategies, monotherapy with L-AmB is not recommended. Although treatment success was reported in one case report with miltefosine monotherapy, more data are needed before recommending this option. There is no evidence supporting other first-line monotherapy regimens.

First-line antifungal combination therapy

Evidence – In the largest case series of lomentosporiosis infections published to date combination antifungal therapy was associated with increased 28-day survival (15/24 survived vs. 4/16 receiving monotherapy)[367]. *In vitro* synergism has been demonstrated with combinations of AmB plus MICA[576], AmB plus pentamidine[577], colistin plus VCZ[578] and particularly VCZ plus TRB [572,579,580]. In several case reports and case series, combination antifungal therapy successfully treated lomentosporiosis with various organ involvement patterns and mixed underlying disease, particularly with VCZ (intravenous 6 mg twice daily loading dose followed by 4 mg twice daily) plus TRB (500 mg daily), plus or minus other antifungals[486]. In one case report, VCZ plus TRB and surgical debridement resulted in suppression of *L. prolificans* osteomyelitis in an immunocompetent woman[556] and in a small case series, 3/3 patients treated with VCZ plus TRB combination therapy survived[162]. In two larger case series, 8/18 (45%) individuals treated with VCZ plus TRB combination therapy were alive at Day 42[11] and 10/16 (63%) who were treated with VCZ plus TRB combination therapy plus or minus other antifungals were alive at Day 28 in another case series[367]; in the latter case series, survival at 84 and 360 days was significantly higher in those who received VCZ plus TRB combination therapy plus or minus other antifungals compared to those receiving other antifungal therapies[367]. Combination therapy with VCZ plus either AmB or MICA has resulted in treatment response and survival in patients with mixed underlying disease in several case series[11,162,367], although outcomes did not differ compared to those treated with VCZ plus TRB combination therapy plus or minus other antifungals. In patients with hematological malignancy in one case series, 2/4 (50%) who were treated with VCZ plus TRB combination therapy survived, while 0/3 survived who received ICZ plus TRB or AmB[469]. In a case series of three patients with CF, combination therapy with VCZ plus MICA, TRB, or inhaled AmB resulted in clinical improvement but not in eradication of the fungus[456]. Surgery as adjunct treatment has been shown to be significantly associated with survival[367]. Resection of surgically amenable lesions is an important adjunct to management of infections caused by *L. prolificans*[581]. Correction of underlying immune deficiencies is also an important adjunct to antifungal therapy.

Recommendations – The guideline group strongly supports first-line VCZ-based combination antifungal therapy for treatment of infections caused by *L. prolificans*, particularly VCZ plus TRB plus or minus other antifungal agents. Combination therapy with VCZ plus either L-AmB or MICA is moderately supported. In patients with hematological malignancy, combination therapy with VCZ plus TRB plus or minus other antifungal agents is also strongly recommended. In patients with CF, combination therapy with VCZ plus MICA, TRB, or inhaled AmB is marginally supported, as available data are too limited to give stronger support to this strategy. There is little evidence supporting other first-line combination therapy regimens.

Other treatment options

Evidence – Surgery in general and surgical debridement has been associated with improved treatment response[367,441]. **(Table 10)**.

Table 10. Other treatment options for *Lomentospora spp.* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Hematopoietic stem cell transplant patients	To cure	VCZ as secondary prophylaxis	C	III	Penteado TID 2018[516]	N=1, failure
Immunocompetent with localized infection	To cure	Surgical debridement	A	III	Wangchinda MMCR 2018[556]	N=1
					Masukane IntMed 2017[582]	N=1, success
Hematological malignancy	To cure in context azole drug-drug interactions	Miltefosine	C	III	Trubiano Mycoses 2014[535]	N=1, success
All	To cure	Surgery (debridement, enucleation, vitrectomy)	A	II	Rodriguez-Tudela MedMycol 2009[441]	N=169, survival associated with surgery
					Jenks CMI 2020[575]	N=7, survival associated with surgery

QoE, quality of evidence; SoR, strength of recommendation, VCZ, voriconazole.

Recommendations – The guideline group strongly recommends the use of surgical debridement where applicable.

Antifungal salvage treatment

Evidence – VCZ monotherapy with TDM was effective in one large case series of 36 patients when VCZ was used for compassionate use or salvage therapy[574]. In a small case series of two patients, both VCZ plus AmB plus PCZ, and TRB plus AmB plus PCZ combinations led to treatment response in both patients[367]. Miltefosine was effective in one case report in a patient with disseminated *L. prolificans* infection and an azole drug-drug interaction[535] **(Table 11)**.

Table 11. Antifungal salvage treatment for *Lomentospora spp.* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To cure	VCZ + TDM	B	IIu	Troke AAC 2008[574]	N=36, 44% success
Any	To cure	PCZ + L-AmB + either VCZ OR TRB	C	III	Jenks CID 2020[575]	N=2, response 2/2

Standard dose unless stated otherwise; L-AmB, liposomal amphotericin B; PCZ, posaconazole; QoE, quality of evidence; SoR, strength of recommendation; TDM, therapeutic drug monitoring; TRB, terbinafine; VCZ, voriconazole.

Recommendations – Although there is limited evidence to support a specific regimen to be used as salvage therapy for invasive lomentosporiosis, the guideline group recommends the use of combination antifungal therapy that should be tailored based on prior antifungal treatment and to the individual patient. VCZ is moderately recommended.

Treatment duration of lomentosporiosis

Evidence – Extended duration of antifungal treatment has been associated with treatment success and/or survival in multiple case reports and case series. In two case series of patients with various underlying diseases and organ involvement patterns, patients who survived received VCZ plus TRB for at least 180 days[367] and antifungal treatment for three to six months in another case series[162]. In one case report of an immunocompetent patient with vertebral osteomyelitis, surgical debridement plus VCZ plus TRB for 180 days resulted in clinical improvement and this patient was maintained on suppressive therapy[535]. In a literature review of immunocompromised adults and children with osteomyelitis, patients were treated for a mean duration of 115 days with a treatment response in 86% of patients[564]. In a multi-center study of CF patients and lomentosporiosis, mean duration of combination therapy was 3.9 months **(Table 12)**[456].

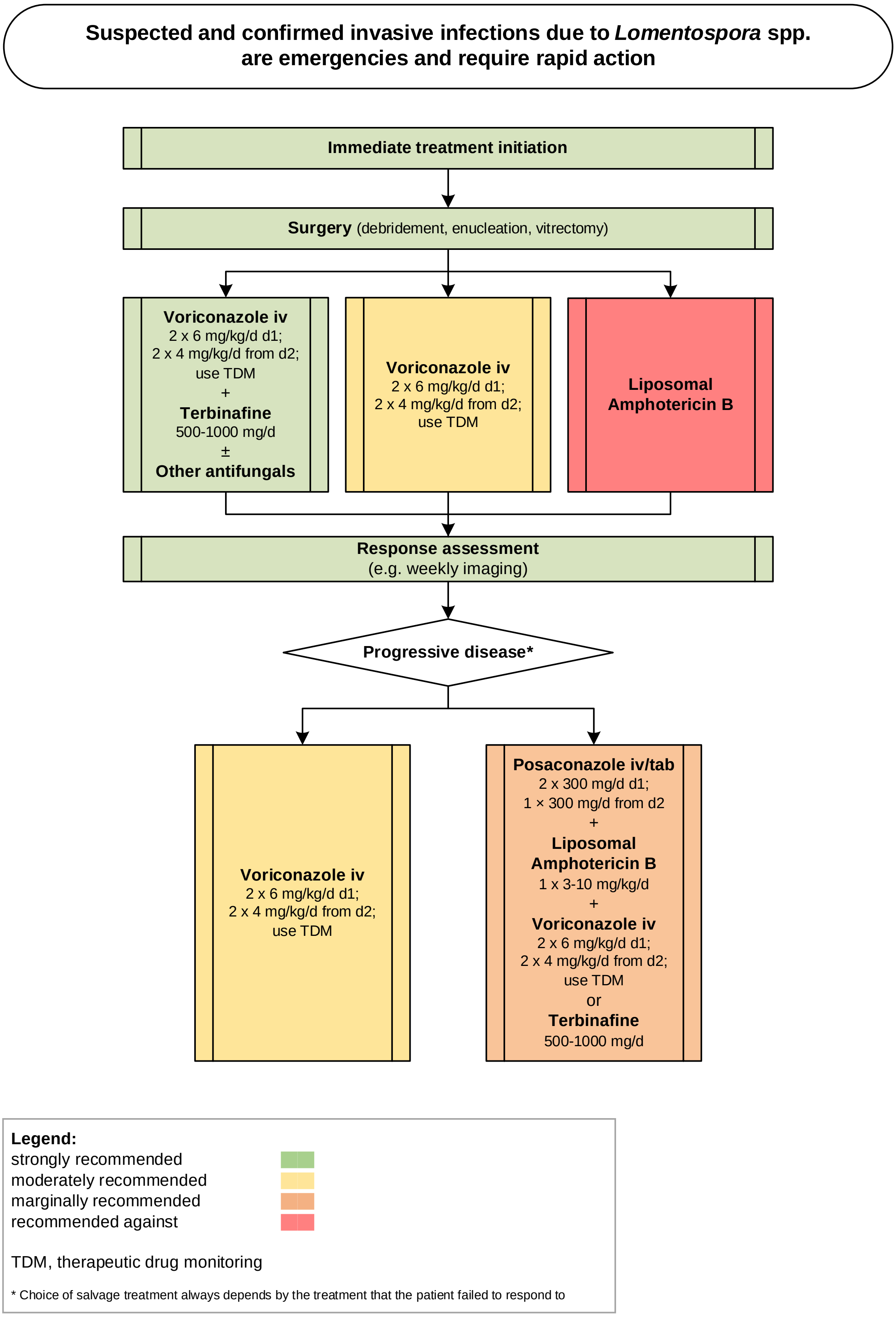
Table 12. Treatment duration for *Lomentospora spp.* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To cure	181 d of therapy with VCZ + TRB	B	III	Jenks CID 2020[575]	IQR 69-332 d
Adults and pediatric immunocompromised patients with osteomyelitis	To cure	Mean duration of 115 d (5 d-730 d) of combined treatment	B	IIr	Taj-Aldeen Medicine 2015[564]	Systematic literature review
Immunocompetent with osteomyelitis	To cure	>180 d of therapy with VCZ and TRB	C	II	Wangchinda MMCR 2018[556]	Case report and literature review
CF patients	To cure	1-14 mo of therapy, mean duration 3.9 months	B	II	Schwarz JCF 2019[456]	N=31, AmB 25 mg qd by inhalation + iv + VCZ 40 mg qd by inhalation + iv
Any	To cure	3-6 mo of therapy	B	II	Jenks IJAA 2018[384]	N=7

Standard dose unless stated otherwise; bid, twice a day; CF, cystic fibrosis; d, days; IQR, interquartile range; iv, intravenous; mo, month(s); qd, once a day; QoE, quality of evidence; SoR, strength of recommendation; TRB, terbinafine; VCZ, voriconazole.

Recommendations – Extended durations of antifungal therapy have been associated with improved outcomes and survival, although no evidence exists to support a pre-specified duration of therapy. It is reasonable to tailor the duration of therapy to the individual patient and consider continuing antifungal therapy until immunological recovery and resolution of all clinical evidence of disease, if possible. A duration of at least 4 to 6 months of combination therapy has been most associated with positive outcomes and thus is moderately recommended **(Figure 9)**.

Figure 9. Optimal treatment pathway for lomentosporiosis in adults when all treatment modalities and antifungal drugs are available



Specific considerations on treatment of lomentosporiosis in children

Evidence – Published case reports and case series show poor outcomes from pneumonia and fungemia caused by *L. prolificans* in immunocompromised children[503,516,583,584]. A larger case series of osteoarticular infections caused by non-*Aspergillus* molds showed a higher incidence of *L. prolificans* bone and joint infections in children compared to adults (35% vs 10%)[564]. In addition, direct inoculation was the main mechanism of infection in 73.5% in children compared to 43.5% in adults[564]. In a recent review of invasive *Lomentospora* (n=22) and *Scedosporium* (n=33) infections in children, surgery and VCZ treatment were associated with improved clinical outcome[584] **(Table 13)**.

Table 13. First-line antifungal therapy for *Lomentospora spp.* infections in children

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To cure	VCZ + other antifungals + surgery for localized infections	A	III	Seidel IJID 2019[585]	N=22
Neuroblastoma	To cure	AmB lipid-based formulations	C	III	Sparrow PHO 1992[583]	N=1, 2.6 yrs, died
Hematological malignancy	To cure	L-AmB 12 mg/kg qd	C	III	Penteado TID 2018[516]	N=1, 14 yrs, failure
					de Lucas EuRadiol 2006[503]	N=1, 18 yrs, died
Any with osteoarticular infections	To cure	Surgery	B	II	Taj-Aldeen Medicine 2015[564]	N=12, mostly trauma/puncture wounds; 9/12 (75%) complete response

Standard pediatric dose unless stated otherwise; AmB, amphotericin B; ICZ, itraconazole; L-AmB, liposomal amphotericin B; qd; once a day; QoE, quality of evidence; SoR, strength of recommendation; VCZ, voriconazole; yrs, years.

Recommendation - Treatment recommendations follow those given for adults, with VCZ (+ TDM) being the backbone of therapy, with improved outcomes reported when combined with TRB, L-AmB or MICA (strong recommendation). Surgery plays a major role and is strongly recommended in the treatment of localized infections.

3. Scedosporiosis

Epidemiology of scedosporiosis

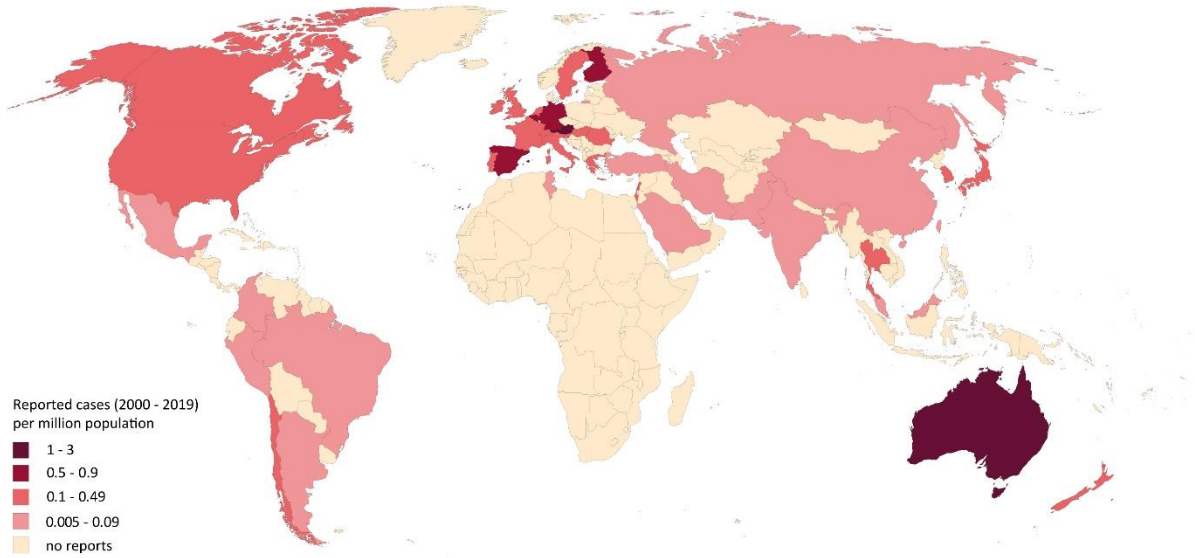
Scedosporium spp. are ubiquitous saprophytes mostly found in temperate areas, with regional differences in species distribution[586,587]. In the clinical setting, the most commonly isolated species are *Scedosporium boydii* and *Scedosporium apiospermum*. *Scedosporium aurantiacum* is isolated to a lesser extent mainly in Australia and Europe[440,445,451,588-590]. Only few cases of infections caused by *Scedosporium dehoogii* have been reported[591-594].

Scedosporium spp. initiates two distinct diseases: mycetoma and scedosporiosis. *Scedosporium* spp. are an important cause of eumycotic mycetoma and the most common cause of this infection in the United States[595]. *Scedosporium* mycetoma usually develops in immunocompetent patients. SOT and treatment for hematological disease are major risk factors for scedosporiosis. Patients predominantly present with pulmonary, cutaneous or cerebral infections[11,445]. Secondary CNS infections may appear without an evident dissemination. Infection may also affect the paranasal sinuses or bones[11,445].

Scedosporium spp. have been recovered from respiratory secretions of patients with chronic pulmonary conditions such as CF, ranking as the second most frequently isolated fungal pathogen after *Aspergillus* spp.[440,456,596]. The significance of *Scedosporium* in this setting is uncertain but may be the first step towards invasive disease[543,590]. Colonization has also been described in cancer patients[444]. Surgery, intravenous drug injection, and repeated corticosteroid injections have also been associated with localized infections. The main route of entry of the pathogen in immunocompetent patients is traumatic inoculation or aspiration of contaminated water. Near drowning-, tsunami-, and earthquake-victims represent a high risk group for developing scedosporiosis[597-600]. Near drowning has been associated with cerebral infection caused by *S. apiospermum* that results from hematogenous spread from the lungs as the primary site of infection[448-455]. CNS infection related to near drowning events caused by *Scedosporium* spp. may also arise from penetration through the cribriform plate with direct invasion of the CNS. Eye infections after traumatic injuries are also common[66,601-605]. Other affected body sides include the spine[448,451,454].

In a US study, *S. apiospermum* accounted for 6% of mold infections and 19% of non-*Aspergillus* infections identified in liver and heart transplant recipients[442]. The incidence of scedosporiosis was 0.93 per 100,000 patient-inpatient days, with a noted increase from 1993 to 2005 in a US cancer center[444]. In Australia, among 137 patients who were monitored for a median of 4 years post-lung transplantation, 13 had fungal infection and 3 of these were caused by *S. apiospermum*[596]. One percent of patients with lung transplantation developed infections caused by *S. apiospermum*[606] **(Figure 10)**.

Figure 10. Worldwide distribution of scedosporiosis (reported cases between 2000 and 2019 per million population)



Cases of severe *Scedosporium*-related infections reported in the medical literature were identified in a PubMed search on November 15, 2019 using the search string "Scedospori* OR Pseudallescheri* OR Lomentospori*" that yielded 1,628 publications. In total, 541 cases were identified from 43 countries[1,82,97,448-453,460,469,473,485,492,514,563,588,589,592-594,596,597,599-601,604,607-83811,300,454,456,524,585,594,598,602,603,605,831-898]. Most cases were reported from the United States (n=146), Australia (n=73), Germany (n=60), India (n=58), Spain (n=41), and Japan (n=28). Australia and Austria (n=10) reported most of the cases per million population. Number of cases reported between 2000 and 2019 are presented as cases per million population per country. The resident population per country was obtained from www.worldometers.info[321].

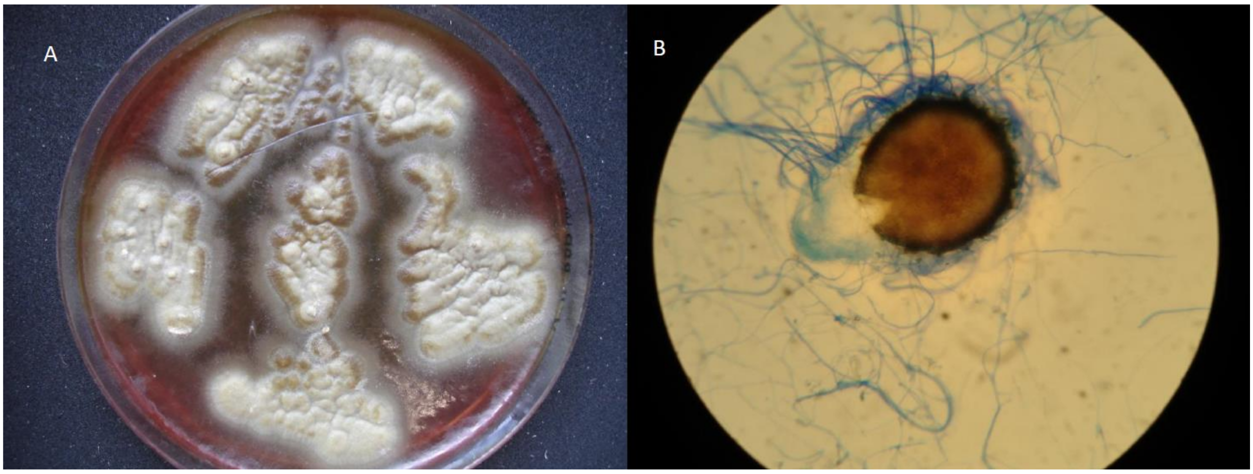
Diagnosis of Scedosporiosis

Diagnosis – Microbiology – Conventional Methods

Evidence – Definitive diagnosis of scedosporiosis is based on culture of the pathogen from infected tissue samples and body fluids from sterile body regions or from blood [13,495,541,542,599,671,760,846,879]. Direct microscopy and histopathology of clinical specimens is important for the diagnosis of a hyalohyphomycosis, while further discrimination based on microscopy is rarely possible[13,462,541,899]. Branching patterns of

Scedosporium spp. often resemble *Aspergillus* spp., with sometimes dichotomously branching septate hyphae seen in tissue, although branching off to the side at a 60° to 70° angle, which is different than the 45° angle seen with *Aspergillus* spp.. In addition, distinctive coremia or an ascocarp as well the presence of pyriform adventitious conidia may indicate *Scedosporium* spp. as the mold. After a few days, the mold colony takes on a tan color and has sporulating structures that differ from *Aspergillu*s spp. See also **Figure 11** for microbiological characteristics.

Figure 11. Microbiological characteristics of Pseudallescheria state of *S. boydii* (owned by co-author V. Arsic-Arsenjevic)



Pseudallescheria state of *S. boydii* growth on blood agar, B fully developed and ruptured cleistothecium, the hallmark of the sexual stage (teleomorph) of this fungus.

Based on >11,600 respiratory tract samples from CF patients, the selective medium *Scedosporium* Selective agar (SceSel+) showed higher isolation rates than the standard medium[543]. Blyth *et al.* found a 90.6% isolation rate for SceSel+ compared with 50% Mycosel and 46.9% for Sabouraud dextrose agar[544]. Other selective fungal culture media that have been successfully used are the inhibitory mold agar (IMA), and brain heart infusion (BHI) agar[546]. Species identification of cultures is achieved by subsequent ITS sequencing[13,495,541,542,599,671,760,846,879] or by macroscopic and microscopic examination of the colonies **(Table 14)**.

Recommendations – The guideline group strongly recommends obtaining infected tissue and body fluids for histological evaluation, direct microscopy, and culture. For respiratory tract samples from CF patients, the guideline group strongly supports the use of selective fungal culture media like SceScel+, IMA or BHI agar. Based on pure cultures, members of the genus *Scedosporium* can rarely be identified by morphology alone.

Diagnosis – Microbiology – Serology

Evidence – Standardized commercial serological tests for the detection of *Scedosporium* spp. infection are lacking[547]. For CF patients, an ELISA test is under development that is based on the detection of mycelial catalase A1 of the *S. apiospermum* complex[900] **(Table 14)**.

Recommendations – There is currently no commercial serological test available.

Diagnosis – Microbiology – Molecular-based

Evidence – Standardized commercial PCR assays are lacking for the diagnosis of scedosporiosis. Various assay formats (oligoarray, multiplex PCR, PCR + reverse line blot hybridization, multiplex + microarray, pan-fungal PCR + ITS sequencing) have been published from different groups in the field mainly based on the ITS region[551-553,901]. The assays from Harun *et al.* [552] and Lu *et al.*[553] aim to discriminate all *Scedosporium* spp. plus *L. prolificans*, while the assays from Bouchara *et al.*[551] and Nagano *et al.*[901] focus on the identification of the *S. apiospermum* complex. All assays have been evaluated on respiratory tract samples of CF patients. Highest sensitivity (100%) and specificity (99.2%) were found for the oligonucleotide array published by Bouchara *et al.* [551] **(Table 14)**.

Recommendations – The guideline group moderately supports the use of the oligonucleotide array published by Bouchara *et al.* [551] for the detection of *S. apiospermum* complex in the sputum of CF patients, and marginally recommends other methods. Future studies are needed to evaluate these tests outside the CF setting.

Diagnosis – Microbiology – Species identification

Evidence – Based on positive cultures, species complex identification is mainly achieved by morphological identification or ITS sequencing. For the identification to the species level, sequencing of both ITS and β-tubulin is required[555,902]. Alternative approaches are: loop-mediated isothermal amplification (LAMP), quantitative real time PCR (qPCR), PCR-based reverse line blot hybridization (PCR-RLB), rolling circle amplification (RCA), repetitive sequence PCR and PCR-ESI-TOF MS, multiplexed PCR and liquid-phase array that allow identification[553,903,904]. Identification and genotyping can be done by repetitive sequence-based PCR[905]. MALDI-TOF MS has been shown to be a reliable method for the identification to the genus level[570] **(Table 14)**.

Recommendations – The guideline group strongly recommends species identification of pure cultures using ITS1-ITS2 and β-tubulin sequencing, and marginally supports identification via alternative molecular methods.

Microbiology – Susceptibility testing

Evidence – *Scedosporium* spp. exhibit high MIC values for AmB, ISAV, ICZ, and fluconazole[906]. Lowest MIC values are found for VCZ, followed by PCZ and the echinocandins (ANID, CASPO, MICA)[151,559,907-909]. Similar results were found using CLSI[561], EUCAST testing[559] and Sensititre® YeastOne® YO10 panels (Trek Diagnostic Systems Ltd.) [151]. All studies found that VCZ was the most effective drug *in vitro* **(Table 14)**.

Recommendations – The guideline group strongly recommends susceptibility testing of *Scedosporium* spp. using Sensititre® YeastOne® YO10 panels (Trek Diagnostic Systems Ltd.), EUCAST or CLSI methodology to inform susceptibility patterns and moderately for clinical decision making, given the fact that clinical breakpoints are not available.

Diagnosis - Pathology

Evidence – Fresh tissue microscopy with KOH treatment in the microbiology laboratory shows hyaline hyphae similar to the hyphae of *Aspergillus* spp., *Fusarium* spp.and other hyalohyphomycetes. Discrimination from other hyalohyphomycetes is therefore difficult, even though *Scedosporium* spp. may show some morphological features in histological findings with H&E or GMS stains such as irregular branching patterns, vascular invasion, and/or intra-tissue conidiation **(Table 14)**.

Recommendations – The guideline group strongly recommends histopathology in the diagnosis of disease, and moderately recommends direct microscopy of biopsies using KOH treatment.

Diagnosis – Imaging

Evidence – Best imaging modality depends on the site of infection[411,910]. In case of suspected bone, spine, and joint infections MRI represents the method of choice for diagnosis of scedosporiosis, with fluorodeoxyglucose (PET)-CT being a suitable alternative imaging modality[411,454,910]. For detection of brain involvement (*e.g.* CNS abscess formations), MRI and, if MRI was not available, CT have been used successfully[448,451,452,597-599]. In CF patients, near drowning victims, and victims of natural disasters, who are at risk for developing pulmonary manifestations of scedosporiosis, chest CT is the imaging modality of choice, while for differentiating colonization from infection in CF patient thorax CT or chest radiograph have been used to detect the abundance of pulmonary infiltrates[445 452] **(Table 14)**.

Table 14. Microbiological, histopathological and imaging diagnostics for *Scedosporium* spp. infections

Population	Intention	Approach	SoR	QoE	Reference	Comment
Microscopy, culture, MIC testing						
Any	To diagnose	Direct microscopy	A	III	Tortorano CMI 2014[13]	
					Cortez CMR 2008[541]	
Any	To diagnose	Culture (species identification by ITS sequencing)	A	III	Tortorano CMI 2014[13]	
					Cortez CMR 2008[541]	
					Lackner Mycoses 2011[542]	
					Denton MMCR 2016[671]	
					Sharma SJKDT 2015[846]	
					Leek CRT 2016[599]	
					Kelly BMCID 2016[495]	Blood culture
					Torres-Sánchez TransProceed 2018[879]	CSF culture
					Loh Cureus 2018[760]	Tissue culture
Any with CF	To diagnose	Culture of respiratory tracts samples on selective media (SceSel+ , inhibitory mold agar or brain heart infusion agar)	A	III	Sedlacek JCF 2015[543]	>11,600 samples, from 2,346 cases, benefit of SceSel+ compared to standard media documented for N=5,000 samples. 150 samples from 42 cases, Scedosporium recovered on SceSel+, Mycosel, and SABD from 90.6%, 50.0%, and 46.9%
					Horré Mycoses 2011[545]	
					Hong JCM 2017[546]	Inhibitory mold agar or brain heart infusion agar.
					Blyth JCM 2010[544]	
Any	To diagnose	Culture and histopathology	A	III	Balandin MMCR 2016[462]	
					Tammer IJID 2011[899]	

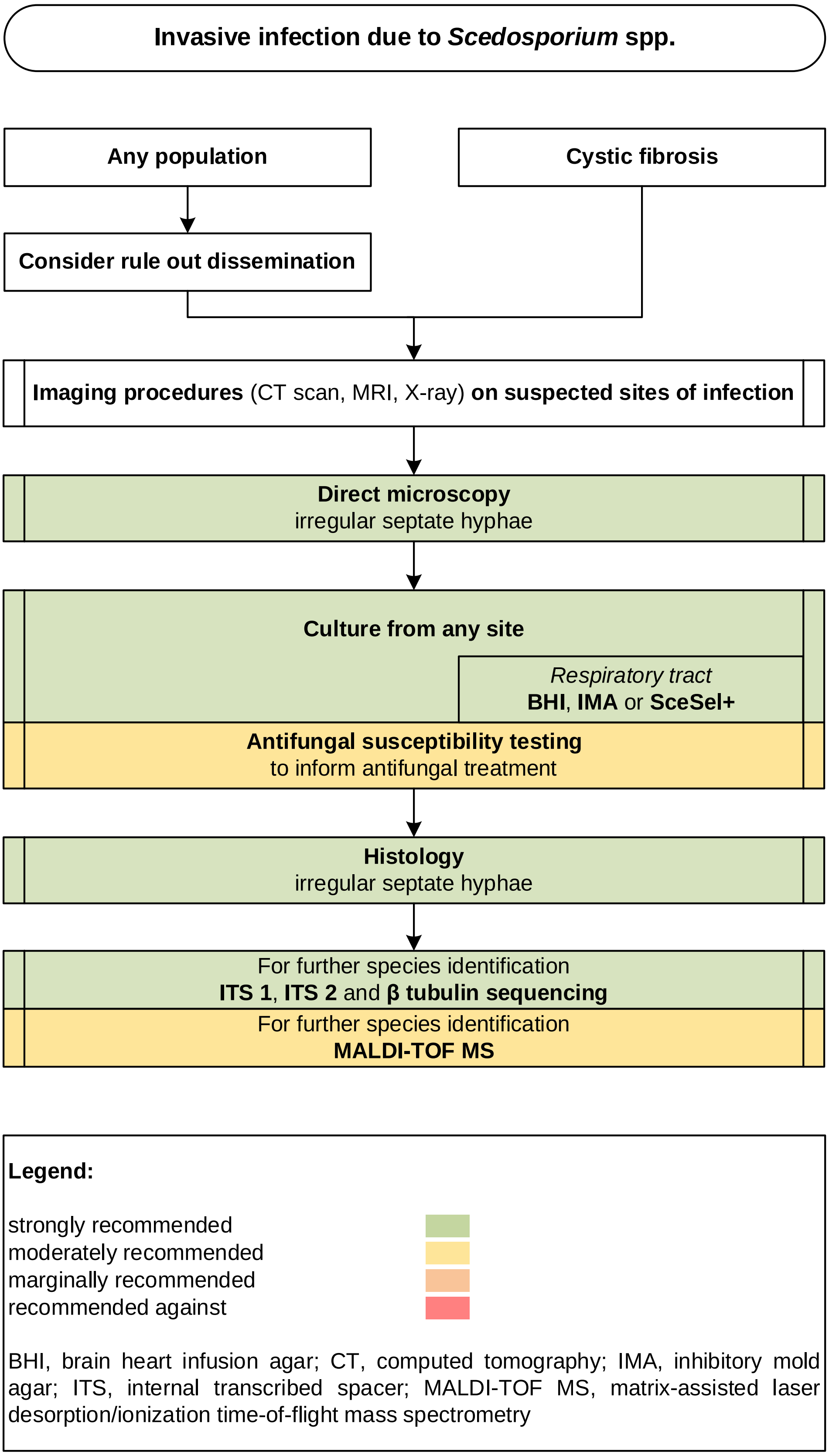
Any	To determine susceptibility	Susceptibility testing with microdilution EUCAST method	A	III	Alastruey-Izquierdo AAC 2018[559]	Most active compound VCZ with MIC50=1 mg/L, followed by echinocandins
Any	To determine susceptibility	Susceptibility testing with CLSI M38-A2 method	A	III	Espinel-Ingroff AJCM 2005[909]	No clinical breakpoints available
Any	To determine susceptibility of *S. apiospermum*	Susceptibility testing with Sensititre® YeastOne® YO10 test (TREK Diagnostic Systems Ltd)	A	IIu	Halliday IJAA 2016[151]	N=14 isolates, VCZ most active antifungal
Any	To inform antifungal treatment	Susceptibility testing with microdilution EUCAST or CLSI method	B	III	Alastruey-Izquierdo AAC 2018[559]	No clinical breakpoints available
Serology assays						
Any with CF	To diagnose	Detection by ELISA of antibodies to mycelial catalase	C	III	Mina CVI 2015[900]	Catalase A1 might be a good candidate for the development of an immunoassay for serodiagnosis of infections caused by the *S. apiospermum* complex in patients with CF. Test not commercially available
Nucleic-acid based assays						
Any with CF	To detect *S. boydii / S. apiospermum*	Oligoarray, with ITS region amplicons hybridized to the array for species identification	B	III	Bouchara JCM 2009[551]	N=57, sputum samples from 39 cases; *S. apiospermum* detected in 16/57 samples vs. 12/57 by culture
Any with CF	To detect *Scedosporium* species and *L. prolificans*	Reverse line blot (RLB) hybridization after group-specific PCR	C	III	Lu Mycoses 2011[554]	N=59, sputum samples from 52 cases; 62.7% of samples were positive by RLB vs. 8.5% by culture
Any with CF	To detect fungal species including *S. apiospermum*	PCR targeting the 18S-ITS1-5.8S-ITS2-28S rRNA gene plus nested PCR	C	III	Nagano MedMycol 2010[901]	N=77, sputum samples from 77 cases; *S. apiospermum* detected in 3/77 samples vs. 2/77 by selective culture
Any with CF	To detect and identify *Scedosporium* spp. and *L. prolificans*	Multiplex PCR, followed by RFLP analysis of ITS region	C	III	Harun JCM 2011[552]	208 sputum samples from 69 cases; Sens. 62%, spec. 97%
Any	To identify	ITS1-ITS2 + beta-tubulin sequencing	A	III	Hedayati MicPath 2019[902]	*S. boydii* in 2/90 and *Scedosporium ellipsoideum* in 1/90 CF patients
Any	To identify	Culture + ITS1-ITS2/beta-tubulin sequencing	A	III	Ziesing MedMycol 2016[555]	*S. apiospermum/ Scedosporium boydii* in 2-3% CF patients, *S. aurantiacum, S. minutisporum* sporadically (N=3,186, over 5-yr period)
Any	To identify	MALDI-TOF MS	B	III	Sitterle CMI 2014[570]	
Any with CF	To identify	ITS sequencing/ microarray	C	III	Schwarz PlOSOne 2017[558]	*S. boydii, S. apiospermum, S. aurantiacum*
Any with CF	To identify and genotype	Repetitive sequence-based PCR	C	III	Matray MedMycol 2016[905]	*S. boydii, S. apiospermum, S. minutisporum, S. aurantiacum, S. ellipsoideum*
Tissue based diagnosis						
Any	Species identification	Direct microscopy of biopsies using KOH treatment	B	III	Ramirez-Garcia MedMycol 2018[445] Kimura PatholInt 2010 [571]	Difficult to distinguish *Scedosporium*-infected tissues from those infected by *Aspergillus* or *Fusarium*, as all of them present hyaline hyphae, regular hyphal septation, and sometimes dichotomous branching. Unique features such as irregular branching patterns or intravascular invasion and intratissue conidiation may help pathologists to diagnose *Scedosporium* mycoses.
Any	To diagnose mold infection	Histopathology of biopsies	A	III	Walts DiagnCyto 2001[911]	
Imaging studies						
Any	To diagnose bone/joint infections	MRI	A	III	Koehler CRM 2014[411] Zimmerli NEJM 2010[910]	
Any		FDG-PET/CT	A	III	Koehler CRM 2014[411]	If MRI is not possible

	To diagnose bone/joint infections				Zimmerli NEJM 2010[910]	
Any with brain lesions / abscesses	To assess clinical manifestations and imaging characteristics	CT scan of the brain	B	IIIt	Berenguer Medicine 1997[562]	N=3
					Uno JIC 2014[536]	N=1
					McKelvie CEO 2001[563]	N=1
Any with brain lesions / abscesses	To assess clinical manifestations and imaging characteristics	MRI of the brain	A	IIIt	Kelly BMCID 2016[495]	N=1
					Ochi IJH 2015[510]	N=1
Any	To diagnose pulmonary infection	CT Thorax	A	III	Nakamura JMCR 2011[452]	
Any	To diagnose pulmonary infection	Chest X-ray	B	III	Nakamura JMCR 2011[452]	
					Ramirez-Garcia MedMycol 2018[445]	
Any with CF	To differentiate colonization from infection	CT Thorax	A	III	Ramirez-Garcia MedMycol2018[445]	

CF, cystic fibrosis; CLSI, Clinical and Laboratory Standards Institute; CSF, cerebrospinal fluid; CT, computed tomography; ELISA, Enzyme-linked Immunosorbent Assay; EUCAST, European Committee for Antimicrobial Susceptibility Testing; FDG, fluorodeoxyglucose; IFD, invasive fungal disease; ITS, internal transcribed spacer; KOH, potassium hydroxide; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; MEC, minimum effective concentration; MIC, minimal inhibitory concentration; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; PET, positron emission tomography; QoE, quality of evidence; RFLP, restriction fragment length polymorphism; RLB, reverse line blot; rRNA, ribosomal ribonucleic acid; SABD Sabouraud Dextrose Agar; SceSel+, *Scedosporium*-selective medium; SoR, strength of recommendation; VCZ, voriconazole; yr, year.

Recommendations – For all patients, the guideline group strongly recommends the use of MRI for the detection of brain abscesses and moderately recommends the use of contrast enhanced CT of the brain, when MRI is not available. For the detection of pulmonary infection, chest CT is strongly supported. For the detection and localization of scedosporiosis and the guided sampling of biopsies and body fluids, the guideline group strongly recommends MRI for the localization of scedosporiosis in bones and/or joints, or FDG-PET CT if MRI is not available. For suspected pulmonary infections the guideline group strongly recommends chest CT, and moderately recommends chest X-ray if chest CT is not available. Diagnostic pathways are displayed in **Figure 12**.

Figure 12. Optimal diagnostic pathway for scedosporiosis, when all imaging and assay techniques are available



Treatment approaches to scedosporiosis

Treatment in Adults

Diagnosis-driven treatment

Evidence – Studies have reported success of diagnosis-driven treatment in lung transplant recipients with *Scedosporium* spp. colonization [493,912], while results were more mixed for empiric treatment in patients after near-drowning accidents [644,728].

Recommendations – In lung transplant recipients with colonization, pre-emptive treatment is moderately recommended. Every attempt to obtain a diagnosis should be made at the time of initiation of therapy, but should not delay therapy. Empiric treatment after near drowning accidents is marginally recommended.

First-line antifungal monotherapy

Evidence – In several studies, outcomes with VCZ based therapy were superior to any formulation of AmB[11,447]. Daily doses administered are started with 6 mg loading IV, followed by 4 mg IV twice daily. *In vitro* clinical resistance to AmB formulations, as well as breakthrough infections, have been reported repeatedly. Use of AmB fromulations should be restricted to settings in which there is no other antifungal therapy available. For the use of ISA, ICZ or PCZ only limited evidence exists[381,440,444,469].

Recommendations – First-line treatment with VCZ is strongly supported across all patterns of organ involvement. Use of AmB formulations is discouraged whenever VCZ is available. The guideline group marginally supports the use of ISA, ICZ or PCZ for first line-treatment.

First-line antifungal combination therapy

Evidence – In multiple studies antifungal combination therapy showed increased efficacy and improved survival compared to monotherapy with AmB[11,444,456,468]. There is a paucity of data evaluating combination therapy vs. VCZ monotherapy **(Table 15)**.

Table 15. First line treatment of *Scedosporium* spp. infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Fever-driven treatment						
Any with hematological malignancy	To cure *S. apiospermum* infection	VCZ	C	III	Girmenia JCM 1998[913]	N=1, died
Diagnosis-driven treatment						
High-risk patients (lung transplant recipients with colonization)	To cure	Pre-emptive treatment	B	IIu	Johnson TID 2014[493]	N=27; 17/20 VCZ and/or others, 9/17 cleared
					Parize TID 2017[912]	N=14, 9/13 received azoles
					Sahi JHLT 2007[833]	N=5
					Tamm TID 2001[914]	N=7
Near-drowning accidents	To cure	Empiric treatment	C	III	Katragkou Mycoses 2007[728]	N=12, mostly AmB
					Kowacs JCP 2004[741]	N=1, AmB + ICZ
					Buzina MedMycol 2006[644]	N=1, CASPO
					Lee AJNS 2018[597]	N=1, AmB, later VCZ
					Chaney SMJ 2004[658]	N=1, AmB
					Mursch CNS 2006[784]	N=1, VCZ, later plus TRB
First-line treatment						
Any	To cure	VCZ iv, step down to oral possible	A	IIu	Seidel CRM 2019[11]	N=137, VCZ N=63, AMB
					Cobo MedMycol 2017[468]	N=10, 6/10 response
					Troke AAC 2008[574]	N=70, 64% response
					Husain CID 2005[447]	N=57, VCZ 6/8 survived vs. AmB 4/20 survived
Any	To cure	VCZ + one or more other antifungals (TRB, MICA, AmB)	B	IIu	Seidel CRM 2019[11]	N=29, VCZ + TRB N=17, VCZ + AmB N=12, benefit of combination unclear
					Cobo MedMycol 2017[468]	N=1, complete response
Any with *S. apiospermum*	To cure	ISA	C	III	Cornely Mycoses 2018[381]	N=2, clinical response 1/2
Any with *S. aurantiacum* infection	To cure	VCZ OR PCZ	C	III	Heath CMI 2009[440]	N=29, low MICs against VCZ and PCZ
Immunocompromised	To cure	AmB lipid formulations	D	IIu	Seidel CRM 2019[11]	N=118, N=50 with AmB, N=30 AmB monotherapy. VCZ d42 mortality 11.3% vs. AmB 58.8% in immunocompromised, higher mortality with AmB mono vs. combination
					Perfect CID 2005[378]	N=8, 1/8 response
Immunocompetent	To cure	AmB lipid formulations alone OR in combination	C	IIu	Seidel CRM 2019[11]	N=90, VCZ d 42 mortality 14% vs. AmB 23.1%
Hematological malignancy	To cure	PCZ +/- AmB lipid formulation	C	III	Lamaris CID 2006[444]	N=4, 3/4 survived (including 2 with PCZ monotherapy)
Hematological malignancy	To cure	AmB lipid formulation + CASPO +/- VCZ	C	III	Lamaris CID 2006[444]	N=7, AmB + CASPO +/- VCZ 2/2 survived vs. AmB + ICZ 0/5 survived
Any with CF and lung infection	To cure	VCZ + either CASPO/MICA iv or inhaled AmB or both	B	III	Schwarz JCF 2018[456]	N=24, 2-drug combi 8/10 response, with 3-drug combi 14/14 response, VCZ mono 1/6 response
		VCZ	C	III		
Any with chronic lung disease	To cure	ICZ	D	III	Cooley EID 2007[469]	N=4, 3/4 died
Any with exogenous endophthalmitis	To cure	VCZ (po/iv, intravitreal), vitrectomy	C	III	Bui JOPT 2016[82]	N=2, success 2/2
					Nochez JOPT 2008[601]	
Any with exogenous endophthalmitis	To cure	AmB iv + intravitreal	D	III	Roy OII 2016[827]	N=2, failure 2/2
					Taylor CEO 2002[602]	
Any with endogenous endophthalmitis	To cure	VCZ iv, oral, intravitreal +/- TRB +/- vitrectomy	B	III	Moloney Retina 2014[915]	N=4, success 4/4
					Jain ArchOphtal 2007[721]	
					Chen CEO 2007[661]	N=2, lack of response due to delayed therapy 2/2
					Sarvat JOI 2007[916]	N=1, success
					Musk JHLT 2006[785]	N=2, success 2/2

Hematological malignancy with endogenous endophthalmitis + other disseminated infections	To cure	AmB iv / intravitreal, VCZ po (delayed treatment)	D	III	McKelvie CEO 2001[563]	N=2, failure 2/2

Standard dose unless stated otherwise; AmB, amphotericin B; bid, twice a day; CASPO, caspofungin; CI, confidence interval; ICZ, itraconazole; ISA, isavuconazole; iv, intravenous; MIC, minimal inhibitory concentration; PCZ, posaconazole; po, orally; QoE, quality of evidence; SoR, strength of recommendation; TRB, terbinafine; VCZ, voriconazole.

Recommendations – There are limited data reporting successful outcomes with antifungal combination therapy with VCZ plus lipid formulation of AmB, VCZ plus TRB, and VCZ plus echinocandins[11,444,456,785]. The guideline group does moderately support VCZ-based antifungal combination therapy for infections caused by *Scedosporium* spp.

Antifungal salvage treatment

Evidence – In general, there are two drug-related reasons for treatment failures, refractory scedosporiosis or toxicity or intolerance to first-line regimens. For the triazole class, hepatic toxicity has the highest prevalence and with AmB formulations renal toxicity may be a limiting factor. Toxicity may be caused by antifungals, or expected due to pre-existing organ damage. Only two drug classes show acceptable efficacy in scedosporiosis, thus salvage treatment mostly means switching to the other class. Successful outcomes have been reported with VCZ after primary treatment failure with lipid formulations of AmB[376], PCZ[917], and after adding an echinocandin[699] to pre-existing VCZ therapy **(Table 16)**.

Table 16. Antifungal salvage treatment for *Scedosporium* spp. infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To cure	VCZ	B	IIu	Perfect CID 2003[376]	N=10, response 3/10
Any	To cure	VCZ + echinocandin + GM-CSF	C	III	Goldman MMCR 2016[699]	N=1 cured after deteriorating on VCZ alone
Any	To cure	ISA	D	III	Cornely Mycoses 2018[381] Marty Mycoses 2018[407]	N=2, failure 2/2
Hematological malignancy with brain abscess	To cure	PCZ	C	III	Mellinghoff CID 2002[917]	N=1, success

Standard dose unless stated otherwise; GM-CSF, granulocyte-macrophage colony-stimulating factor; ISA, isavuconazole; PCZ, posaconazole; QoE, quality of evidence; SoR, strength of recommendation; VCZ, voriconazole.

Recommendations – The guideline group moderately recommends VCZ, and marginally PCZ or adding an echinocandin to VCZ monotherapy for salvage treatment.

Other treatment options

Evidence – Other treatment options include surgery and discontinuation/reduction of immunosuppressive drugs **(Table 17)**.

Table 17. Other treatment options for *Scedosporium* spp. infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Immunocompromised patients with subcutaneous scedosporiosis	To cure	Discontinuation of cyclosporine and prednisone followed by 2 wk levofloxacin iv without antifungal treatment	C	III	Li Mycopathol 2017[593]	N=1, success
Any with eye infection	Survival	Surgery	B	III	Seidel CRM 2019[11]	N=47, surgery not associated with better survival
Iv, intravenous; QoE, quality of evidence; SoR, strength of recommendation; wk, week(s)						

Treatment duration for scedosporiosis

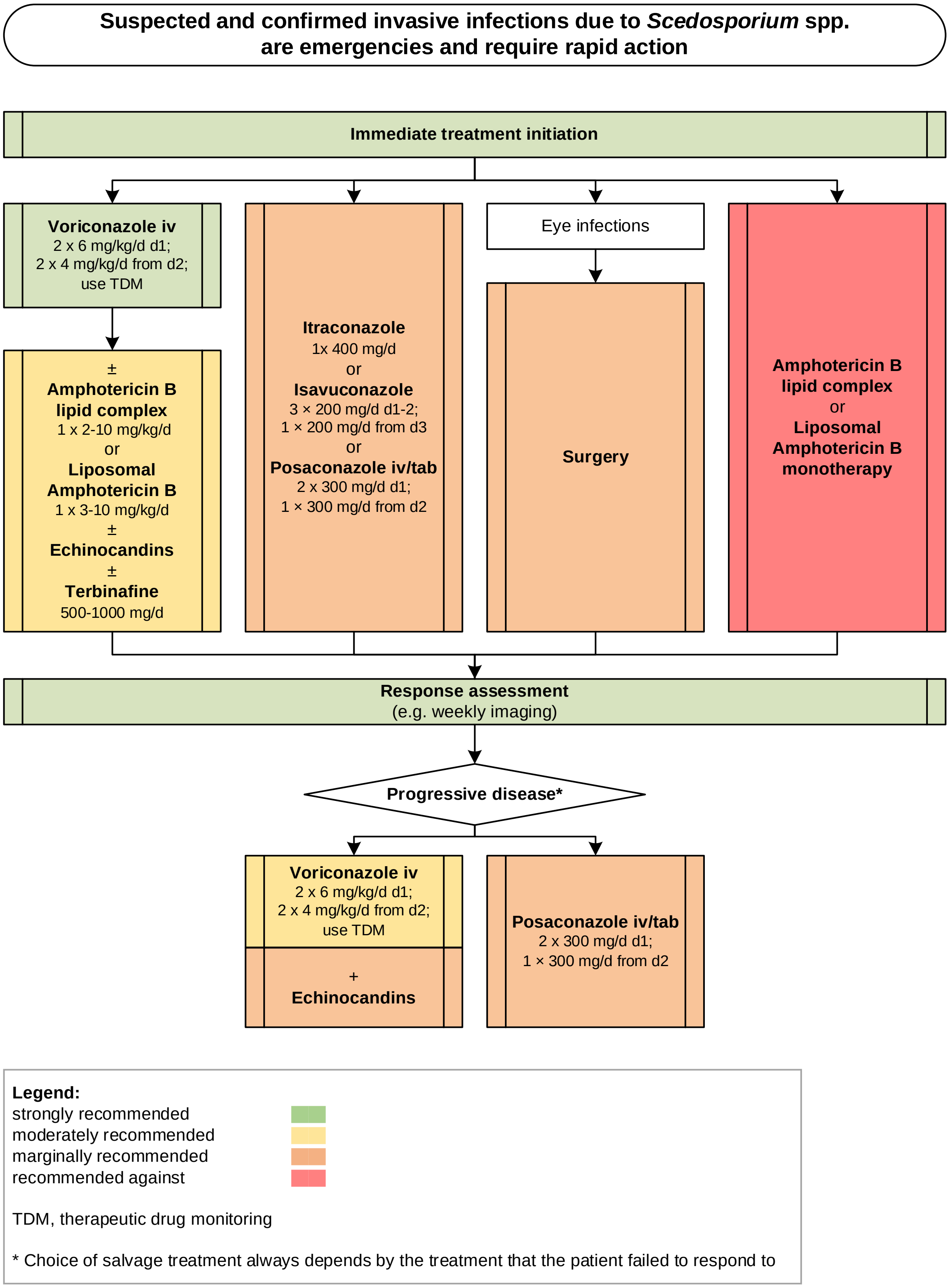
Evidence – The duration of therapy necessary to treat scedosporiosis is unknown. In general, weeks to months of therapy are given. If the underlying immunodeficiency resolves (*e.g.*, diabetes is controlled, neutropenia definitively resolved, or immunosuppression can be tapered or stopped), therapy can be continued until resolution of signs and symptoms **(Table 18)**.

Table 18. Treatment duration for *Scedosporium* spp. infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
CNS infections	To cure	VCZ for >3 mo	B	III	Schwartz Infection 2011[918]	*Aspergillus* spp. 63%, *Scedosporium* spp. 18%, duration (mean) 93 d (1-1.128)
Any with fungal osteoarticular infections	To cure	VCZ for > 3 mo	B	III	Kumashi CMI 2006[742]	*Aspergillus fumigatus* N=2, non-*fumigatus Aspergillus* spp. N=8, non-specified *Aspergillus* spp. N=3, *Fusarium* spp. N=6, Zygomycetes N=5, *S. apiospermum* N=2, *Exserohilum* spp. N=1, Duration: 5 mo (median 3 mo; range 11 d to 18 mo)
Standard dose unless stated otherwise; CNS, central nervous system; d, day(s); mo, month(s); QoE, quality of evidence; SoR, strength of recommendation, VCZ, voriconazole.						

Treatment pathways for adults are displayed in **Figure 13**.

Figure 13. Optimal treatment pathway for scedosporiosis <u>in adults</u> when all treatment modalities and antifungal drugs are available



Specific considerations on treatment of scedosporiosis in children

Evidence – The clinical presentation of scedosporiosis in immunocompromised children is comparable to that observed in adult patients, with a high rate of disseminated disease. Pulmonary and CNS scedosporiosis in near-drowning patients is an important characteristic of *Scedosporium* spp., and the association of invasive fungal disease and near drowning seems to be unique to scedosporiosis[728]. Reported outcomes for disseminated diseases are dramatically poor, both in immunocompromised and immunocompetent patients[919]. In a recent review of invasive *Lomentospora* (n=22) and *Scedosporium* (n=33) infections in children VCZ use and surgery were associated with improved clinical outcome[584]. Favourable outcome for localized infections in immunocompetent children treated with VCZ have been reported also in other studies[664,860,920] **(Table 19)**.

Table 19. Therapy in children for *Scedosporium* spp. infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
First-line antifungal therapy						
Any	To cure	VCZ +/- other antifungals + surgery for localized infections	A	III	Seidel IJID 2019[585]	N=22
Immunocompetent	To cure	VCZ 8-9 mg/kg qd iv, use of TDM	A	III	Stripelis MedMycol 2009[860]	N=1, 10 yrs, success
					Cruysmans PID 2015[664]	N=1, 7 yrs, success
					Salamat IJPO 2015[920]	N=1, 6 yrs, success
Any	To cure	VCZ iv + TRB 25 mg qd po	C	III	Whyte PID 2005[540]	N=1, 8 yrs, survived
					Tintelnot MedMycol 2009[533]	N=1, 9 yrs
Hematological malignancy	To cure	L-AmB (N=11, 9 died)	C	IIIt	Caira Haematol 2008[919]	Literature review, N=52, median age 47 (3-79): *S. apiopsermum* N=15, 7/15 died (1 adult, died); *L. prolificans* N=37, 33/37 died
		D-AmB (N=24, 21 died)	C	IIIt		
		D-AmB + 5FC (N=2, 2 died)	C	IIIt		
		D-AmB + azoles (N=9, 6 died)	C	IIIt		
		Azoles (N=6, 4 died)	C	IIIt		
Hematological malignancy	To cure	Surgery + azole	**C**	IIIt	Issakainen MedMycol 2010[720]	N=1, 14 yrs, success
Hematological malignancy with endogenous endophthalmitis + disseminated	To cure	VCZ 8 mg/kg qd iv, 100 µg intravitreal, TRB 125 mg qd, CASPO 50 mg qd, vitrectomy, surgical debridement	C	III	Chiam JAAPOS 2013[467]	N=1, success
Any with exogenous endophthalmitis	To cure	VCZ po, intravitreal 2x 200 µg, vitrectomy	C	III	Zarkovic IntOphthalmol 2007[603]	N=1, success
Any with chronic granulomatous disease	To cure	VCZ (OR ICZ)	C	III	Jabado CID 1998[921]	N=2, + surgery, 2/2 survived
Salvage antifungal therapy						
Hematological malignancy	To cure	L-AmB (N=2, 2 died)	D	III	Caira Haematol 2008[919]	Literature review, N=52, median age 47 (3-79): *S. apiopsermum* N=15, 7 died (1 adult, died); *L. prolificans*, N=37, 33 died
		L-AmB + VCZ (N=1, died)	C	IIIt		
		L-AmB + ICZ (N=1, survived)	C	IIIt		
		ICZ (N=4, 3 died)	C	IIIt		
		VCZ (N=1, died)	D	IIIt		
		PCZ (N=1, survived)	C	IIIt		
		VCZ + TRB (N=1, survived)	C	IIIt		

Standard pediatric dose unless stated otherwise; 5-FC, 5-fluorocytosine; bid, twice a day; CASPO, caspofungin; d, day(s); D-AmB, amphotericin B deoxycholate; CASPO, caspofungin; ICZ, itraconazole; iv, intravenous; L-AmB, liposomal amphotericin B; PCZ, posaconazole; po, orally; qd, once a day; QoE, quality of evidence; SoR, strength of recommendation; TDM, therapeutic drug monitoring; TRB, terbinafine; VCZ, voriconazole; yrs, years.

Recommendations – Treatment recommendations follow those given for adults. VCZ (+ TDM) is the first-line treatment of infections due to members of the genus *Scedosporium*. Surgery for localized disease is strongly recommended. Additional measures to reduce the immunosuppression should be considered.

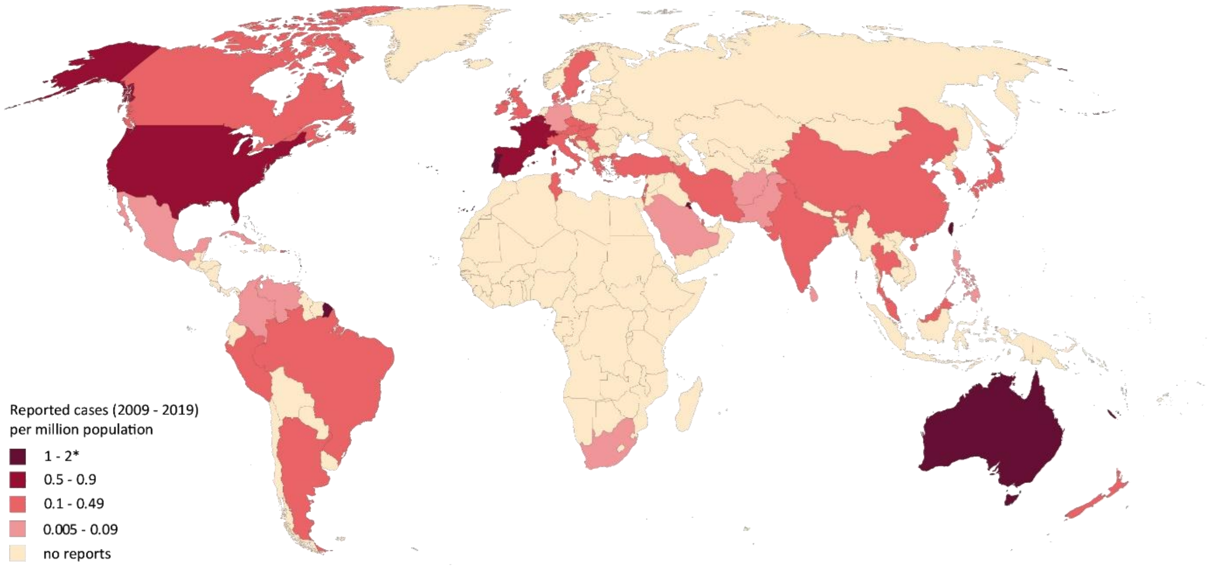
4. Phaeohyphomycosis

Epidemiology of phaeohyphomycosis

Phaeohyphomycosis (Greek: phaeo = dark) is caused by a heterogeneous group of melanized dematiaceous fungi that have a worldwide distribution and are found in soil, wood and decaying matter. Clinically important species causing systemic infections belong to the genera *Alternaria, Aureobasidium, Bipolaris, Chaetomium, Cladophialophora, Cladosporium, Curvularia, Exophiala, Exserohilum, Fonsecaea, Helminthosporium, Lomentospora* (see **lomentosporiosis**), and *Ochroconis*[922]. The term phaeohyphomycosis has been introduced to separate these infections from the clinically and pathologically distinct chromoblastomycosis or mycetoma that are also caused by melanized fungi[923]. Phaeohyphomycosis occurs more frequently in male than female individuals and, unlike other mold infections, commonly occurs in immunocompetent patients, presenting as keratitis, subcutaneous, rhinosinusitis, allergic bronchopulmonary, but sometimes also as invasive pulmonary or severe cerebral infections with fungemia, with recent research indicating that previously unrecognized CARD9 immunodeficiency is present in many patients developing severe infections who were previously thought to be immunocompetent[924,925]. Eye and subcutaneous phaeohyphomycosis usually follow a traumatic injury or surgery, without apparent underlying immune deficiency[251,923], in contrast, a history of previous trauma is rarely found in immunocompromised patients [926]. If left untreated subcutaneous lesions slowly increase in size to form abscesses. Eye infections are frequently caused by *Alternaria*, *Curvularia*, *Exserohilum*, or *Helminthosporium* spp.. In skin infections, commonly associated genera are *Alternaria*, *Bipolaris*, *Exophiala,* and *Phialophora*[248,922,927-929]. Brain infections are comparably common, may present as brain abscess, meningitis, or encephalitis[925], and are mainly caused by *Cladophialophora bantiana*, a neurotropic fungus that caused severe infections also in immunocompetent patients, with a considerable number of cases described in India[924,928,930]. However, other fungi, such as *Rhinocladiella mackenziei*, *Chaetomium strumarium*, *Verruconis gallopava* and *Exophiala dermatitidis* in immunocompromised hosts and *Exserohilum rostratum* in a recent outbreak in the United States are also well decribed as causing CNS phaeohyphomycosis[925,931-933]. *Bipolaris* and *Curvularia* are associated with fungal sinusitis with brain invasion, a clinical form that is becoming more common[922]. Melanized molds can cause endocarditis after valve replacement, mediastinitis following surgery, and peritonitis in patients on continuous peritoneal dialysis[934-938]. Disseminated phaeohyphomycosis is mostly associated with immunocompromising or debilitating disease and is thought to originate in the lung after inhalation of the fungal agent[923,929,939].

The prevalence of phaeohyphomycosis varies between regions, patient population and etiological agent. Cerebral phaeohyphomycosis occurs worldwide, but most cases have been reported from the United States, mostly in immunocompromised patients. Iatrogenic meningitis and other infections related to epidural injections of corticosteroids have been reported in two recent US outbreaks traced to environmental contamination at compounding pharmacies. During 2012, 754 cases of infection and 64 deaths were confirmed among the 13,534 people potentially exposed to contaminated lots of methylprednisolone[932]. Cerebral phaeohyphomycosis has also been frequently reported from India, particularly affecting immunocompetent individuals[940]. In India, *Alternaria* and *Curvularia* accounted for 7% of mold-related keratitis in a 10-year study[248]. In a multicentre study, 9.4% of fungal infections in liver and heart transplant recipients were related to phaeohyphomycosis, affecting sinuses, lung and CNS[442] **(Figure 14)**.

Figure 14. Worldwide distribution of phaeohyphomycosis (reported cases between 2009 and 2019 per million population)



Cases of phaeohyphomycosis reported in the medical literature were identified in a PubMed search on October 31, 2019 using the search string (Phaeohyphomycosis OR *Acrophialophora* OR *Alternaria* OR *Anthopsis* OR A*rnium* OR *Arthrinium* OR *Aureobasidium* OR *Bipolaris* OR *Botryodiplodia* OR *Botryomyces* OR *Chaetomium* OR *Chrysonilia* OR *Cladophialophora* OR *Cladosporium* OR *Cladorrhinum* OR *Coniothyrium* OR *Corynespora* OR *Curvularia* OR *Cyphellophora* OR *Dichotomophthora* OR *Dichotomophthoropsis* OR *Dissitimurus* OR *Drechslera* OR *Exophiala* OR *Wangiella* OR *Exserohilum* OR *Fonsecaea* OR *Hormonema* OR *Hortaea* OR *Lecytophora* OR *Leptosphaeria* OR *Medicopsis* OR *Microsphaeropsis* OR *Myceliophthora* OR *Mycocentrospora* OR *Mycoleptodiscus* OR *Nattrassia* OR *Neoscytalidium* OR *Neurospora* OR *Nigrograna* OR *Nodulisporium* OR *Ochroconis* OR *Oidiodendron* OR *Onychocola* OR *Papulaspora* OR *Periconia* OR *Phaeoacremonium* OR *Phaeosclera* OR *Phaeotheca* OR *Phaeotrichoconis* OR *Phialemonium* OR *Phialophora* OR *Phillostica* OR *Phoma* OR *Didymella* OR *Phomopsis* OR *Phyllostictina* OR *Pleurophoma* OR *Pleurophomopsis* OR *Pleurostoma* OR *Polycytella* OR *Pseudomicrodochium* OR *Pyrenochaeta* OR *Ramichloridium* OR *Rhinocladiell*a OR *Rhytidhysteron* OR *Sarcinomyces* OR *Scytalidium* OR *Taeniolella* OR *Tetraploa* OR *Thermomyces* OR *Trematosphaeria* OR *Trichomaris* OR *Ulocladium* OR *Veronaea* OR *Verruconis*) AND (case [Title/Abstract] OR patient [All Fields] OR report [Title/Abstract] OR infections OR invasive OR fungemia OR blood OR disseminat*) NOT Chromoblastomycosis[Title/Abstract] NOT mycetoma [Title/Abstract]) that yielded 3,325 publications. In total, 935 cases were identified from 55 countries [97,138,666,926,934,936,939,941-111697,138,666,926,934,936,939,941-11158,163,173,178,489,518,749,935,937,1117-1343251,310,319,607,834,863,890,929,938,1344-1468]. Most cases were reported from India (n>200), China (n=188), United States (n=162), Spain (n=44), and Japan (n=41). Most infections were related to species of the genera *Alternaria* (>300), *Curvularia*, *Exophiala* (~100 each), *Exserohilum* (~70), *Cladophialophora*, *Bipolaris* (~50 each), *Phaeoacremonium*, *Cladosporium*, *Fonsecaea*, *Aureobasidium* (~20 each). Number of cases reported between 2009 and 2019 are presented as cases per million population per country. The resident population per country was obtained from www.worldometers.info[321]. *One case each was reported from French Guiana, Martinique and New Caledonia (>2 cases per million population between 2009 and 2019)[957,1092,1272].

Diagnosis of phaeohyphomycosis

Diagnosis – Microbiology – Conventional Methods

Evidence – Diagnosis relies on histopathology and careful gross and microscopic examination of cultured strains, which show dark colonies with usually darkly pigmented septate hyphae with widely variable conidia and conidiophores, respectively[14,1131,1354]. Pleomorphism that is seen in dematiaceous organisms on histopathology is the most specific finding in microscopy. The Fontana-Masson stain helps to make melanin visible in dematiaceous molds that may appear pale in H&E and other stains, and helps to differentiate melanized elements of phaeohyphomycetes from other mold structures in tissue samples[14] **(Table 20)**.

Recommendations – The guideline group strongly recommends histological evaluation and culture from clinical samples.

Diagnosis – Microbiology – Serology

Evidence – There are no simple serological or antigen diagnostic tests for infections caused by phaeohyphomycetes, mainly due to the huge diversity of these pathogens. BDG and GM tests may cross-react with some melanized fungi, though neither has been proven useful for diagnosis of phaeohyphomycosis in general[518] **(Table 20)**.

Recommendations – While the guideline group marginally supports serology on a case by case basis, there is currently no serological test that can be recommended.

Diagnosis – Microbiology – Molecular-based

Evidence – ITS1/ITS2 targeting oligonucleotide probes or PCR followed by sequencing on DNA extracted directly from sputum, tissue samples or sinus aspirates were occasionally applied successfully, showing a higher sensitivity than culture[551,901,926]. However, much more data is needed for routine use of this approach. Possible contamination should be carefully considered due to the ubiquitous nature of dematiaceous fungi **(Table 20)**.

Recommendations – Based on case reports, direct analysis of clinical samples using oligonucleotide arrays or universal PCR followed by sequencing can only marginally be supported.

Diagnosis – Microbiology – Species identification

Evidence – Morphological identification may be complicated by limited sporulation of causative pathogens. Identification to species level is performed by ITS1/ITS2 or D1/D2 sequencing[1144,1354,1399,1469,1470] and/or MALDI-TOF MS analysis[1077,1471-1474] of strains cultured from tissue or blood samples. The usefulness of MALDI-TOF MS is highly dependent on the use of enriched databases **(Table 20)**.

Recommendations – The guideline group strongly recommends identification to species level by ITS1/ITS2 or D1/D2 sequencing and moderately by MALDI-TOF MS analysis of cultured strains.

Microbiology – Susceptibility testing

Evidence – The relevance of susceptibility testing is not yet fully defined, as breakpoints have not been established by CLSI or EUCAST, and there is a limited correlation between *in vitro* MICs and clinical outcomes. VCZ[1181,1475] or PCZ[1254] are the most active drugs when tested by broth microdilution. A number of genera showed good *in vitro* susceptibility to ICZ, PCZ, VCZ, and AmB using Sensititre® YeastOne® YO10 panel[151] **(Table 20)**.

Recommendations – Susceptibility testing is strongly recommended for identifying susceptibility patterns, and moderately for guiding treatment.

Diagnosis - Pathology

Evidence – Histopathological examination of tissue samples may lead to diagnosis[1476] or provides important diagnostic information[1477] **(Table 20)**.

Recommendations – The guideline group strongly recommends histopathological examination of tissue samples.

Diagnosis – Imaging

Evidence – Chest CT scan was the most common abnormal radiographic study in transplant recipients suffering from phaeohyphomycosis[1477]. Cranial CT/MRI is indicated for evaluation of possible CNS infection **(Table 20)**.

Table 20. Microbiological, histopathological and imaging diagnostics for phaeohyphomycetes/dematiaceous fungi/black fungi infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Microscopy, culture, MIC testing						
Any	To diagnose	Direct microscopy	A	III	Chowdhary CMI 2014[14]	Usually darkly pigmented
					Hoelett MMCR 2019[1478]	
Any	To diagnose	Culture, species identification by morphological characteristics or ITS sequencing	A	III	Chowdhary CMI 2014[14]	Use Fontana-Masson stain avoid H&E stain
					Hoelett MMCR 2019[1478]	
					Sato IntMed 2019[1354]	
Any	To diagnose	Histopathology and culture	A	III	Ito ADV 2017[1144]	Subcutaneous infections: Delayed growth (12-15 d). Culture confirmed by molecular methods
					Shi Dermatopathol 2017[1479]	
					Taj-Aldeen MedMycol 2010[1399]	CNS infections: Delayed growth (12-15 d) in most cases. Culture confirmed by molecular methods
					Koo MedMycol 2010[1184]	
					Cristini JCM 2010[1469]	
					Garazoni MedMycol 2008[1470]	
					Revankar OFID 2017[518]	N=99, Diagnosis confirmed by culture in 97/99 (98%). Using ITS sequencing, the CDC further identified 5 isolates to the species level, 2 unknown isolates were identified, and 1 isolate initially identified as *Phialophora verrucosa* was determined to be *Pleurostomophora richardsiae*. Histopathology showed granulomatous inflammation and/or fungal elements in 49 of 99 (49%) cases.

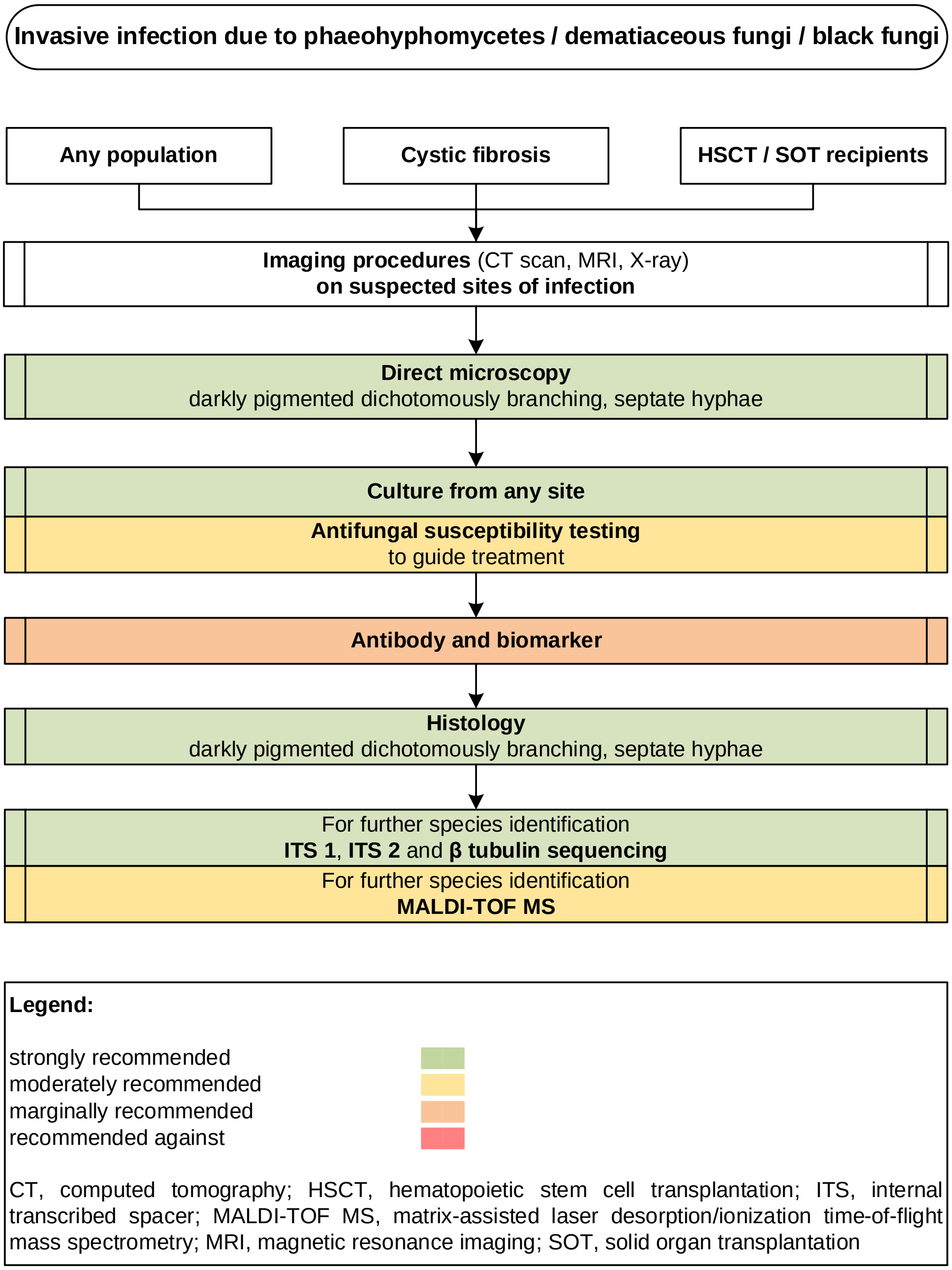
					Kondori FEMSML 2015[1472]	
					Ozhak-Baysan MedMycol 2015[1473]	
					Singh JCM 2017[1474]	
					Fernandez BJID 2017[1077]	
GVHD	To diagnose	Blood culture	A	III	Sato IntMed 2018[1354]	
Osteomyelitis and septic arthritis	To diagnose	Biopsy culture + histopathology	A	III	Lang BMCID 2018[1200]	N=1
Any	To identify susceptibility pattern	Sensititre® YeastOne® YO10 panels	A	IIu	Halliday IJAA 2016[151]	MIC90 AmB 1 µg/ml, ICZ 0.12 µg/ml, PCZ 0.25 µg/ml and VCZ 0.5 µg/ml had good *in vitro* activity against nine genera of dematiaceous fungi
Any	To identify susceptibility patterns	CLSI testing of isolates	A	III	Revankar OFID 2017[518]	N=16 isolates, extended spectrum azoles and TRB most active antifungals
Any	To guide treatment of *Alternaria malorum* infection	Culture and molecular identification	B	III	Mirhendi MedMycol 2013[1254]	MICs PCZ 0.063 µg/ml, AmB 0.125 µg/ml, ICZ 0.125 µ g/ml, VCZ 1 µg/ml,FCZ 32 µg/ml. MECs ANID 0.016 µg/ml, CASPO 0.25 µg/ml
Any	To guide treatment of *E. dermatitidis* infection	Culture and molecular identification	B	III	Klasinc Mycopathol 2019[1181]	MICs for 48h (µg/ml): ANID (8 µg/ml); FCZ (4 µg/ml); PCZ (0.25 µg/ml); VCZ (<0.016 µg/ml); ISA (0.125 µg/ml); ICZ (1 µg/ml); AmB (2 µg/ml)
Immunosuppressed patients	To guide treatment of *Exophiala oligosperma* infection	Culture and molecular identification	B	III	Rimawi MMCR 2013[1475]	MICs AmB 0.5 µg/ml, MICA 0.25 µg/ml, PCZ 0.03 µg/ml, and VCZ 0.125 µg/ml
Serology assays						
Transplant patients	To diagnose	Serum GM or BDG	C	III	Revankar JFungi 2015[1480]	GM and BDG occasionally may be cross-reactive with this group of fungi, but this is not consistent
Allergic bronchopulmonary aspergillosis	To detect fungi in sinus aspirates	Serum IgE level	C	III	Chowdhary MedMycol 2011[1481]	Peripheral eosinophilia and elevated total serum IgE level may indicate Allergic bronchopulmonary aspergillosis
Nucleic-acid based assays/MALDI-TOF MS						
Transplant patients	To diagnose	DNA sequencing from tissue	C	III	Ferrandiz-Pulido Mycoses 2019 [926]	N=11; DNA sequencing confirmed the presence of *Alternaria* spp. (8 cases), *Cladosporium cladosporioides*, *Microsphaeropsis arundinis* and *E. oligosperma*
Any with CF	To detect in sputum	PCR + ITS1-ITS2 sequencing	C	III	Nagano MedMycol 2010[901]	1 each of *E. dermatitidis,* and *Cladosporium* spp. were detected using the PCR assay compared to 3, and 0 respectively by selective culture
Any with CF	To detect in sputum *Acrophialophora fusispora, E. dermatitidis*	Oligoarray, developed with probes designed according to ITS1/ITS2 sequence data	C	III	Bouchara JCM 2009[551]	Correct identification to species level of all *A. fusispora* and all but one strain of *E. dermatitidis.* It detected the presence of *E. dermatitidis* in two sputum samples whereas culture was negative in both samples.
Any with allergic fungal rhinosinusitis	To detect fungi in sinus aspirates	PCR assays using universal fungal primers (ITS 3-ITS4), followed by *Bipolaris* primers (Bipol A73 + B572)	C	III	El-Morsy JLO 2010[1482]	A comparison of culture with PCR assays; Cultures yielded 30 dematiaceous fungi in 68 samples. Universal fungal PCR assay yielded 68/68 positive results, no further fungal identification was performed. *Bipolaris* PCR assay gave 27 positive results. PCR assays gave positive results in 4 of 10 control samples without sinusitis. This study did not include an in-house validation of the assays used.
Any with fungal meningitis or other infections linked to contaminated	To detect *E. rostratum* and other in CSF	PCR using broad-range primers targeting ITS2 region vs. *E. rostratum* -specific primers	C	II	Gade JCM 2015[1483]	*E. rostratum* DNA was detected in 28% of 413 cases (mostly from CSF samples). The *E. rostratum* -specific PCR assay was more sensitive than

methylprednisolone acetate						the broad-range PCR assay and when compared to culture. *Cladosporium* DNA was detected in CSF of one case.
Any	Species identification from culture	Species identification by ITS or D sequencing	A	III	Revankar OFID 2017[518]	N=99, Diagnosis confirmed by culture in 97/99 (98%). Using ITS sequencing, the CDC further identified 5 isolates to the species level, 2 unknown isolates were identified, and 1 isolate initially identified as *P. verrucosa* was determined to be *P. richardsiae*. Histopathology showed granulomatous inflammation and/or fungal elements in 49 of 99 (49%) cases.
Any	Species identification from culture	Species identification by MALDI-TOF MS	B	III	Fraser JCM 2017[1471]	MALDI-TOF MS for species identification form strains cultured from tissue
Tissue-based diagnosis						
SOT recipients	To diagnose	Histology	A	IIu	Schieffelin TID 2014[1476]	N=27, 4 diagnosed by histological appearance alone
SOT or HSCT transplant recipients	To diagnose	Histopathology	A	IIu	McCarthy MedMycol 2015[1477]	N=56, histopathology added to the diagnostic information in 15 patients with 13 (86.7%) of those being skin specimens
Any with allergic rhinosinusitis	To diagnose	Histopathology	A	III	Montone HNP 2016[1484]	Microscopic examination in allergic fungal rhinosinusitis reveals eosinophilic mucin
Imaging studies						
SOT or HSCT transplant recipients	To diagnose	Chest CT, chest X-ray, cranial CT, cranial MRI	A	IIu	McCarty MedMycol 2015[1477]	N=56, Chest CT most common abnormal radiographic study, assisting in the diagnosis of 24 patients

ANID, anidulafungin; BAL, bronchoalveolar lavage; CASPO, caspofungin; CDC, Centers for Disease Control; CF, cystic fibrois; CLSI, Clinical and Laboratory Standards Institute; CNS, central nervous system; CSF, cerebrospinal fluid; CT, computed tomography; d, day(s); DNA, deoxyribonucleic acid; FCZ, fluconazole; HSCT, hematopoietic stem cell transplantation; ICZ, itraconazole; ISA, isavuconazole; ITS, internal transcribed spacer; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; MEC, minimum effective concentration; MICA, micafungin; MIC, minimal inhibitory concentration; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; PCZ, posaconazole; QoE, quality of evidence; SoR, strength of recommendation; SOT, Solid organ transplantation; TRB, terbinafine; VCZ, voriconazole.

Recommendations – The guideline group strongly recommends chest CT scan and cranial CT/MRI in the case of suspected lower respiratory tract and CNS infection, respectively **(Figure 15)**.

Figure 15. Optimal diagnostic pathway for phaeohyphomycosis, when all imaging and assay techniques are available



Treatment approaches to phaeohyphomycosis

Treatment in adults

Targeted first-line antifungal therapy

Evidence – The use of either VCZ or lipid formulations of AmB (including combinations particularly for disseminated infections) has successfully treated phaeohyphomycosis cases with various organ involvement patterns[378,518,932,1322,1477,1485-1487]. There are reports of successful treatment with CASPO or PCZ mono- or combination therapy[518,1477,1486,1487].

In several case series of CNS phaeohyphomycosis, D-AmB or lipid formulations of AmB as well as - more recently - VCZ (alone or in combination with lipid formulations of AmB) were the most commonly successfully used agents[518,923,924,940]. VCZ alone (n=301) or in combination with L-AmB (n=143) has been successfully used in the outbreak associated with *E. rostratum* contaminated methylprednisolone injections[932]. Large case series report the successful use of either ICZ or VCZ (both sometimes in combination with surgery) for cutaneous or subcutaneous phaeohyphomycosis, with smaller case-series reporting similar success rates for PCZ, and case reporting of successful use of TRB[518,926,1119,1476,1488-1491]. ICZ has also been most frequently used to successfully treat chromoblastomycosis[1492].

ISA has been used successfully in first-line treatment for *Exserohilum* or *Curvularia* infections but not for infections due to *Cladophialophora* spp. or *Cladosporium* spp.[381].

Intravenous or intravitreal AmB (with or without VCZ, 5-fluorocytosine (5-FC) or vitrectomy) has been the mainstay of the treatment for patients with endogenous or exogenous endophthalmitis with inconsistencies in treatment outcomes reported[518,952,1030,1082,1390,1453,1493-1499] **(Table 21)**.

Table 21. First-line antifungal therapy for phaeohyphomycetes/dematiaceous fungi/black fungi infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To cure	AmB lipid formulations	B	IIu	Perfect CID 2005[378]	N=4 with *Curvularia,* response/cure 3/4 (75%)
					Ben-Ami CID 2009[1486]	N=9, response 6/9
					Bagga MMCR 2019[1500]	N=1, success
					McCarty MedMycol 2015[1477]	N=56, L-AmB N=20, combination therapy N=15, overall death N=14
Any with *Clado-phialphora* or *Cladosporium* infection	To cure	ISA	D	IIu	Cornely Mycoses 2018[381]	N=2, *C. bantiana* + *Cladosporium* spp., response 0/2
Any with *Exserohilum* or *Curvularia* infection	To cure	ISA	C	IIu	Cornely Mycoses 2018[381]	N=2, *E. rostratum* and *Curvularia lunata*, response 2/2

Any with disseminated infection	To cure	AmB lipid formulation + VCZ / PCZ / ICZ OR echinocandin OR all three +/- TRB +/- 5-FC	B	III	Ben-Ami CID 2009[1486]	N=27, AmB lipid formulation + VCZ/PCZ N=10 OR + echinocandin N=14 OR all three N=3; combination therapy 22/27 response, monotherapy 13/19 response
					Hagiya JIC 2019[1501]	N=1, *E. dermatitidis*, L-AmB + VCZ, failure
					Chalkias TID 2014[1010]	N=1, *E. dermatitidis*, L-AmB + VCZ, success
					Edathodu JCM 2013[1502]	N=1, *Triadelphia pulvinata*, VCZ + L-AmB, failed
					McCarty MedMycol 2015[1477]	N=56, VCZ N=24, L-AmB N=20, CASPO N=7; combination therapy N=15, 14/56 died
					Qiu JIC 2019[1503]	N=1, *Phialophora* spp., success
					Santos CMI 2017[1488]	N=2
					Qureshi ClinTransplant 2012[1322]	N=10, *V. gallopava*, AmB lipid formulations or D-AmB + VCZ or ICZ, 8/10 survived
					Revanker OFID 2017[518]	N=26, response d30 31%, mortality d30 38%. Combination 5/16 response vs. monotherapy 3/10 response. 18/26 died, 11/16 combination vs. 7/10 monotherapy.
Any	To cure	VCZ	B	III	Ben-Ami CID 2009[1486]	N=3, response 2/3
					Pundhir IJSTDAIDS 2016[1504]	N=1, *Scolecobasidium* spp., response
					Vasquez CID 2017[1487]	N=14, 13/14 survived
					McCarty MedMycol 2015[1477]	N=56, VCZ N=24, combination therapy N=15, 14/56 died
					Revanker OFID 2017[518]	N=26, 9/26 VCZ monotherapy, including 2 with endocarditis (both died)
Any	To cure	CASPO	C	III	Ben-Ami CID 2009[1486]	N=4, response 3/4
					McCarty MedMycol 2015[1477]	N=56, CASPO N=7, combination N=15, 14/56 died
Any with disseminated infection	To cure	VCZ / PCZ + echinocandin OR TRB	B	III	Vasquez CID 2017[1487]	N=2, 2/2 failed
					Thomas MMI 2018[1490]	N=1, + surgery, failed
					Revanker OFID 2017[518]	N=26, 16/26 combination, 2/4 endocarditis patients survived
Any	To cure	PCZ	C	III	Ben-Ami CID 2009[1486]	N=1, response
					Moran TID 2019[1269]	N=1, *V. gallopava*, success
Any	To cure	D-AmB	D	IIIt	Sribenjalux TID 2019[1385]	N=1, *P. richardsiae*, success
					Chhabra IJDR 2013[1023]	N=1, *Alternaria alternata,* + surgery, success
SOT patients with cavitary native lung nodules	To cure	VCZ + CASPO	C	III	Shah TID 2019[1363]	N=1, *Phaeoacremonium parasiticum*, success
Phaeohyphomycosis of the CNS	To cure	Lipid based AmB formulations	B	III	Revanker CID 2004[924]	N=109, AmB N=59, L-AmB N=8, 5-FC + AmB N=24, ICZ +/- AmB N=18, +/- surgery, overall death N=66
					Chakrabarti MedMycol 2015[940]	N=113, *C. bantiana,* D-AmB 48.7%, AmB + 5-FC 15.3%, lipid preparations of AmB 14.2%, none associated with better outcome
					Doymaz Mycoses 2015[1505]	N=1, *Fonsecaea monophora*, success
					Thomas MMI 2018[1490]	N=1, fatal outcome
					Howlett MMCR 2019[1131]	N=1, *C. bantiana*, failure
Phaeohyphomycosis of the CNS	To cure	VCZ +/- L-AmB	B	III	Dobias FMicrobiol 2018[1062]	N=1, *F. monophora*, success
					Gopalakrishnan IJMM 2017[1104]	N=2, *C. bantiana*, success 2/2
					Rosow TID 2011[1341]	N=1, *Bipolaris spicifera*, success
					Santos CMI 2017[1488]	N=1, success
					Jung JKNS 2014[1160]	N=1, + surgery, success
					Gadgil JCN 2013[1088]	N=1, *Curvularia* spp., + surgery, success

					Mukhopadhyay JMM 2017[1198]	N=1, *C bantiana,* failure
					Mohammadi Mycoses 2018[1260]	N=1, *R. mackenziei*, failure
					Revanker OFID 2017[518]	N=6, + surgery in 4/6, 2/6 survived
					Taj-Aldeen MedMycol 2010[1399]	N=1, *R. mackenziei*, failure
	To cure	D-AmB +/- surgery	D	III	Revanker CID 2002[923]	Review, N=72, D-AmB N=62, L-AmB N=3, 5-FC + AmB N=5, Azole + AmB N=10, +/- surgery, 57/72 died. D-AmB 14/62 (23%) survived
					Badali JCM 2011[973]	N=1, *Thielavia subthermophila*, failure
					Sládeková JMMCR 2014[1506]	N=1, *C. bantiana,* failure
Any with *E. rostratum*	To cure	L-AmB + PCZ / ICZ +/- surgery	C	III	Katragkou MedMycol 2014[1485]	Review, N=48, 13/32 combination therapy
Any with *E. rostratum*	To cure	VCZ +/- L-AmB	B	III	Smith NEJM 2013[932]	N=444, VCZ monotherapy N=301, combination VCZ + L-AmB N=143
Any with cutaneous / subcutaneous phaeohyphomycosis	To cure	ISA	C	III	Dalla GTID 2019[1507]	N=1*, A. alternata,* success
		PCZ +/- surgical debridement	B	III	Los-Arcos TID 2019[1508]	N=1, *Medicopsis romeroi*, success
					Crawford TID 2015[1509]	N=1, *M. arundinis*, success
					Thomas MMI 2018[1490]	N=1, +surgery, success
					Revanker OFID 2017[518]	N=32, PCZ N=10, response d30 79%, mortality d30 3%, follow-up response 84%, 11/32 partial response, 16/32 complete response
					Crabol PLOSNTD 2014[1491]	N=5, response 5/5
Any with cutaneous / subcutaneous phaeohyphomycosis	To cure	ICZ +/- surgery	A	IIu	Schieffelin TID 2014[1476]	N=24, excision N=22, ICZ N=19, VCZ N=2, no antifungal therapy N=3
					Santos CMI 2017[1488]	N=51, surgical excision w/o antifungals N=21, ICZ N=30, success 51/51
					Ogawa Mycopathol 2016[1489]	N=6, 2 *Exophiala* and 3 *Fonsecaea*; ICZ N=4; ICZ + surgery N=2, success
					Revanker OFID 2017[518]	N=32, ICZ N=13, surgery N=6, response d30 79%, mortality d30 3%, follow-up response 84%
					Ferrándiz-Pulido Mycoses 2018[926]	N=6, response 3/6; failure 3/6
					Chan SMJ 2014 [1510]	N=1, *M. romeroi*, success
					Bohelay Mycoses 2016[1511]	N=1, *Exophiala spinifera*, success
					Sharma Mycopathol 2016[1512]	N=1, *M. romeroi,* success
					Khader IJDVL 2015[1513]	N=1, *C. bantiana*, success
					Furudate CRD 2012[1514]	N=1, *Phaeoacremonium rubrigenum*, success
					Chander Mycopathol 2016[1515]	N=2, *Rhytidhysteron rufulum*, success 1/2
					Chhonkar IJPS 2016[1516]	N=3, success 2/3
					Mittal IJD 2014[1517]	N=1, *Cladophialophora carrionii*, success
					Parente Mycoses 2011[1518]	N=2, *E. jeanselmei* and *C. carrionii*, success 2/2
					Sang MedMycol 2011[1519]	N=1, *Veronaea botryosa*, success
					Pereira IJDVL 2010[1520]	N=1, *Cladophialophora boppii*, success
					Crawford TID 2015[1509]	N=1, *M. arundinis*, success
					Gunathilake JMM 2013[1521]	N=1, *C. lunata*, success
					Machmachi MedMycol 2011[1522]	N=1, *Falciformispora tompkinsii*, success
					Nolêto CMI 2019[1523]	N=1, *Phoma (Peyronellaea)* spp., success
					Haridasan TID 2017[1524]	N=7, no response to ICZ alone, 6/7 lesions surgically excised

					Michelon DOJ 2014[1525]	N=1, *Alternaria* spp., success
Any with cutaneous / subcutaneous phaeohy-phomycosis	To cure	VCZ +/- surgery	A	IIu	Galipothu IJMM 2015[1526]	N=1, order *Pleosporales*, success
					Revanker OFID 2017[518]	N=41, surgery N=20; systemic anti-fungals N=40. VCZ 26 (65%),VCZ combi 12 (30%), response d30 53%, mortality d30 12%, follow-up re-sponse 68%
					Revanker OFID 2017[518]	N=32, VCZ N=9
					Ferrándiz-Pulido Mycoses 2018[926]	N=11, *Exophiala*; partial response
					Balla JCP 2015[1527]	N=1, *Curvularia* spp., success
					Los-Arcos TID 2019[1508]	N=1, SOT, *M. romeroi*, success
					Brokalaki TransProceed 2012[1528]	N=1, *V. gallopava*, success
					Lief TID 2011[1529]	N=2, *E. jeanselmei*, mixed response
					Desoubeaux JMM 2013[1530]	N=1, *E. jeanselmei*, success
					Crabol PLOSNTD 2014[1491]	N=8, response in 5/8; 2/3 switched to PCZ salvage treatment
					Crawford TID 2015[1509]	N=1, *M. arundinis*, success
					Vermeire DMID 2010[1531]	N=2, *A. alternata* and *Curvularia* spp., success 2/2
Any with cutaneous / subcutaneous phaeohy-phomycosis	To cure	TRB +/-VCZ / ICZ	C	III	Mohammed AJM 2019[1532]	N=1, *E. jeanselmei*, success
					Thomas MMI 2018[1490]	N=2, success 1/2
					Ogawa Mycopathol 2016[1489]	N=1, success
		Ketoconazole + surgery	D	III	Radhakrishnan IJMM 2010[1533]	N=1, *E. spinifera*, failure
Any with subcutaneous chromoblasto-mycosis	To cure	ICZ +/- FCZ OR 5-FC +/- sur-gery	B	III	Bao AJTMH 2018[1534]	N=1, *F. monophora*, success
					Mouchalouat IJD 2011[1492]	N=15, ICZ N=6, ICZ + FCZ N=5, sur-gery N=4, success 12/15 (80%)
					Label MMCR 2018[1535]	N=1, *F. monophora*, success
					Gomes ABD 2014[1536]	N=1, *Fonsecaea pedrosoi*, success
					Antonello RIMT 2010[1537]	N=1, success
		TRB + local thermotherapy	C	III	Shi MMCR 2016[1538]	N=1, *F. monophora*, success
		PCZ + surgery	C	III	Dupont TID 2010[1539]	N=1, *C. carrionii*, success
Intravenous drug user with endophthalmitis	To cure	AmB intravitreal, iv, VCZ iv + vitrectomy	D	III	Fox JOII 2016[1082]	N=1, Pleurostoma *richardsiae*, fail-ure
Post-op patients with exogenous endophthal-mitis	To cure	AmB intraocular, VCZ intra-ocular, po + vitrectomy	D	III	Alex MMCR 2013[952]	N=1, *C. lunata*, failure
Post-op patients with exogenous endophthal-mitis	To cure	5-FC, AmB intravitreal, topi-cal	C	III	Kaushik AJO 2001[1498]	N=1, C. *lunata* cultured, success
Post-op patients with exogenous endophthal-mitis	To cure	VCZ topical, intravitreal, FCZ po + vitrectomy	C	III	Homa Mycopathol 2018[1540]	N=1, *E. dermatitidis*, success
Any with exogenous endophthalmitis	To cure	AmB intravitreal +/- iv +/- subconjunctival + vitrec-tomy	D	III	Clamp RCBR 2014[1030]	N=1, *E. dermatitidis*, failure
					Hofling-Lima AJO 1999[1493]	N=2, *E. jeanselmei*, failure
					Margo AJO 1990[1496]	N=1, *E. dermatitidis*, failure
					Sun MedMycol 2010 [1390]	N=1, *P. verrucosa*, failure
Any with endogenous endophthalmitis	To cure	AmB intravitreal, iv, FCZ po +/- intravitreal +/- topical +/- vitrectomy	C	III	Rao Retina 2004[1541]	N=1, *Alternaria* spp, outcome was VA hand movements
					Pavan AJO 1993[1497]	N=1, *Bipolaris hawaiiensis*, success
Any with endogenous endophthalmitis	To cure	AmB intravitreal, iv + vitrec-tomy	C	III	Zayit-Soudry AJO 2005[1494]	N=1, *Phialemonium curvatum*, suc-cess
					Weinberger MedMycol 2006[1542]	N=1, *P. curvatum*, failure
Any with endogenous endophthalmitis	To cure	AmB intravitreal, topical, iv, FCZ intravitreal, topical, sys-temic, VCZ intravitreal, sys-temic + vitrectomy	C	III	Wu RCBR 2011[1453]	N=1, *Cladosporium* spp., success
Any with endogenous endophthalmitis	To cure	VCZ	C	III	Dogra IJO 2018[1543]	N=1, *Lecythophora* spp., success

Standard dose unless stated otherwise; 5-FC, 5-fluorocytosine; AmB, amphotericin B; CASPO, caspofungin; CNS, central nervous system; CSF, cerebrospinal fluid; d, day(s); D-AmB, amphotericin B deoxycholate; FCZ, Fluconazole; ICZ, itraconazole; ISA, isavuconazole; KCZ, ketoconazole; L-AmB, liposomal amphotericin B; PCZ, posaconazole; qd, once a day; QoE, quality of evidence; SoR, strength of recommendation; SOT, solid organ transplantation; TRB, terbinafine; VA, visual acuity; VCZ, voriconazole.

Recommendation – Lipid formulations of AmB alone or in combination with a triazole and/or echinocandin and VCZ monotherapy are all moderately supported as first-line treatment across all patterns of organ involvement, including the CNS. For CNS infection due to *C. bantiana*, the addition of 5-FC is marginally supported. Specifically for disseminated infections combination therapy with VCZ or PCZ plus an echinocandin or TRB is a moderately supported alternative. The use of D-AmB is discouraged whenever better tolerated lipid formulations of AmB are available.

For *E. rostratum* infections, first-line treatment with VCZ (with or without L-AmB) is moderately supported, while the guideline group marginally supports the use of combination therapy with L-AmB and another azole (with or without surgery). ISA is marginally supported as first-line treatment for *Exserohilum* or *Curvularia* infections, but the group recommends against the use of ISA for infections due to *Cladophialophora* spp. or *Cladosporium* spp. In patients with cutaneous or subcutaneous phaeohyphomycoses the guideline group strongly supports the use of VCZ or ICZ as first line treatment, with a moderate recommendation for PCZ and marginal recommendations for ISA or TRB.

Salvage antifungal therapy

Evidence – ISA has been successfully used as salvage therapy in patients infected with *Alternaria* spp. (n=1) and *Curvularia* spp. (n=1)[381]. PCZ and VCZ have also been successfully used for salvage treatment[926,1491] **(Table 22)**.

Table 22. Antifungal salvage treatment for phaeohyphomycetes/dematiaceous fungi/black fungi infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To cure	ISA	B	IIu	Cornely Mycoses 2018[381]	*N=1, Alternaria* spp., response
					Cornely Mycoses 2018[381]	*N=1, Curvularia* spp., response
Any	To cure	PCZ	B	III	Meriden MedMycol 2012[1544]	N=1, *V. gallopava*, success
					Secníková DermaTeraphy 2014[1545]	N=1, *A. alternata*, success
					Crabol PLOSNTD 2014[1491]	N=2, success 2/2
Any with cutaneous / subcutaneous phaeohyphomycosis	To cure	VCZ +/- TRB + surgical debridement	B	III	Kulkarni ECT 2017[1546]	N=1, *M. romeroi*, success
					Garcia-Reyne TID 2011[1547]	N=1, *Diaporthe longicolla*, success
					Ferrándiz-Pulido Mycoses 2018[926]	N=3, success 3/3
Immunocompromised patients with subcutaneous phaeohyphomycosis	To cure	Intralesional L-AmB	C	III	Mahajan IJD 2014[1548]	N=1, *Rhytidhysteron* spp., success

Immunocompromised patient with chromoblastomycosis	To cure	L-AmB, ICZ, intralesional L-AmB	C	III	Tawade Cutis 2018[1549]	N=1, *Cladosporium carrionii*, success
		ICZ + TRB + local heat therapy	C	III	Tan Mycopathol 2015[1550]	N=1, *F. monophora*, partial response
Immunocompetent patient with recurrent infection	To cure	ICZ	C	III	Geltner Infection 2015[1551]	N=1, *V. gallopava*, relaps after discontinuation
					Maquiné RSBMT 2019[1552]	N=1, *C. bantiana*, failure
Any with chromoblastomycosis	To cure	VCZ	C	III	Criado JDT 2011[1553]	N=3, *F. pedrosoi,* partial response
Standard dose unless stated otherwise; ICZ, itraconazole; ISA, isavuconazole; L-AmB, liposomal amphotericin B; QoE, quality of evidence; SoR, strength of recommendation; TRB, terbinafine; VCZ, voriconazole.						

Recommendation – ISA, PCZ or VCZ are recommended with moderate strength (BIII) for salvage treatment of phaeohyphomycosis.

Other treatment

i) Surgical/medical interventions

Evidence – In several case reports or series**,** surgical interventions (*i.e.* surgery, cryosurgery, cryotherapy, laser therapy, heat therapy or potassium iodide) were performed to contain localized cutaneous infection or reduce infectious burden in advanced phaeohyphomycosis cases[926,1119,1476,1486,1488,1554-1559]. The surgery involved either debridement of the skin and soft tissue, resection of subcutaneous or pulmonary nodules, or drainage of brain abscess. For phaeohyphomycosis cases with cerebral abscess, complete excision with administration of antifungal therapy was documented[930,940,1145,1260,1560-1563]. Complete excision of lesions was shown to be critical for successful management of *C. bantiana*-related CNS infection [1564]. The use of surgical intervention in addition to systemic corticosteroids for patients with allergic fungal sinusitis to reduce symptoms has been noted as well[1565]. For patients with allergic bronchopulmonary mycosis, surgical intervention alone for reducing symptoms was reported[1565] **(Table 23)**.

Table 23. Other treatment options for phaeohyphomycetes/dematiaceous fungi/black fungi infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any with localized cutaneous infection or subcutaneous nodule	To cure	Surgery	A	IIu	Ferrandiz-Pulido Mycoses 2018[926]	N=11, surgery N=6
					Haridasan TID 2017[1119]	N=7
					Schiffelin TID 2017[1566]	N=17
					Guarro JCM 2003[1554]	N=2
					Ben-Ami CID 2009[1486]	N=14, 11/14 survived
					Santos CMI 2017[1488]	N=51, complete excision w/o antifungals N=21, partial debridement 16/30 with antifungals (53.3%), 51/51 cured
	To cure		C	III	Yang MedMycol 2012[1555]	N=1

Any with subcutaneous nodule		Cryotherapy, laser therapy, heat therapy or potassium iodide			Gugnani MedMycol 2006[1556]	N=1
					Torres-Rodriguez ArchDermatol 2005[1557]	N=1
Any with chromoblastomycosis	To cure or reduce infectious burden in advanced cases	Cryosurgery	B	IIu	Bonifaz Mycoses 2001[1558]	N=51
					Castro IJD 2003[1559]	N=22
Any with cerebral abscess	To cure	Surgery + antifungal therapy	A	IIu	Mohammadi Mycoses 2018[1260]	N=1
					Kantarcioglu MedMycol 2017[1560]	N=85
					Doymaz Mycoses 2015[1505]	N=1
					Nandedkar AJN 2015[1567]	N=1
					Jung JKNS 2014[1160]	N=1
					Gadgil JCN 2013[1088]	N=1
					Delfino MedMycol 2006[1561]	N=1
					Jabeen CID 2011[1145]	N=6
					Garg NeurolIndia 2007[930]	N=10
					Chakrabarti MedMycol 2015[940]	N=114, *C. bantiana,* complete excision 52/114 (46.4%), partial excision 41/114 (36.7%)
Any with cerebral abscess	To cure	Adding 5-FC to antifungal treatment	C	III	Chakrabarti MedMycol 2015[940]	N=113, *C. bantiana*
Any with allergic sinusitis	To reduce symptoms	Surgery + systemic corticosteroids	A	IIu	Rinaldi DMID 1987[1565]	N=5
Any	To cure	G-CSF or GM-CSF	C	III	Ben-Ami CID 2009[1486]	N=39, response 16/19 for G-CSF or GM-CSF
Any with allergic sinusitis	To reduce symptoms and corticosteroids	Omalizumab	C	III	Gan AJO 2015[1568]	N=7, unclear how many of those phaeohyphomycosis
Allergic bronchopulmonary mycosis	To reduce symptoms	Surgery	C	III	Halwig ARRD 1985[1562]	N=1
					Rinaldi DMID 1987[1565]	N=5
					Chowdhary MedMycol 2012[1563]	N=1
					Chowdhary MedMycol 2011[1569]	N=1, pediatric patient
Solid organ transplant recipients with cutaneous phaeohyphomycosis	To cure	Surgical excision / debridement	A	IIu	Santos CMI 2017[1488]	N=51, complete excision w/o antifungals N=21, partial debridement 16/30 (53.3%), 51/51 cured

5-FC, 5-fluorocytosine; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICZ, itraconazole; QoE, quality of evidence; SoR, strength of recommendation.

Recommendation – For localized cutaneous infections or subcutaneous nodules, the guideline group strongly supports a complete surgical removal whenever possible. The group strongly supports the use of surgery in addition to systemic antifungal therapy or corticosteroids for patients with cerebral abscess or allergic fungal sinusitis, respectively.

ii) Augmentation of host response

Evidence – G-CSF or GM-CSF has been added to antifungal treatment in a case series that involved 39 cases of proven or probable phaeohyphomycosis[1486].

Recommendation – The guideline group marginally supports G-CSF or GM-CSF to augment host response against phaeohyphomycosis.

Treatment duration

Evidence – There is no standard treatment duration for phaeohyphomycosis, with durations ranging from weeks to months. A median duration of treatment with a variety of antifungal agents (*i.e.* VCZ, PCZ, ICZ, AmB or TRB) was reported as 50 to 73 days in all patients[518] while in patients with underlying malignancy and infection with *E. dermatitidis*, the duration of successful treatment with triazoles ranged between 7 and 64 days[1487]. Among SOT recipients, an average treatment duration of 10 months[1476] was noted, ranging from 3 to 18 months[1488] **(Table 24)**.

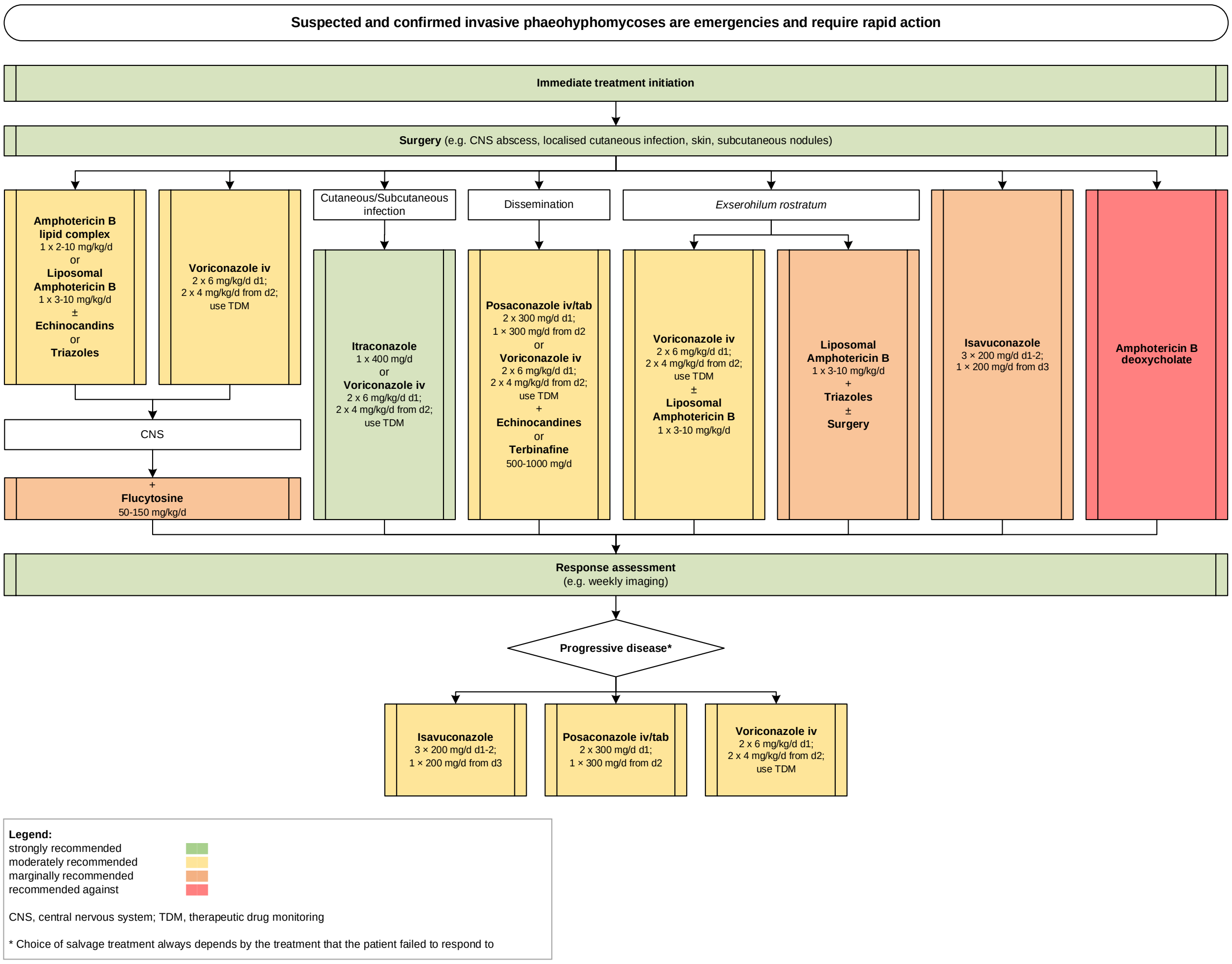
Table 24. Treatment duration for phaeohyphomycetes/dematiaceous fungi/black fungi infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
SOT recipients with cutaneous infection	To cure	ICZ for 3–18 mo	B	III	Schieffelin TID 2014[1476]	N=24; Average treatment among 17 survivors 10 mo (range 6–27 mo)
					Santos CMI 2017[1488]	N=30, ICZ for 3-18 mo
Any	To cure	Long term treatment with VCZ, PCZ, ICZ, AmB, 5-FC	B	III	Revankar OFID 2017[518]	N=99, median duration 50–73 d (range 2–915 d)
Any	To cure	ICZ for a median of 50–73 d for local infections, VCZ for more severe infections +/- TRB or AmB	C	III	Revanker OFID 2017[518]	Local superficial infection: median duration 73 d (range 1–915 d). Local deep infection: median duration 50 d (range 3–710 d). Disseminated infection: median duration treatment 61 d (range 2–720 d).
Hemato-oncological patients with *E. dermatitidis* bloodstream infections	To cure	*E. dermatitidis:* VCZ for 7–64 d	C	III	Vasquez CID 2017[1487]	*E. dermatitidis*, duration of successful treatment range 7-64 d

5-FC, 5-fluorocytosine; AmB, amphotericin B; d, day(s); ICZ, itraconazole; PCZ, posaconazole; mo, month(s); QoE, quality of evidence; SoR, strength of recommendation; SOT, Solid organ transplant; TRB, terbinafine; VCZ, voriconazole.

Recommendation – The guideline group moderately supports treatment until all signs and symptoms of infection have resolved. The treatment duration is determined by clinical response regardless of the type of antifungal agents administered **(Figure 16).**

Figure 16. Optimal treatment pathway for phaeohyphomycosis <u>in adults</u> when all treatment modalities and antifungal drugs are available



Specific considerations on treatment of phaeohyphomycosis in children

Evidence - There are minimal data in children on the treatment of phaeohyphomycosis[924]. All reported pediatric cases had CNS involvement **(Table 25)**.

Table 25. First-line antifungal therapy in children for phaeohyphomycetes/dematiaceous fungi/black fungi infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Immunocompetent	To cure	ABLC 3-6 mg/kg qd + 5-FC, then ABLC 6 mg/kg qd + ICZ 6 mg/kg qd	C	III	Chang JCN 2009[1570]	N=1, 3 yrs, cerebral abscesses, *E. dermatitidis,* failure
Immunocompetent	To cure	L-AmB, VCZ 200 mg bid	C	III	Alabaz MedMycol 2009[1571]	N=1, 8 yrs, systemic infection, *E. dermatitidis*, failure
Hematological patients	To cure	L-AmB + surgery	C	III	Bay RCI 2017[1572]	N=1, 8 yrs, nasal vestibule infection, *Curvularia spicifera*, success
					Saint-Jean CJIDMM 2007[1573]	N=1, 3 yrs, cutaneous infection, *E. rostratum*, success

HSCT	To cure	PCZ 150 mg qd	C	III	Tanuskova JMMCR 2017[1574]	N=1, 8 yrs, lung infection, *E. dermatitidis*, lung, failure
HSCT	To cure	ANID 1.5 mg/kg qd+ VCZ 7 mg/kg qd for 5 d, then ANID 1.5 mg/kg qd + L-AmB	C	III	El Feghaly MMCR 2012[1575]	N=1, 2 yrs, fungemia, *Graphium basitruncatum*, failure
Any	To cure	Various including AmB, surgery, combination L-AmB plus 5-FC	C	IIu	Revankar CID 2004[924]	N=15 children, CNS; most frequent *C. bantiana* and *Ramichloridium mackenziei*, 5/15 survived: AmB + 5-FC + (partial) excision N=2, AMB N=1, lipid AMB + ICZ + aspiration/excision N=2
Standard pediatric dose unless stated otherwise; 5-FC, 5-flucytosine; ANID, anidulafungin; AmB, amphotericin B; ABLC, Amphotericin B Lipid Complex; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; bid, twice a day; CNS, central nervous system; d, day(s); HSCT, hematopoietic stem cell transplantation; ICZ, itraconazole; L-AmB, liposomal amphotericin B; PCZ, posaconazole; qd, once a day; QoE, quality of evidence; SoR, strength of recommendation; VCZ, voriconazole; yrs, years.						

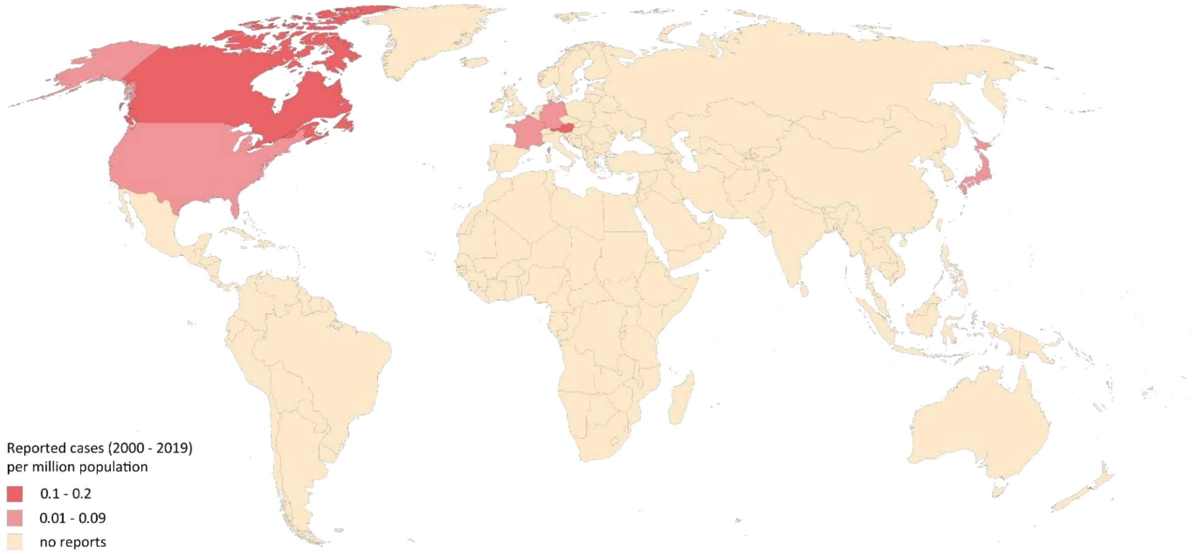
Recommendation - Consideration should be given to prescribe an antifungal with sufficient CNS penetration. Combination therapy containing lipid formulations of AmB is moderately recommended, as is surgery.

5. Rasamsonia

Epidemiology of infections caused by *Rasamsonia* spp.

Rasamsonia is a new genus introduced in 2011 comprising 11 thermotolerant species that were formerly classified in the genera *Geosmithia*, *Penicillium,* or *Talaromyces*[1576]. *Rasamsonia* has rarely been reported as the causative pathogen of fungal infections in humans, and most of these reports originate from northern countries and high-resource settings[10]. *Rasamsonia* can colonize the respiratory tract of patients with CF with variable clinical significance[1577,1578]. Infections caused by *Rasamsonia argillacea* (formerly known as *Geosmithia argillacea*), *Rasamsonia piperina* and *Rasamsonia aegroticola* have been reported mainly in severely ill patients with chronic granulomatous disease or underlying malignancy, and those undergoing hematopoietic stem cell transplantation or lung transplantation[1034,1579-1585]. Diagnosis can be misleading as *R. argillacea* is morphologically similar to *Penicillium* spp. and *Paecilomyces* spp. and misidentification has been frequently reported [1579-1581,1586]. *Rasamsonia*-related infections predominantly affect the lungs and may disseminate to adjacent organs or to the CNS[1580,1585] **(Figure 17)**.

Figure 17. Worldwide distribution of infections caused by *Rasamsonia* spp. (reported cases between 2000 and 2019 per million population)



Cases of severe *Rasamsonia*-related infections reported in the medical literature were identified in a PubMed search on November 15, 2019 using the search string "*Rasamsonia* OR *Geosmithia* OR *Penicillium argillaceum*" that yielded 126 publications. Twenty eight cases have been reported from six countries since 2000[10,1034,1577,1579-1585,1587-1590]. Most cases were reported from Germany (n=8), Canada (n=7) and the United States (n=6). The number of cases reported between 2000 and 2019 is presented as cases per million population per country. The resident population per country was obtained from www.worldometers.info[321].

Diagnosis of Rasamsonia infections

Diagnosis – Microbiology – Conventional Methods

Evidence - Cultures using yeast extract-peptone-dextrose agar, Sabouraud dextrose agar or potato flakes agar can achieve the highest diagnostic value for clinical samples obtained from sterile body sites. Incubation at 28–30°C for up to four weeks has been reported[10]. For superficial and respiratory tract samples, clinical signs and symptoms are important to differentiate between colonization/contamination and infection[1579-1583,1586,1587,1591-1593]. Mucous sputum samples should be pretreated with a mucolytic agent before culture, but these pretreatments may cause false negative GM antigen levels[1594,1595] **(Table 26)**.

Recommendation - The guideline group strongly recommends culture from clinical samples.

Diagnosis – Microbiology – Serology

Evidence – GM cross-reacts with *Rasamsonia* spp. and positive results have been described from BALF and serum [1585] **(Table 26)**.

Recommendations – The guideline group moderately supports GM testing from BALF and serum.

Diagnosis – Microbiology – Molecular-based

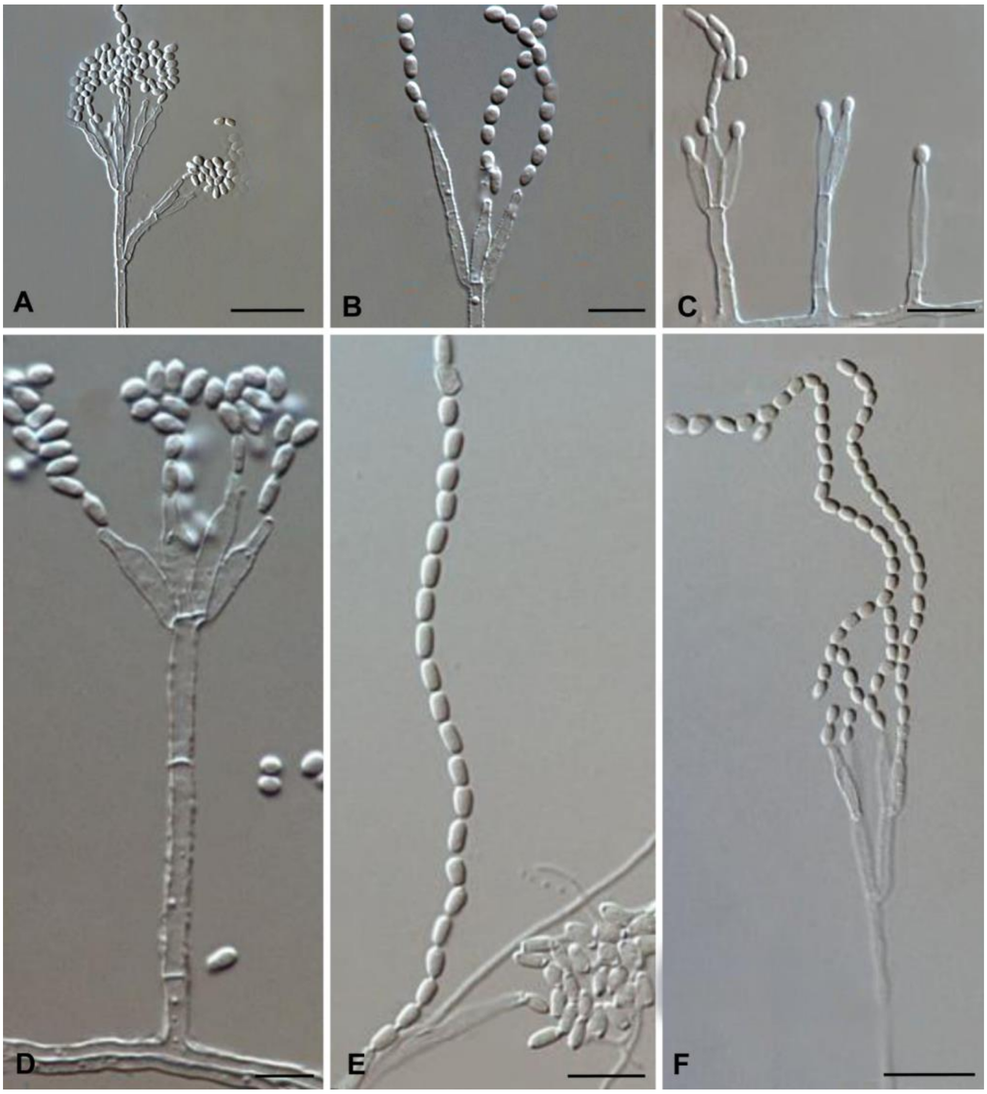
Evidence - Direct fungal detection by real-time PCR of respiratory samples has been reported in CF patients[1596] **(Table 26)**.

Recommendation - The guideline group moderately recommends specific real-time PCR for detection of *Rasamsonia* spp. in respiratory samples from CF patients.

Diagnosis – Microbiology – Species identification

Evidence - Identification to the genus level can be done by microscopic examination. *Rasamsonia* spp. overall resembles *Paecilomyces* spp. and *Penicillium* spp., but *Rasamsonia* spp. differs from *Paecilomyces* spp. in having more regular branched conidiophores with distinct rough-walled structures, and from *Paecilomyces* spp. and *Penicillium* spp. by the shape of the conidia which are cylindrical. See also **Figure 18** with microscopic morphology from the Atlas of Clinical Fungi project[19] **(Table 26)**.

Figure 18. Microscopic morphology of *Rasamsonia* spp. [19]



Panel A-B, *R. argillacea,* phialides with conidia in chains; **Panel C-D**, *Rasamsonia eburnea*, with erect conidiophores and monoverticillate, later becoming biverticillate penicilli producing ellipsoidal or ovoidal, conidia; **Panel E-F**, *R. piperina*, phialides with conidia in chains. Scale bars = 10 µm.

Accurate identification to the species level requires ITS/β-tubulin sequencing[555,1577,1580,1581,1591,1593,1597-1599]. Genotyping can be achieved by repetitive sequence-based PCR and random amplification of polymorphic DNA[1581,1599] **(Table 26)**.

Recommendation - The guideline group strongly recommends to perform microscopy of cultures, followed by ITS/β-tubulin gene sequencing for species identification.

Diagnosis – Microbiology – Susceptibility testing

Evidence - Antifungal susceptibility testing according to EUCAST or CLSI guidelines may be useful to determine susceptibilities[1580,1583,1587,1590,1591]; however, the absence of interpretive breakpoints warrants caution when utilizing MICs to guide treatment **(Table 26)**.

Recommendation - The guideline group strongly recommends that antifungal susceptibility testing should be performed for epidemiological purposes, while susceptibility testing to guide the choice of antifungal therapy is moderately recommended.

Diagnosis – Microbiology – Pathology

Evidence - Histological examination of PAS, H&E, GMS stained tissue biopsy sections is important for ascertaining fungal structures[1583,1587,1593] **(Table 26)**.

Recommendation - The guideline group strongly recommends that histology should be performed whenever possible.

Diagnosis – Microbiology – Imaging studies

Evidence – Diagnostic imaging studies of the affected organ/systems (CT for thorax, CT or MRI for brain) can delineate the extent of involvement of the infection[1580,1582-1584,1587]. Invasion of adjacent structures as shown by CT examination has been reported[1580,1582-1584,1587] **(Table 26)**.

Table 26. Microbiological, histopathological and imaging diagnostics for *Rasamsonia* infections

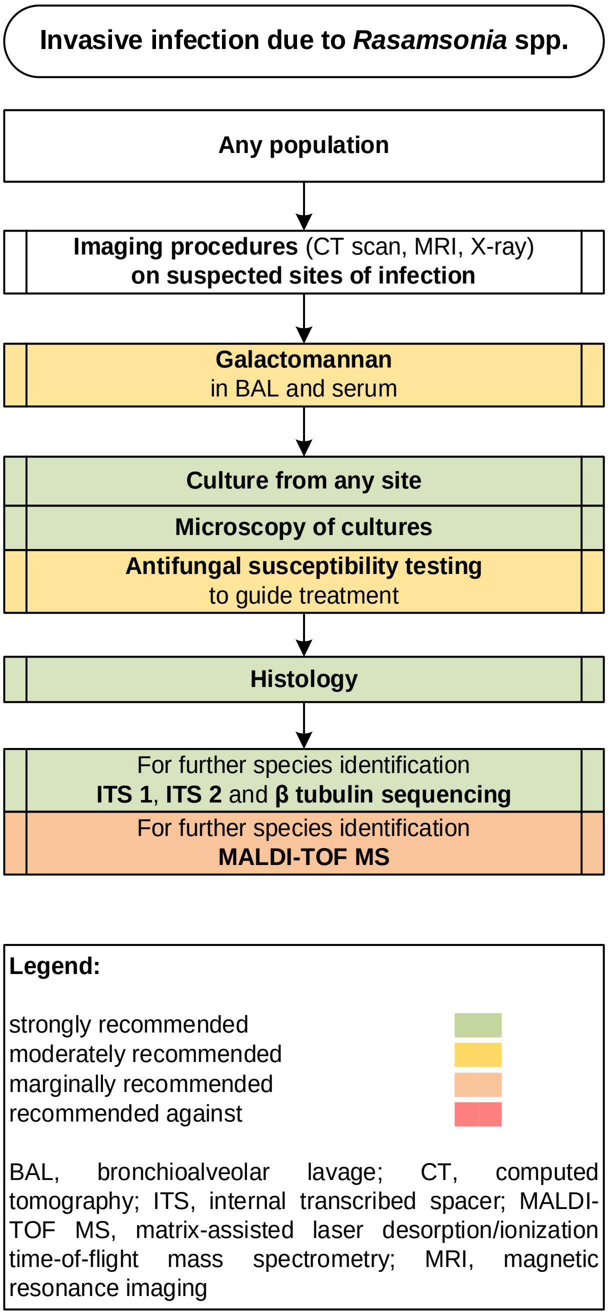
Population	Intention	Intervention	SoR	QoE	Reference	Comment
Microscopy, culture, MIC testing						
Any	To diagnose	Histology (GMS & HE)	A	IIIu	Machouart JCM 2011[1583]	
					Doyon JCM 2013[1587]	
					Sohn ALM 2013[1593]	
Any	To diagnose	Culture, SDA 28-30°C, ≤4 wk	A	IIIu	Abdolrasouli Mycoses 2018[1578]	
					De Ravin CID 2011[1580]	
					Doyon JCM 2013[1587]	
					Hong JCF 2017[1581]	
					Ishiwada Mycopathol 2016[1582]	
					Machouart JCM 2011[1583]	
					Matos Mycoses 2015[1592]	
					Sohn ALM 2013[1593]	
Any with CF / chronic granulomatous disease	To diagnose	Culture – selection of YPDA, SDA, PFA	A	III	Giraud JCM 2010[1591]	
					Giraud FM 2013[1586]	
					Babiker MMCR 2019[1579]	
Any with CF	To diagnose	Culture, sputum with mucolytic, homogenize, serial dilution, SDA + 100mg/L chloramphenicol, 28-30°C, ≤4 wk	A	IIIu	Masoud-Landgraf MedMycol 2014[1594]	
					Abdolrasouli Mycoses 2018[1578]	
Any	To diagnose	Microscopy of cultures	A	III	Hong JCF 2017[1581]	
					Giraud JCM 2010[1591]	
Children/neonates	To diagnose	Culture, gastric aspiration of sputum	B	III	Fujita TJEM 2019[1588]	Acid resistant
Chronic pulmonary disease with colonization	To diagnose underlying disease	Test for CF	C	III	Grenouillet Mycopathol 2018[1600]	Colonization indicative of CF
Any with CF	To identify susceptibility patters	Antifungal susceptibility testing according to EUCAST or CLSI	B	III	Giraud JCM 2010[1591]	
					Hong JCF 2017[1581]	
					Doyon JCM 2013[1587]	
					Machouart JCM 2011[1583]	
					Houbraken JCM 2013[1598]	
Any	To guide treatment	Antifungal susceptibility testing of *R. argillacea*	B	III	De Ravin CID 2011[1580]	
Serology assays						
Any	To diagnose	GM testing (Platelia, Bio-Rad) in BAL and serum	B	III	Valentin BMT 2012[1585]	
					Cumming DMID 2007[1601]	
					Machouart JCM 2011[1583]	
					Sohn ALM 2013[1593]	
Nucleic-acid based assays/MALDI-TOF MS						
Any	To diagnose from any sample	Molecular sequencing (ITS1, ITS2, β-tubulin),	B	III	Hong JCF 2017[1581]	
Any	To diagnose from autopsy tissue	PCR/ESI-TOF-MS	B	III	Giraud FMB 2013[1602]	
CF	To detect in respiratory secretions	Real-time PCR	B	II	Steinman NMNI 2014[1596]	Not *R. eburnea*
CF	To diagnose from sputum	Oligoarray, ITS2	C	III	Bouchara JCM 2009[551]	N=20 fungal species, sens. 100% and spec. 99.2%, *Rasamsonia emersonii* included
Any	To identify	MALDI-TOF MS	C	IIIu	Barker MedMycol 2014[1597]	Only *R. argillacea*
Any	To identify	ITS1-ITS2/beta-tubulin sequencing	A	IIu	Houbraken JCM 2013[1598]	
					De Ravin CID 2011[1580]	
					Hong JCF 2017[1581]	
					Barker MedMycol 2014[1597]	
					Marguet MMCR 2012[1577]	
					Sohn ALM 2013[1593]	
					Giraud JCM 2010[1591]	
CF	To identify	Culture + ITS1-ITS2/beta-tubulin sequencing	A	III	Ziesing MedMycol 2016[555]	
Any	To identify	Morphology, molecular genetics (beta-tubulin and calmodulin genes, ITS analysis)	A	IIu	Houbraken JCM 2013[1598]	Differs from *Paecilomyces* by more regular branching

Any with CF	To identify and genotype	Repetitive sequence-based PCR	C	III	Mouhajir JCM 2016[1603]	
Any	To genotype	RAPD	C	III	Guevara-Suarez JCM 2016[1604]	
Tissue-based diagnosis						
Any	To diagnose	Histology; PAS, HE, GMS stainings	A	III	Hong JCF 2017[1581]	
					Machouart JCM 2011[1583]	
Any with CF	To establish definitive diagnosis	Autopsy	A	III	Hong JCF 2017[1581]	
					Valentin BMT 2012[1585]	
Imaging studies						
Any with CGD	To detect	Chest CT	A	III	De Ravin CID 2011[1580]	N=5
Any	To detect contiguous spread & dissemination	Chest CT, cranial CT	A	III	De Ravin CID 2011[1580]	
					Ocak RCR 2019[1584]	
					Doyon JCM 2013[1587]	
					Ishiwada Mycopathol 2016[1582]	
					Machouart JCM 2011[1583]	

AmB, amphotericin B; BAL, bronchoalveolar lavage; CF, cystic fibrosis; CGD, chronic granulomatous disease; CLSI, Clinical and Laboratory Standards Institute; CT, computed tomography; ESI-TOF MS, Electrospray Ionization time of flight mass spectrometry; EUCAST, European Committee for Antimicrobial Susceptibility Testing; GM, Galactomannan; GMS, Grocott-Gomori's methenamine silver; HE, hematoxylin-eosin; ICZ, itraconazole; ITS, internal transcribed spacer; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; MIC, minimal inhibitory concentration; PAS, periodic acid-Schiff; PCR, polymerase chain reaction; PCZ, posaconazole; PFA, potato flakes agar; QoE, quality of evidence; SDA, Sabouraud Dextrose agar; SoR, strength of recommendation; TRB, terbinafine; VCZ, voriconazole; wk, week(s); YPDA, yeast extract-peptone-dextrose agar.

Recommendation - The guideline group strongly recommends performing radiological examinations to delineate the extent of involvement of the infection **(Figure 19)**.

Figure 19. Optimal diagnostic pathway for *Rasamsonia* infections, when all imaging and assay techniques are available



Treatment approaches to *Rasamsonia* infections

Treatment in Adults

Evidence - Evidence for the treatment of invasive infections due to *Rasamsonia* spp. is sparse, with currently only 23 cases available in the literature[10]. Colonization, specifically in CF patients, was reported much more frequently. However, mortality reaches upwards of 40% in invasive infections, so treatment should not be delayed for this significant pathogen[10]. Overall, high MICs have been reported for triazoles, while echinocandins show best *in vitro* susceptibility[1577,1579-1583,1585,1587,1590]. CASPO and MICA have been used successfully[1579,1584,1587,1605]; successful use of combination therapy with an echinocandin plus PCZ or L-AmB has also been reported[1579,1584,1605]. Surgery is another important cornerstone of treatment of localized infections[1579,1580,1587]. Secondary prophylaxis with PCZ has been reported[1577,1580,1583,1587] **(Table 27)**.

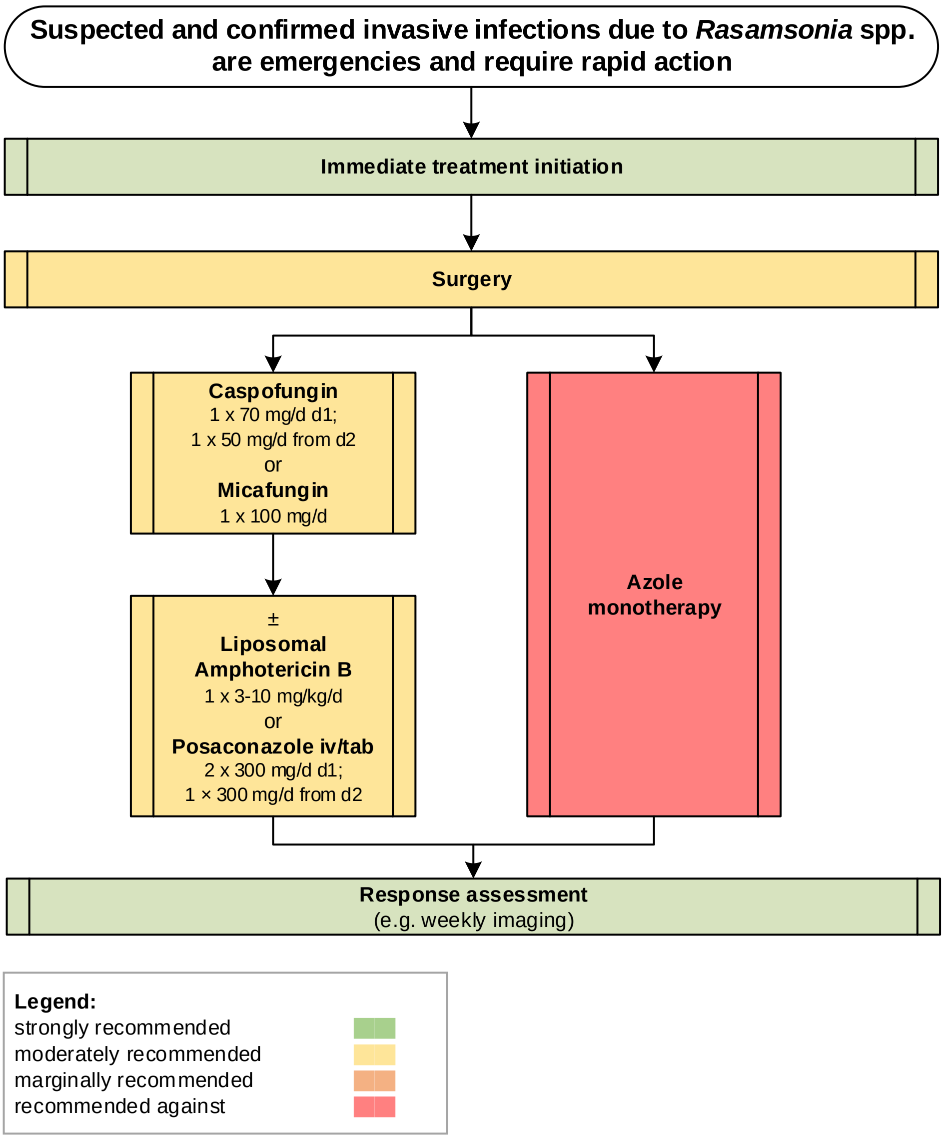
Table 27. Therapy for *Rasamsonia* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
First-line antifungal therapy						
Any, any with CGD	To cure and avoid treatment failure/death	Avoid azole monotherapy	A	IIu	De Ravin CID 2011[1580]	Azoles lead mostly to failure, MICs VCZ and occasional ICZ >8-16, PCZ usually >4
					Marguet MMCR 2012[1577]	
					Machouart JCM 2011[1583]	
					Valentin BMT 2012[1585]	
					Doyon JCM 2013[1587]	
					Ishiwada Mycopathol 2016[1582]	
					Hong JCF 2017[1581]	
					Babiker MMCR 2019[1579]	
					Steinmann AAC 2016[1590]	*In vitro* study in CF; Echinocandins with highest activity; resistance to azoles
Any	To cure	CASPO OR MICA +/- PCZ	B	III	Abdolrasouli Mycoses 2018[1578]	N=1, CF, response to CASPO
					Doyon JCM 2013[1587]	N=1, MICA + PCZ, response
HSCT with GVHD	To cure	CASPO + L-AmB +/- PCZ	B	III	Valentin BMT 2012[1585]	N=1, initial response, then deterioration; died
					Ocak RCR 2019[1584]	N=1, partial clinical response
HSCT	To cure	L-AmB	C	III	Corzo-Leon Mycoses 2015[1034]	N=1, died
Antifungal salvage treatment						
Any with CF	To cure	Echinocandin	C	III	Abdolrasouli Mycoses 2018[1578]	N=1, clinical stable
Any with CGD	To cure	Empirical treatment with PCZ, L-AmB and CASPO	C	III	Ocak RCR 2019[1584]	
					Babiker MMCR 2019[1579]	
Other treatment options						
Any with CGD	To cure	Surgery	B	III	De Ravin CID 2011[1580]	N=4, 1/4 died
					Babiker MMCR 2019[1579]	N=1, success
Immunocompetent	To cure	Surgery / resection of infected (graft) material	B	III	Doyon JCM 2013[1587]	N=1, success
Treatment duration						
Any with CGD	To cure	Prolonged treatment / Secondary prophylaxis	B	IIu	Machouart JCM 2011[1583]	N=9, 5/9 survived, 4/9 prolonged treatment/secondary prophylaxis up to 6 yrs
					De Ravin CID 2011[1580]	
Immunocompetent	To cure	Prolonged treatment / Secondary prophylaxis	B	III	Doyon JCM 2013[1587]	N=1, 13 mo of PCZ, success

Standard dose unless stated otherwise; CASPO, caspofungin; CF, cystic fibrosis; CGD, chronic granulomatous disease; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; ICZ, itraconazole; L-AmB, liposomal amphotericin B; MICA, micafungin; mo, months; PCZ, posaconazole; QoE, quality of evidence; SoR, strength of recommendation; TRB, terbinafine; VCZ, voriconazole.

Recommendations - The guideline group strongly recommends to avoid azole monotherapy due to reports of clinical failure and high MICs. The guideline group moderately recommends primary treatment with an echinocandin, or a combination of an echinocandin with either L-AmB or PCZ. Surgery, whenever possible is moderately recommended for treatment of *Rasamsonia* infections. Treatment duration depends on the affected site, clinical response and underlying condition but can be up to several months. Secondary prophylaxis is moderately recommended **(Figure 20)**.

Figure 20. Optimal treatment pathway for *Rasamsonia* infections <u>in adults</u> when all treatment modalities and antifungal drugs are available



Specific considerations on treatment of Rasamsonia infections in children

Evidence – Invasive infections caused by *Rasamsonia* spp. have been described as a complication occurring in children with CGD[1580]. Breakthrough infections while on mold-active azole prophylaxis have been reported, but treatment with MICA has been associated with favorable outcome [1402,1580,1588] **(Table 28)**.

Table 28. First-line antifungal therapy in children for *Rasamsonia* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any with CGD	To cure	MICA+VCZ	B	III	Ishiwada Mycopathol 2015[1582]	N=1, 16 yrs, mixed infection with *Aspergillus nidulans*
Any	To cure	MICA	B	III	Fujita TJEM 2019[1588] De Ravin CID 2011[1580] Tanuskova JMM 2017 [1402]	N=3 CGD, 3/3 survived; N=1 Allo SCT; survived
Any with CF	To cure and improve lung function	Secondary prophylaxis	B	III	Marguet MMCR 2012[1577]	N=1, long-term prophylaxis with PCZ + intermittent echinocandin improved FEV1

Standard pediatric dose unless stated otherwise; CF, cystic fibrosis; CGD, chronic granulomatous disease; d, day(s); FEV1, forced expiratory volume in one second; iv, intravenous; MICA, micafungin; po, orally; QoE, quality of evidence; SoR, strength of recommendation; VCZ, voriconazole; wk, week(s); yrs, years.

Recommendations – Echinocandins with or without another antifungal are moderately recommended as first-line treatment in children, in line with recommendations in adults. Triazole monotherapy should be avoided. Surgery is moderately recommended if feasible and infection is not disseminated.

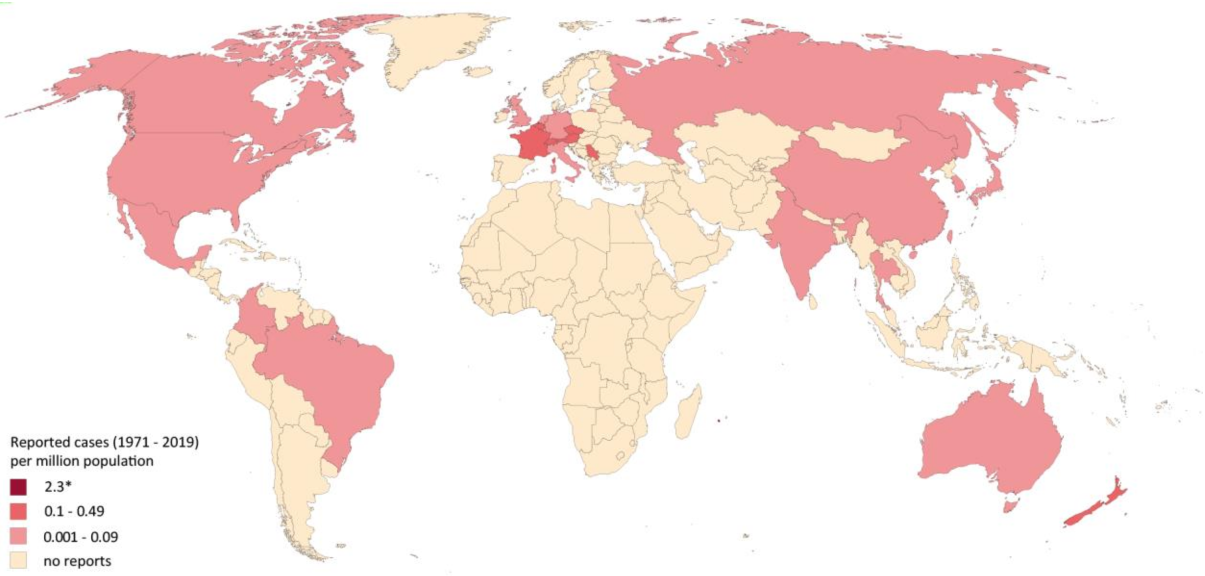
6. *Schizophyllum* and other basidiomycetes

Epidemiology of infections caused by *Schizophyllum* spp. and other basidiomycetes

Basidiomycetes, such as *Schizophyllum commune*, *Coprinopsis cinerea* (formerly *Hormographiella aspergillata)* and *Phanerochaete chrysosporium* (formerly *Sporotrichum pruinosum*) are often found in decaying matter. Despite their worldwide distribution invasive infections due to these fungal agents are rare. In humans, *S. commune* accounts for the majority of infections caused by these organisms, the vast majority of which presents as pulmonary disease or sinusitis[1606-1610]. *C. cinerea* is the second most prevalent basidiomycete causing mainly infections of the lung[1611,1612]. Eye infections have also been identified[1613-1615]. There are only few reports of infections affecting other organs such as brain, spine or peritoneum[1616-1618]. Fungemia is uncommon[1619,1620].

Co-infection with other molds has often been reported, possibly because affected patients are critically ill or severely immunocompromised and thus are susceptible to concomitant infections[1611]. True prevalence of these infections is likely underestimated, as microbiological methods of species identification are rather difficult due to lack of asexual reproduction[1621] **(Figure 21)**.

Figure 21. Worldwide distribution of infections caused by *Schizophyllum* spp. and other basidiomycetes (reported cases between 1971 and 2019 per million population)



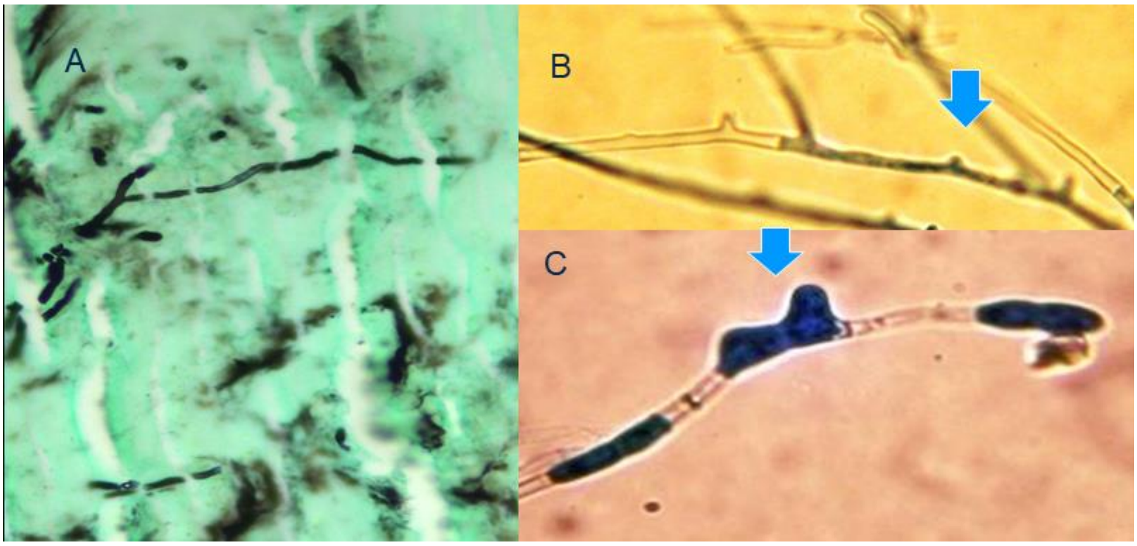
Cases of infections caused by *Schizophyllum*, *Coprinopsis* and other basidiomycetes reported in the medical literature were identified in a PubMed search on October 15, 2019 using the search string "(*Trametes OR Lenzites* OR *Lophotrichus* OR *Schizophyllum* OR Ustilago OR *Coprinus* OR *Coprinopsis* OR *Hormographiella aspergillata* OR *C. cinereus*) AND (infection OR case report OR report [title/abstract] OR case [title/abstract] OR abscess OR fungemia OR blood OR invasive)" excluding reports on plants and animals that yielded 748 publications. Reports of 103 cases of rare basidiomycetes-related infections have been identified from 27 countries, 88 since the year 2000[239,1034,1116,1209,1606,1607,1609,1611-1674]. *S. commune* and *C. cinerea* accounted for 73% of the cases. Infections due to *P. chrysosporium*, *Schizophyllum radiatum*, *Ustilago* spp. and other basidiomycetes were found sporadically in the literature. Most cases were reported from the United States (n=11) and India (n=6). In Singapore and Hong Kong one case each has been reported since 1971. The number of cases reported between 1971 and 2019 is presented as cases per million population per country. The resident population per country was obtained from www.worldometers.info[321]. *Two cases of infections caused by *Trametes polyzona* were reported from La Réunion (2.3 cases per million population between 1971 and 2019)[1639].

Diagnosis of infections caused by *Schizophyllum* and other basidiomycetes

Diagnosis – Microbiology – Conventional Methods

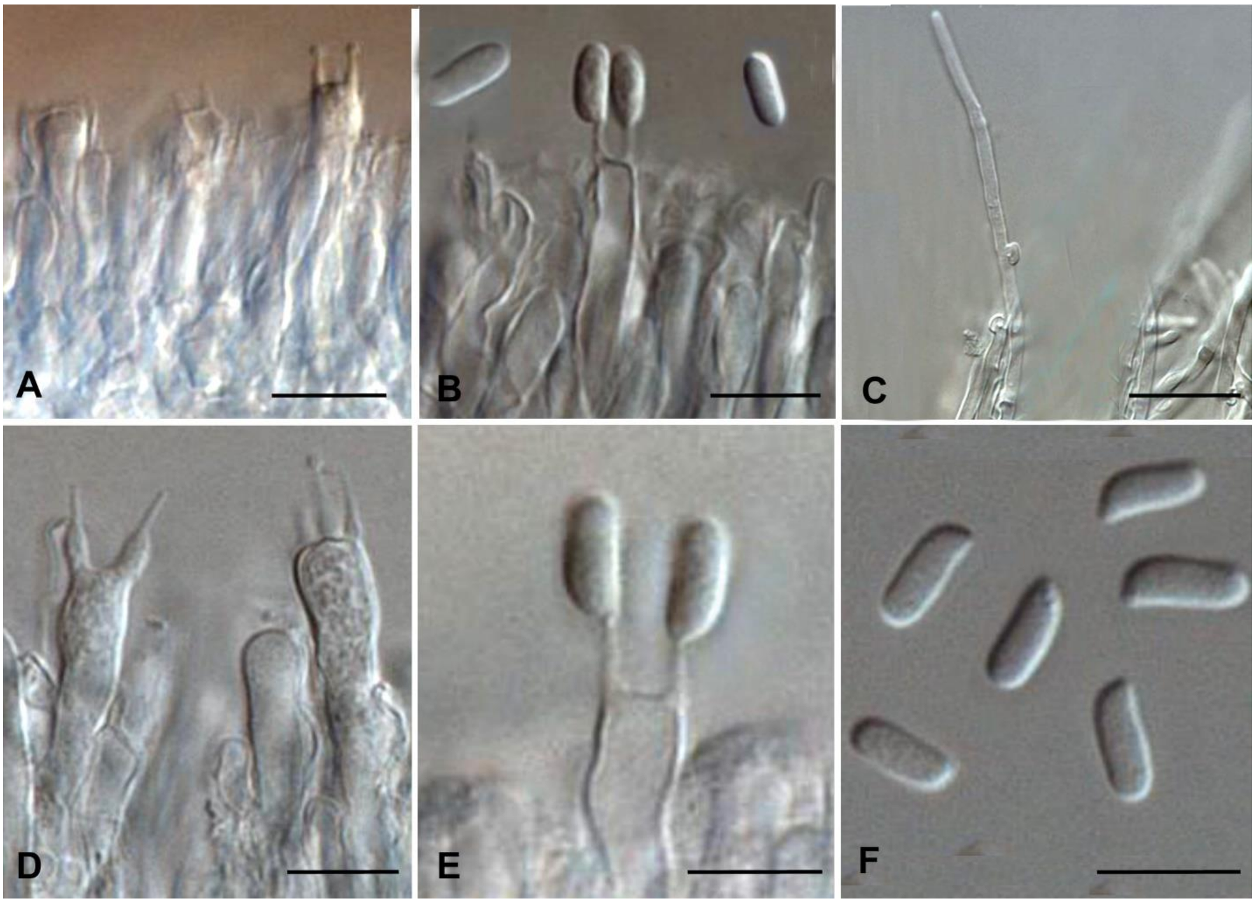
Evidence – Culture is mandatory for species identification and antifungal susceptibility testing, but many basidiomycetes are sterile and do not sporulate in the laboratory[1621]. In microscopy the hyphal clamp connections with spicules suggest *S. commune*[1609]. See also **Figure 22 and Figure 23** for microscopic morphology from the Atlas of Clinical Fungi project[19].

Figure 22. Microbiological characteristics of *S. commune* (owned by co-author V. Arsic-Arsenjevic)



Panel A, GMS stain; **Panel B-C,** Spicula (blue arrows) in lactophenol cotton blue stain, microscopy x 400 and x 1000.

Figure 23. Microscopic morphology of *Schizophyllum* spp. [19]



Panels A-C, *S. commune*, palisade of basidia located on the gills, basidiospores, hyphae with clamp connections. **Panels D-F**, *S. radiatum*, basidia producing basidiospores and basidiospores. Scale bars = 10 µm.

For non-sterile samples, such as respiratory tract specimens, distinguishing among genuine infection, colonization, and contamination is important **(Table 29)**.

Recommendation - The guideline group strongly recommends that tissue microscopy and culture should be performed when possible.

Diagnosis – Microbiology – Serology

Evidence – In general, basidiomycetes lack GM and BDG in their cell walls, but cases with elevated BDG have been reported[1646,1675-1677]. A positive GM test may suggest co-infection with *Aspergillus* species[1612,1644,1646,1665,1675,1676]. *S. commune* infection may lead to false-positive cryptococcal antigen testing and may result in misdiagnosis[1631] **(Table 29)**.

Recommendation – Serological testing is not recommended as part of the diagnostic evaluation for infections caused by *Schizophyllum* and other basidiomycetes.

Diagnosis – Microbiology – Molecular-based

Evidence – PCR sequencing of cultured strains and of formalin-fixed paraffin-embedded tissue samples can be useful for detection of basidiomycetes such as *H. aspergillata* and *Phellinus undulatus*[1641] **(Table 29)**.

Recommendation - The guideline group moderately recommends PCR from formalin-fixed paraffin-embedded tissue samples.

Diagnosis – Microbiology – Species identification

Evidence – Definite identification requires PCR of fungal isolates followed by ITS and/or D1/D2 sequencing for this heterogeneous group of fungi[1606,1609,1613,1641,1644,1646,1665,1666,1672,1673,1675-1679]. MALDI-TOF MS has been reported to be useful for identification of *S. commune*[1606] **(Table 29)**.

Recommendation – While species identification of basidiomycetes may be difficult due to incomplete public databases and rareness of reference strains the guideline group strongly recommends that ITS and/or D1/D2 sequencing should be performed on the culture isolate for species identification, and marginally recommends MALDI-TOF MS for the same purpose.

Diagnosis – Microbiology – Susceptibility testing

Evidence – Antifungal susceptibility testing is useful to determine susceptibilities[1646,1680-1682]; however, the absence of interpretive breakpoints warrants caution when utilizing MICs to guide treatment **(Table 29)**.

Recommendation - The guideline group strongly recommends that antifungal susceptibility testing should be performed for identifying susceptibility patterns, while susceptibility testing to guide the choice of antifungal therapy is moderately suggested.

Diagnosis –Pathology

Evidence – Histological examination of PAS and/or GMS stained tissue biopsy sections are important for ascertaining fungal invasion, but are often inconclusive and may confound basidiomycetes with *Aspergillus* spp.[1613,1627,1634,1641,1646,1666,1672,1675,1676,1679] **(Table 29)**.

Recommendation – The guideline group moderately recommends that histopathology should be performed when possible.

Diagnosis – Microbiology – Imaging studies

Evidence – Most diagnostic imaging studies involve infections caused by *Hormographiella* spp.. CT scan is useful to detect pulmonary and sino-orbito-cerebral infections[1641] and MRI has been used to detect CNS involvement[1641,1677] **(Table 29)**.

Table 29. Microbiological, histopathological and imaging diagnostics of infections caused by *Schizophyllum* spp.and other basidiomycetes

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Microscopy, culture, MIC testing						
Any	To diagnose	Microscopy of tissue biopsy	A	III	Chowdhary JCM 2013[1632] Hoenigl Mycoses 2013[1609] Saha Mycopathol 2013[1614] Toya IJH 2013[1683] De Ravin JCM 2014[1679] Verma Mycopathol 2014[1684] Shigemura Infection 2015[1676] Lim AJD 2017[1685] Jain JMM 2019[1613] Nanno TID 2016[1646]	Observation of tissue invasion by fungi. No definite identification of species provided. Occasionally spicules on the hyphae may be seen in biopsy samples from lung and sinuses. Culture and identification are mandatory for species identification.
Any	To diagnose	Culture	A	III	Friman SJID 2006[1686] Chowdhary JCM 2013[1632] Hoenigl Mycoses 2013[1609] Saha Mycopathol 2013[1614] Toya IJH 2013[1683] Chan JCM 2014[1631] De Ravin JCM 2014[1679] Verma Mycopathol 2014[1684] Shigemura Infection 2015[1676] Hardin Mycopathol 2017[1640] Lim AJD 2017[1685] Heiblig Mycoses 2015[1641] Surmont MedMycol 2002[1666]	Always need to distinguish between genuine infection, colonization and contamination specifically in respiratory specimens. Other molds co-exist in respiratory specimens culture. Multiple cultures may be necessary. Complementary with tissue biopsy. Basidiomycete colonies in culture are white cottony to fluffy, fast-growing, with yellow- brown reverse. Basidiomycetes are sensitive to cycloheximide, allowing them to be distinguished from *Coccidioides* spp. Many basidiomycetes are sterile and will not sporulate in the laboratory, although some develop clamp connections or spicules on the hyphae, and some isolates may produce basidiocarps (after exposure of the culture plate to alternating cycles of light and darkness). Definitive species identification requires ITS and or LSU sequencing
Any	To diagnose *S. commune*	Morphology	C	IIIu	Michel MedMycol 2016[1606]	Fungus usually does not sporulate under classical culture conditions
Any	To guide treatment and correlate MICs with outcome for basidiomycetes	Antifungal susceptibility testing with CLSI method	Bu	IIIu	Singh JCM 2013[1660]	MIC AmB < 1 µg/ml, VCZ < 0.25 µg/ml
Any	To determine MICs of *S. commune, Bjerkanderaadusta* and *Coprinus* spp.	Antifungal susceptibility testing	A	IIu	Gonzalez AAC 2001[1680]	MIC90 AmB 0.5 µg/ml, ICZ 0.125 µg/ml, PCZ 0.5 µg/ml, VCZ 0.5 µg/ml for *S. commune*, *Bjerkandera adusta* and *Coprinus* spp., FCZ and 5-FC higher MIC90 but within achievable concentrations in serum
Any	To determine MICs of *C. cinerea* (formerly *H. aspergillata*) and *Coprinellus domesticus* (formerly *Hormographiella verticillata).*	Antifungal susceptibility testing	A	IIu	Gené AVL 1996[1687] Verweij JCM 1997[1672] Nanno TID 2016[1646]	MICs miconazole 0.6 to 5.0 µg/ml, ICZ 0.07 to 0.6 µg/ml, and KCZ 0.2 to 1.6 µg/ml. Resistant to FCZ 20 to 80 µg/ml and 5-FC 322 to 0.322 µg/ml, susceptibility to AmB variable 0.07 to 4.6 µg/ml. All strains of *C. domesticus* susceptible to AmB; 4/7 of *C. cinerea* resistant 2.3 to 4.6 µg/ml
					Abuali JCM 2009[1623]	N=1, *C. cinerea,* resistant to echinocandins and AmB, but susceptible to azoles
Any	To determine MICs of *S. commune*	Antifungal susceptibility testing	A	IIu	Chowdhary AAC 2013[1681]	Low geometric mean MICs of AmB 0.29 µg/ml, ISA 0.19 µg/ml, ICZ 0.2 µ g/ml, VCZ 0.24 µg/ml,. High geometric mean MICs of FCZ 19.39 µg/ml and 5-FC

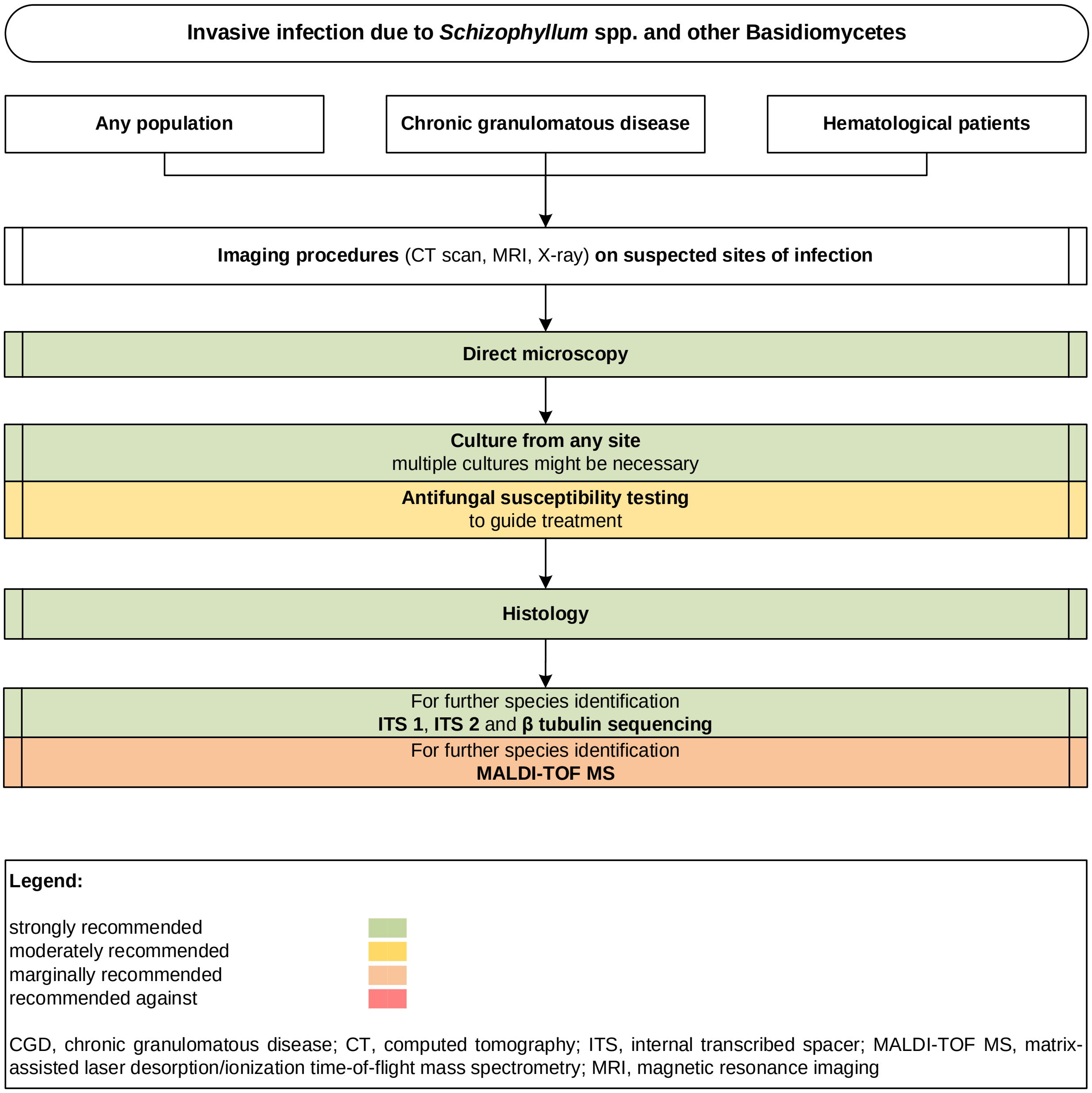
						17.28 g/ml. 5/8 cases of ABPM treated with ICZ had no recrudescence
Any	To determine MICs of *S. commune + S. radiatum*	Antifungal susceptibility testing	A	IIu	Siqueira JCM 2016[1662]	Geometric mean MICs AmB 0.29 µg/ml, CFG 0.58 µg/ml and TBF 0.79 µg/ml, ICZ 1.67 µg/ml and PCZ 2.93 µg/ml. *S. radiatum* showed higher GM MICs for all the antifungals than *S. commune*, especially for ICZ and PCZ
Any	To determine MICs of *Inonotus/Phellinus* spp.	Antifungal susceptibility testing, E-test	A	III	Davis PIDJ 2007[1634] Sutton JCM 2005[1667] Ramesh JCI 2014[1682]	Case reports
Serology assays						
Any	To diagnose	GM	D	III	Suarez JCM 2011[1665] Lagrou JMM 2005[1644] Nanno TID 2016[1646] Godet Mycopathol 2017 Haidar Mycoses 2017[1675] Shigemura Infection 2015[1676]	Not used for detecting invasive infection with basidiomycetes. Positive GM can suggest co-infection with *Aspergillus* spp.*;* ELISA cross-reactivity with *C. cinerea* or *Inonotus/Phellinus* spp. cannot be excluded
Any	To diagnose	BDG	D	III	Chauhan LabMed 2017[1616] Nanno TID 2016[1646] Haidar Mycoses 2017[1675] Shigemura Infection 2015[1676] Koncan JMBT 2016[1688]	N=3 with elevated BDG, but in general basidiomycetes lack GM and BDG in their cell walls
S. commune empyema thoracis	To diagnose	Cryptococcal Ag latex agglutination	D	III	Chan JCM 2014[1631]	Cross-reactivity with *Cryptococcal* antigen test leading to misdiagnosis
Nucleic-acid based assays/MALDI-TOF MS						
Any	To diagnose *C. cinierea*	PCR	B	III	Heiblig Mycoses 2015[1641]	PCR from formalin-fixed paraffin-embedded tissue samples. Described for *C. cinerea.*
Any	To identify *S. commune*	MALDI-TOF MS	C	III	Michel MedMycol 2016[1606]	Homemade reference spectra library must be used
Any	To identify *S. commune*	ITS sequencing	A	IIu	Won ALM 2012[1689] Michel MedMycol 2016[1606] Buzina JCM 2001[1678]	
Any	To identify *Hormographiella* spp.	ITS sequencing	A	III	Verweij JCM 1997[1672] Lagrou JMM 2005[1644] Suarez JCM 2011[1665] Jain JMM 2019[1613] Nanno TID 2016[1646] Correa Martinez NMI 2017[1690] Heiblig Mycoses 2015[1641] Surmont MedMycol 2002[1666] Chauhan LabMed 2019[1616]	N=10, all case reports
Any	To identify *Hormographiella* spp.	D1/D2 sequencing	A	III	Nanno TID 2016[1646]	
Any	To identify *Inonotus/Phellinus* spp.	ITS, D1/D2 sequencing	B	III	De Ravin JCM 2014[1679] Haidar Mycoses 2017[1675] Shigemura Infection 2015[1676] Williamson JMM 2011[1673]	Case reports, species identification difficult besides sequencing due to incomplete public database/small amount of reference strains
Tissue-based diagnosis						
All	To diagnose	Histopathology of biopsy tissue; PAS and GMS stainings	C	III	Bojic Mycoses 2013[1627] Jain JMM 2019[1613] Nanno TID 2016[1646] Verweij JCM 1997[1672] Correa Martinez NMI 2017[1690] Heiblig Mycoses 2015[1641] Surmont MedMycol 2002[1666]	*Hormographiella* spp.: microscopic examination often inconclusive, only suggestive, may confound with *Aspergillus* spp.

Any	To diagnose	Microscopy of tissue biopsy	A	III	Chowdhary JCM 2013[1632]	Observation of tissue invasion by fungi. No definite identification of species provided. Occasionally spicules on the hyphae may be seen in biopsy samples from lung and sinuses. Culture and identification are mandatory for species identification.
Hematological/allogeneic SCT patients with respiratory symptoms or persistent neutropenic fever	To diagnose	Lung biopsy with histopathology (bronchoscopic or transthoracic)	A	III	Nanno TID 2016[1646] Surmont MedMycol 2002[1666]	
Any with CGD	To diagnose	Biopsy of inflamed tissue with histopathology and stainings	C	III	Davis PIDJ 2007[1634] De Ravin JCM 2014[1679] Haidar Mycoses 2017[1675] Shigemura Infection 2015[1676]	Case reports
Imaging studies						
Hematological/allogeneic SCT patients with respiratory symptoms or persistent neutropenic fever	To detect pulmonary infection and assess imaging characteristics of *Hormographiella* spp. infection	Chest (HR-) CT	A	III	Suarez JCM 2011[1665] Bojic Mycoses 2013[1627] Lagrou JMM 2005[1644] Nanno TID 2016[1646] Godet Mycopathol 2017[1612]	N=5
Hematological/allogeneic SCT patients	To detect sino-orbito-cerebral infection and assess imaging characteristics of sinusitis caused by *Hormographiella* spp.	CT Sinuses	A	III	Heiblig Mycoses 2015[1641]	Case report
Hematological patients with neurologic symptoms/seizures	To detect CNS involvement of *Hormographiella* spp. infection	MRI	A	III	Chauhan LabMed 2019[1616] Nanno TID 2016[1646] Heiblig Mycoses 2015[1641]	Case reports
Any with CGD	To detect granulomatous inflammation and assess imaging characteristics	MRI	B	III	Davis PIDJ 2007[1634] De Ravin JCM 2014[1679] Ramesh JCI 2014[1682] Haidar Mycoses 2017[1675]	Case reports: soft tissue / osteomyelitis due to *Tropicoporus tropicalis*

5-FC, 5-fluorocytosine; ABPM, allergic bronchopulmonary mycosis; AFST, antifungal susceptibility testing; AmB, amphotericin B; BDG, Beta-D-Glucan; CGD, chronic granulomatous disease; CLSI, Clinical and Laboratory Standards Institute; CT, computed tomography; ELISA, Enzyme-linked Immunosorbent Assay; FC, fluorocytosine; FCZ, fluconazole; GM, Galactomannan testing; GMS, Grocott-Gomori's methenamine silver; HR, high-resolution; ICZ, itraconazole; ITS, internal transcribed spacer; ISA, isavuconazole; KCZ, ketoconazole; LSU, large subunit; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; MIC, minimal inhibitory concentration; MRI, magnetic resonance imaging; PAS, periodic acid-Schiff; pat., patient; PCR, polymerase chain reaction; PCZ, posaconazole; QoE, quality of evidence; SCT, stem cell transplant; SoR, strength of recommendation; TRB, terbinafine; VCZ, voriconazole.

Recommendation – The guideline group strongly recommends HR chest CT and CT of the sinuses and MRI of the brain to delineate the extent of involvement of the infection **(Figure 24).**

Figure 24. Optimal diagnostic pathway for infections caused by *Schizophyllum* spp. and other basidio-mycetes, when all imaging and assay techniques are available



Treatment approaches to infections caused by *Schizophyllum* spp. and other basidiomycetes

Treatment in Adults

First line treatment for infections caused by S. commune

Evidence – For treatment of infections caused by *S. commune*, data are primarily obtained from case reports and small case series. Infections with systemic spread and proven or probable CNS involvement have primarily been treated with L-AmB, with step down therapy to oral azoles (*e.g.*, PCZ) after clinical improvement and stabilization of the patient[1609,1620,1652]. In patients with pulmonary infections including bronchopneumonia and pulmonary fungal balls VCZ, ICZ and FCZ have led to clinical improvement or cure[1621,1670,1681] **(Table 30)**.

Table 30. First-line antifungal therapy for infections caused by *Schizophyllum* spp. and other basidiomycetes

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To cure *S. commune* infection	L-AmB; optional: step down to PCZ	B	IIIt	Oliveira PLOSNTD 2017[1620]	N=1, success
					Hoenigl Mycoses 2013[1609]	N=1, brain abscess, step down to PCZ solution, success
					Rihs JCM 1996[1652]	N=1, brain abscess, success
Patients with pulmonary fungal ball	To cure	ICZ + tapering doses of systemic glucocorticoids	C	III	Chowdhary Mycoses 2013[1621]	N=1, success
Any	To cure invasive pulmonary diseases/ bronchopneumonia due to *S. commune*	VCZ	C	III	Chowdhary AAC 2013[1681]	N=2, success 2/2
					Tullio MedMycol 2008[1670]	
Any	To cure invasive pulmonary diseases/ bronchopneumonia due to *C. cinerea*	Echinocandins	D	III	Lagrou JMM 2005[1644]	N=4, progression while on CASPO/MICA
					Suarez JCM 2011[1665]	
					Chauhan LabMed 2019[1616]	
					Nanno TID 2016[1646]	
					Conen CMI 2010[1611]	
		Avoid echinocandins	A	III	Lagrou JMM 2005[1644]	N= 7, high MICs to echinocandins, progression under treatment
					Suarez JCM 2011[1665]	
					Chauhan LabMed 2019[1616]	
					Nanno TID 2016[1646]	
					Godet Mycopathol 2017[1612]	
					Ramesh JCI 2014[1682]	
					Conen CMI 2010[1611]	
Hematological malignancies patients	To cure *C. cinerea* infection	L-AmB iv +/- inhalative L-AmB OR VCZ 4 mg/kg bid after loading dose	B	IIIt	Godet Mycopathol 2017[1612]	N=1, poor response to L-AmB but rapid improvement after addition of nebulized L-AmB
					Corzo-Leon Mycoses 2015[1034]	N=1, bIFI under VCZ prophylaxis, treatment with L-AmB, died
					Heiblig Mycoses 2015[1641]	N=1, stable disease
					Surmont MedMycol 2002[1666]	N=1, improvement
Hematological malignancies patients	To cure pulmonary infection	D-AmB, ICZ	D	III	Verweij JCM 1997[1672],	N=1, failure
Hematological malignancies patients	To cure disseminated infection	VCZ iv	C	III	Conen CMI 2010[1611]	N=3, stable disease 1/3, success 2/3
					Koncan JMBT 2016[1688]	N=1, success
					Heiblig Mycoses 2015[1641]	N=1, stable disease
Any	To cure respiratory diseases, ranging from saprobic colonization to fungal pneumonia	ICZ	C	III	Chowdhary JCM 2013[1632]	N=4, *Emmia lacerata*, outcome unknown
Patients with CGD	To cure	VCZ	C	III	Ramesh JCI 2014[1682]	N=1, *T. tropicalis*, success
Immunodeficiency patients	To cure disseminated infection	L-AmB, VCZ	D	III	Friman Scand JID 2006[1686]	N=1, *Phlebia tremellosa*, failure
Patients with CGD	To cure	L-AmB 7.5 mg/kg qd, followed by L-AmB + ISA	C	III	Haidar Mycoses 2017[1675]	N=1, *T. tropicalis,* success

Standard dose unless stated otherwise; bid, twice a day; bIFI, breakthrough invasive fungal infection; CASPO, caspofungin; CGD, chronic granulomatous disease; d, day(s); D-AmB, amphotericin B deoxycholate; ISA, isavuconazole; iv, intravenous; L-AmB, liposomal amphotericin B; MICA, micafungin; MIC, minimal inhibitory concentration; PCZ, posaconazole; po, orally; qd, once a day; QoE, quality of evidence; SoR, strength of recommendation; VCZ, voriconazole.

Recommendations – The guideline group moderately recommends first-line treatment with L-AmB (with the option to step down to PCZ later) based on descriptive case reports for infections with *S. commune*. Primary treatment with VCZ is marginally supported and may be used in patients intolerant to L-AmB.

First line treatment for infections caused by C. cinerea

Evidence - First line treatment for *infections caused by C. cinerea* is difficult to establish due to limited clinical data but *in vitro* data highlight that AmB and VCZ MICs are lower compared to those of CASPO and FCZ [1612,1691]. Echinocandins should be avoided for the treatment of these infections due to higher MICs as well as reports of progression of disease in patients receiving echinocandins[1611,1612,1644,1646,1665,1677]. L-AmB with dosages of 3 – 10 mg/kg has been used to treat *C. cinerea*-related infections and has led to cure in some patients. In cases of pulmonary infection caused by *C. cinerea* and nephrotoxicity associated with high-dose systemic L-AmB treatment, addition of inhaled L-AmB may allow dose reduction of systemic L-AmB and cure[1612]. VCZ may also be used as an alternative to L-AmB[1034] in cases where there are contraindications to L-AmB as well as for step down treatment after clinical improvement under L-AmB treatment[1611].

Recommendations – Despite limited literature on the treatment of these infections, first-line treatment with systemic L-AmB +/- inhaled L-AmB or VCZ is moderately recommended for *C. cinerea*-related infections in patients with hematological malignancy. No data are available for other patient cohorts. Use of parenteral VCZ as first-line treatment is marginally recommended, whereas the guideline group recommends against the use of echinocandins.

First line treatment for infections caused by other filamentous basidiomycetes

Evidence - The relevance of *Emmia lacerata* (formerly *Ceriporia lacerate)* as a human pathogen remains unclear. In case series, *E. lacerata* was found to be a colonizer of the airways but also a cause for fungal pneumonia[1632,1660]. *In vitro* data showed the lowest MICs for PCZ and ISA compared to FCZ, 5-FC and echinocandins[1632,1660]. Data on treatment is limited to three patients who received ICZ (n=2) or VCZ (n=1). Outcome is known for only one patient who received ICZ 200 mg tid and improved clinically[1632].

Infections caused by *Tropicoporus tropicalis* (formerly *Phellinus tropicalis*) have been reported exclusively in patients with CGD causing pneumonia, abscesses, brain lesions or osteomyelitis[1634,1667,1675,1682]. Reported MICs were low for AmB and all triazoles, whereas higher MICs were found for FCZ [1634,1675,1682]. Several different antifungal strategies have been published. Most included L-AmB either in combination with VCZ or ISA[1634,1675,1692]. VCZ monotherapy was used in one patient successfully[1682]. Surgical treatment with resection of infected areas or drainage of abscess formations were performed in the majority of cases. Interestingly, the majority of *T. tropicalis*-related infections occurred while patients were on ICZ prophylaxis [1634,1667,1682].

A single case of *Phlebia tremellosa* (formerly *Merulius tremellosus*) related infection in an immunocompromised host was published[1686]. Despite sequential therapy with L-AmB and VCZ the patient ultimately died.

Recommendations – Based on *in vitro* susceptibility data and a single case report, ICZ treatment is recommended marginally for the treatment of *E. lacerata* (formerly *Ceriporia lacerata*) infections. For patients with CGD and infection due to *T. tropicalis* the guideline group marginally recommends first line treatment with L-AmB +/- VCZ or ISA. VCZ monotherapy is an alternative option (marginally recommended).

Salvage treatment for basidiomycetes

Evidence – Few breakthrough infections with *C. cinerea* in patients with hematological malignancy have been reported. Different antifungal strategies including surgery have been used for management of these infections. However, the majority of patients had a fatal outcome despite salvage treatment because of progression of the infection or the underlying disease. Salvage treatment with L-AmB (5-10 mg/kg) alone or in combination with VCZ and surgical debridement was associated with clinical and radiological improvement or cure in some of these patients[1641,1665]. The addition of inhaled L-AmB (25 mg tiw) to systemic L-AmB led to cure and reduced nephrotoxicity in a patient[1675] **(Table 31)**.

Table 31. Antifungal salvage treatment for infections caused by *Schizophyllum* spp. and other basidiomycetes

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Hematological malignancies patients	To cure pulmonary infection with *C. cinerea*	L-AmB	B	III	Suarez JCM 2011	N=2, success 1/2
					Conen CMI 2010[1611]	N=2, success 2/2
Hematological malignancies patients	To cure *C. cinerea* infection	L-AmB 5-10 mg/kg + VCZ iv/PCZ +/- surgical debridement	C	III	Conen CMI 2010[1611]	N=2, improved when PCZ was added to L-AmB + VCZ
					Heiblig Mycoses 2015[1641]	N=1, improved
Hematological malignancies patients	To cure *C. cinerea* infection	VCZ 4 mg/kg iv bid after loading	B	III	Conen CMI 2010[1611]	N=2, stable 1/2, improved 1/2
Hematological patients/allo SCT patients	To cure pulmonary infection and reduce toxicity	Add nebulized AmB 25 mg tiw	C	III	Godet Mycopathol 2017[1612]	N=1, stable disease
Hematological malignancies patients	To cure *C. cinerea* infection	Echinocandin	D	III	Conen CMI 2010[1611]	N=1, failure
Patients with CGD	To cure	ISA + dose-reduced L-AmB	C	III	Haidar Mycoses 2017[1675]	N=1, response

Standard dose unless stated otherwise; bid, twice a day; CGD, chronic granulomatous disease; ISA, isavuconazole; iv, intravenous; L-AmB, liposomal amphotericin B; PCZ, 106osaconazole; QoE, quality of evidence; SCT, stem cell transplantation; SoR, strength of recommendation; tiw, three times a week; VCZ, voriconazole.

Recommendations – Use of L-AmB (5-10 mg/kg) or parenteral VCZ are moderately recommended for salvage treatment of *C. cinerea*-related infections in hematological patients. Combination of both +/- surgical debridement is marginally recommended as is the combination of ISA with a reduced dose of L-AmB. The guideline group recommends against the use of echinocandins for basidiomycetes.

Other treatment options for basidiomycetes

Evidence – There is a single case report of a patient with biphenotypic acute leukemia developing *C. cinerea*-related infection during induction chemotherapy while on primary prophylaxis[1665]. In this patient L-AmB 5 mg/kg was successfully used as secondary prophylaxis during allogeneic HSCT for a total of 45 days. This patient did not suffer from relapsing disease and was considered cured from her IFI. In CGD patients with basidiomycetes infection, cultures from infected sites may be positive for years[1692]. However, they should not be classified as contaminants but should be considered as the causative agent in this setting[1675]. Secondary prophylaxis with VCZ or PCZ was used in two patients for several years without recurrence of infection[1692].

For deep-seated basidiomycete infections surgical intervention may be of benefit for the patient. Case reports of successful treatment of such infections including surgery (debridement, excision of lung nodules, wedge resection, excision of infected tissue), plus antifungal treatment, highlight the potential benefits of surgery in this setting[1611,1641,1673,1676]. However, surgery was not able to control fungal infection in a patient with post-surgical eye infection or in an allogeneic HSCT patient with pulmonary infection who showed progress of disease after resection of the lung nodules[1612,1613]. Thus, surgery and antifungal treatment should always be combined if feasible **(Table 32)**.

Table 32. Other treatment options for infections caused by *Schizophyllum* spp. and other basidiomycetes

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Hematological patients planned for allo-SCT	Secondary prophylaxis	L-AmB or inhaled AmB	C	IIIu	Suarez JCM 2011[1665]	
Patients with CGD	Secondary prophylaxis	VCZ	C	III	Ramesh JCI 2014[1682]	Case reports, CGD patients kept on VCZ for several months without recurrence or deterioration
					Nguyen JACI 2009[1692]	
					Shigemura Infection 2015[1676]	
Patients with CGD	To cure *Inonotus/Phellinus* spp. infection	Do not consider as contamination	C	III	Haidar Mycoses 2017[1675]	Literature review, fungus was misinterpreted as contaminant, but should be considered as causative in this specific setting
Post-surgical eye infection	To cure	Surgery/vitrectomy	C	III	Jain JMM 2019[1613]	N=1, TPK + vitrectomy + systemic and topical antifungals
Sinusitis	To cure	Surgical debridement	B	III	Heiblig Mycoses 2015[1641]	N=1, improved
Pulmonary infection	To cure	Surgical excision of lung nodules/wedge resection	B	III	Godet Mycopathol 2017[1612]	N=1, Progress of infection after surgery
					Conen CMI 2011[1611]	N=2, success 1/2, stable disease 1/2
Skin/subcutaneous infection	To cure	Surgical excision of infected tissue	B	III	Shigemura Infection 2015[1676]	
					Williamson JMM 2011[1673]	N=1, *Fuscoporia undulatus*, cure

AmB, amphotericin B; CGD, chronic granulomatous disease; L-AmB, liposomal amphotericin B; QoE, quality of evidence; SCT, stem cell transplantation; SoR, strength of recommendation; TPK, total penetrating keratoplasty; VCZ, voriconazole.

Recommendations – Secondary prophylaxis for hematological patients during allogeneic HSCT with L-AmB is marginally recommended. In CGD patients with basidiomycete infections secondary prophylaxis with VCZ or PCZ is marginally recommended. Surgical resection of infected tissue and debridement are moderately recommended whenever feasible.

Treatment duration

Evidence - Treatment duration has been determined on a case-by-case basis and depends on the extent of surgery, the organs involved, the pathogen involved, status of underlying disease and ongoing immunosuppression. For CNS infections a treatment duration of 6 weeks or more has been reported[1609,1652], while for fungal rhinosinusitis with mucosal and/or bone invasion a treatment duration of >2 months has been reported[1606]. For bone infections a treatment duration of up to several years has been published[1692] **(Table 33)**.

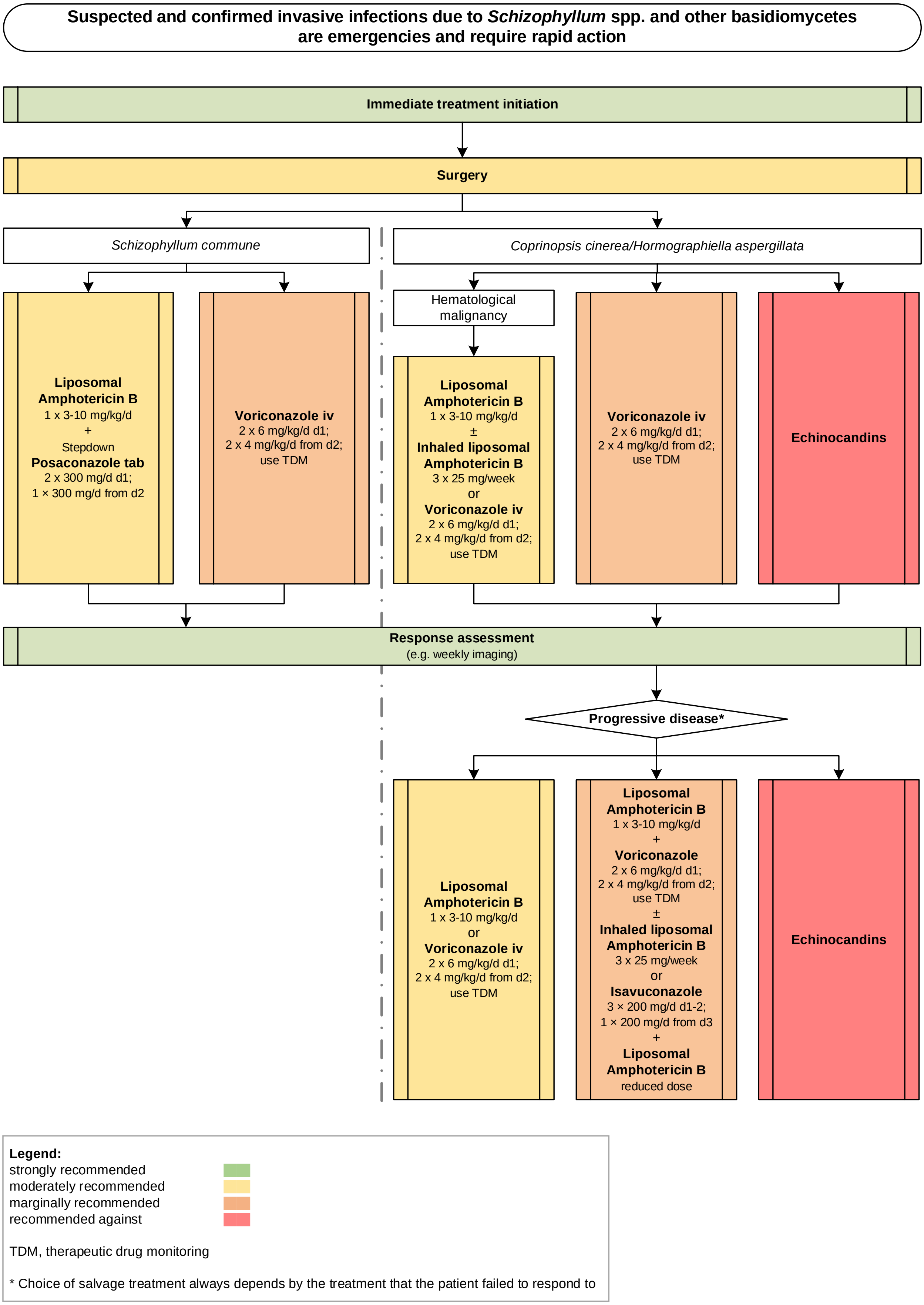
Table 33. Treatment duration for infections caused by *Schizophyllum* spp. and other basidiomycetes

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any with CNS involvement	To cure	6 wk or more of therapy	B	III	Hoenigl Mycoses 2013[1609] Rihs JCM 1996[1652]	N=2, duration determined case-by-case
Fungal rhinosinusitis with mucosal and/or bone invasion	To cure	2 mo or more	C	III	Michel MedMycol 2016[1606]	Treatment duration determined case-by-case

CNS, central nervous system; mo, month(s); QoE, quality of evidence; SoR, strength of recommendation; wk, week(s)

Recommendation – Treatment duration should be determined on a case by case basis. For all types of CNS infections a treatment duration of 6 weeks or more is moderately recommended[1609,1652]. For fungal rhinosinusitis with mucosal and/or bone invasion a treatment duration of 2 months or more is marginally recommended[1606] **(Figure 25)**.

Figure 25. Optimal treatment pathway for infections caused by *Schizophyllum* spp. and other basidiomycetes in adults when all treatment modalities and antifungal drugs are available



Specific considerations on treatment of infections caused by Schizophyllum spp. and other basidiomycetes in children

Evidence – Pediatric data is limited only to case reports **(Table 34)**.

Table 34. Therapy in children for *Schizophyllum* spp. and other basidiomycetes infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
First-line antifungal therapy						
BMT (Immunocompromised) with invasive basidiomycosis	To cure	VCZ iv	B	III	Lim AJD 2017[1685]	N=1, 22 mo, *Earliella scabrosa*, failure
Any	To cure	L-AmB 7.5-10 mg/kg qd, VCZ iv, surgical debridement	B	III	Heiblig Mycoses 2015[1641]	N=1, 19 yrs, stable disease
AML	To cure	No treatment	D	III	Abuali JCM 2009[1623]	N=1, 14 yrs, fatal outcome
Antifungal salvage treatment						
Children/Young adults with CGD	To cure	VCZ 200 mg po bid +/- other antifungals	B	III	Ramesh JCI 2014[1682]	N=1, 24 yrs, *T. tropicalis* retropharyngeal abscess, survived
					Sigemura Infection 2015[1676]	N=1, 23 yrs, *Phellinus mori,* VCZ + MICA, survived
					Davis PIDJ 2007[1634] (molecular work presented by Sutton JCM 2005[1667])	N=1, 21 yrs, paraspinal abscess and sacral osteomyelitis, *T. tropicalis,* survived
					De Ravin JCM 2014[1679]	N=1, 10 yrs, *Phellinus* spp., paravertebral abscess, + surgery, survived
Children with soft tissue infection	To cure	PCZ 100 mg qd + alternate regimen of PCZ 100 mg/200 mg for consolidation 2 wk after clinical improvement	C	III	Correa Martinez NMI 2017[1690]	N=1, *C. cinerea*

Standard pediatric dose unless stated otherwise; AML, acute myeloid leukemia; bid, twice a day; BMT, bone marrow transplant; CGD, chronic granulomatous disease; iv, intravenous; L-AmB, liposomal amphotericin B; MICA, micafungin; PCZ, posaconazole; po, orally; qd, once a day; QoE, quality of evidence; SoR, strength of recommendation; VCZ, voriconazole; yrs, years.

Recommendations - According to data in adults, the recommendation for therapy includes VCZ alone or in combination with L-AmB (moderate recommendation).

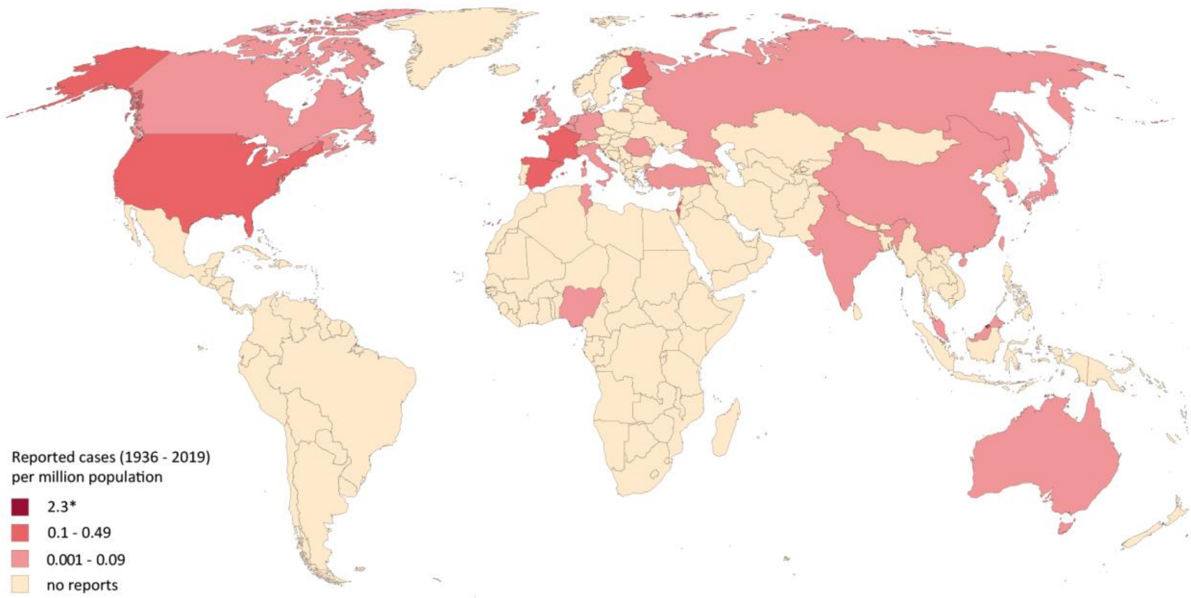
7. Scopulariopsis

Epidemiology of infections caused by *Scopulariopsis* spp.

Scopulariopsis is found in soil and plant material with a worldwide distribution[1693]. Some species have teleomorphs, which are classified in the genus *Microascus*[1694]. *Scopulariopsis brevicaulis* is the most relevant species in humans [1695]. Single cases of infections caused by *Scopulariopsis acremonium*, *Scopulariopsis brumptii* and *Scopulariopsis candida* have been reported, mostly from Western Europe and North-

America[1696-1698]. *Scopulariopsis* is typically associated with onychomycosis or other superficial infections[1699,1700]. Severe *Scopulariopsis*-related systemic infections have been reported, mainly in immunosuppressed patients with underlying malignancy, HSCT and SOT recipients, affecting lungs, paranasal-sinuses and soft tissues[1589,1697,1698,1701,1702]. Infections have rarely occurred in immunocompetent patients following traumatic injuries or surgery affecting the eye or deep soft tissue[1703-1705]. Dissemination to the CNS or other organs and fungemia have been noted in severely ill patients[1706,1707]. Heart infections due to *Scopulariopsis* have been described mainly in patients who underwent prosthetic valve implantation[1708,1709]. In a retrospective study in a US Cancer Center, ~2% of fungal infections of the brain in bone marrow transplant patients were caused by *Scopulariopsis*[1710]. The National Institutes of Health reported that in patients with positive bronchoalveolar lavage BDG tests ~3% of confirmed fungal infections were due to *Scopulariopsis*[1711]. In patients post-lung transplantation an incidence of 0.2% was reported in a center in Spain[606] **(Figure 26)**.

Figure 26. Worldwide distribution of infections caused by *Scopulariopsis* spp. (reported cases between 1936 and 2019 per million population)



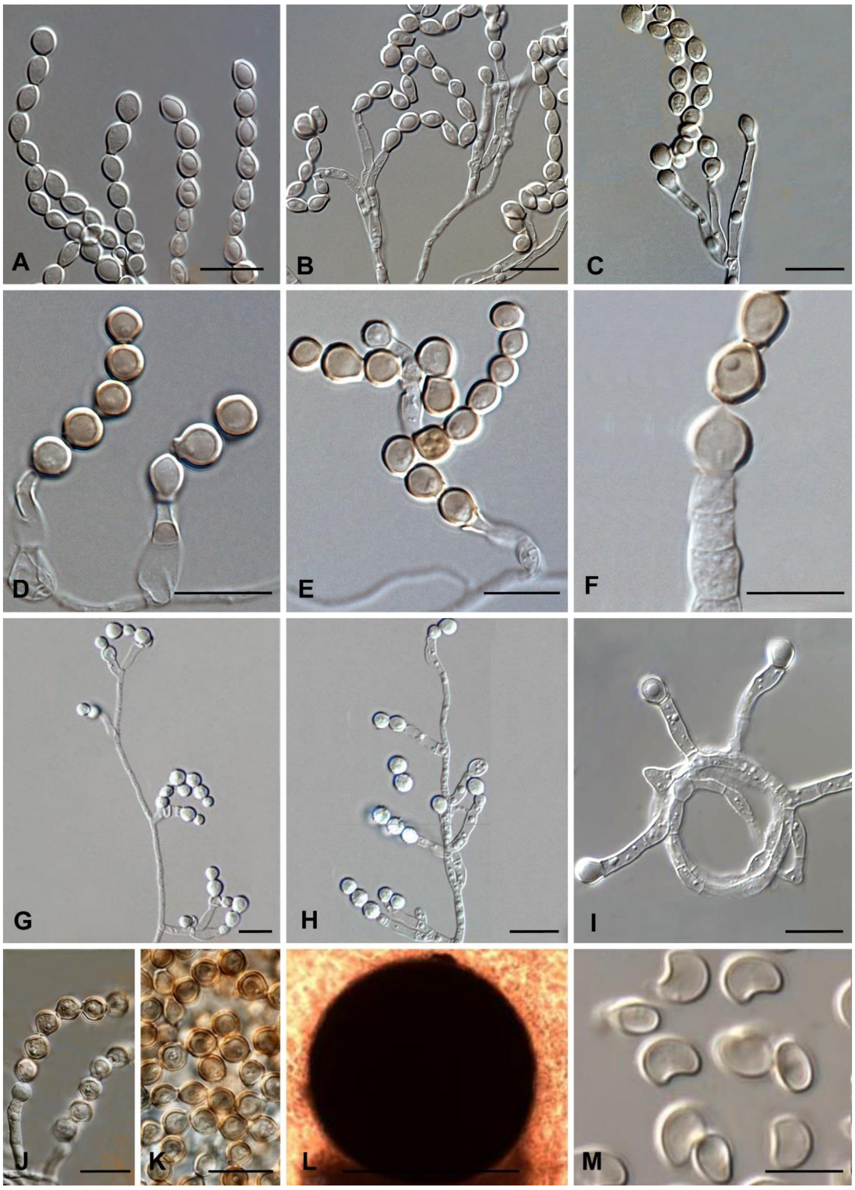
Cases of *Scopulariopsis*-related infections reported in the medical literature were identified in a PubMed search on October 30, 2019 using the search string "*Scopulariopsis* OR *Microascus*" that yielded 554 publications. In total, 86 cases were identified from 24 countries, 61 since the year 2000[606,643,720,1107,1116,1589,1696-1698,1701-1764]. Most cases were reported from the United States (n=41), France (n=10) and Spain (n=5). The number of cases reported between 1936 and 2019 is presented as cases per million population per country. The resident population per country was obtained from www.worldometers.info[321]. *One case was reported from Brunei Darussalam (2.3 cases per million population between 1936 and 2019)[1752].

Diagnosis of Scopulariopsis

Diagnosis – Microbiology – Conventional Methods

Evidence – The definitive diagnosis of *Scopulariopsis*-related infections has traditionally relied on the isolation of *Scopulariopsis* spp. from infected tissue or body fluids, with histological findings showing dichotomously branched septate hyphae, and culture confirmation of *Scopulariopsis* spp.[1706,1732,1751,1765]. See also **Figure 27** with microscopic morphology from the Atlas of Clinical Fungi project[19].

Figure 27. Microscopic morphology of *Scopulariopsis* spp. [19]



Panel A-C, *Scopulariopsis koningii*, conidiogenous cells single or in groups, cylindrical or with slightly swollen basal parts, conidia spherical to ovoidal; **Panel D-F**, *Scopulariopsis asperula*, conidiophores with annellated conidiogenous cells and conidia; **Panel G-H**, *S. brevicaulis*, conidiogenous cells and conidia; Panel I, *Scopulariopsis flava*, conidiogenous cells and conidia; **Panel J-K**, *Scopulariopsis fusca*, conidiogenous cells arising from aerial hyphae, single or in groups; **Panel L-M**, *S. candida*, ascomata spherical, black, with pore in the apical papilla, ascospores heart-shaped in lateral view. Scale bars = 10 µm.

Culture is essential, and histological findings alone are insufficient for the diagnosis of these infections as *Scopulariopsis* spp. can be difficult to distinguish from *Aspergillus* spp., *Fusarium* spp., and *Scedosporium* spp. by histology and morphological appearance alone[1746] **(Table 35)**.

Recommendations – The guideline group strongly recommends that infected tissue or body fluids be obtained for culture and also microscopic/histological evaluation, when possible.

Diagnosis – Microbiology – Serology

Evidence – In multiple case reports, *Aspergillus* GM and BDG have been negative [1711,1732,1766] **(Table 35)**.

Recommendations – *Aspergillus* GM and BDG are not recommended as part of the diagnostic evaluation for *Scopulariopsis*-related infections.

Diagnosis – Microbiology – Molecular-based

Evidence – PCR assays have been developed that can identify *Scopulariopsis* isolates to the genus level[1767] and species level[1706,1726,1732,1764,1766], in two reports PCR has been performed directly on sputum samples[1706,1768] **(Table 35)**.

Recommendations – The guideline group marginally supports the use of molecular-based diagnostic tests to diagnose *Scopulariopsis*-related infections, if available.

Diagnosis – Microbiology – Species identification

Evidence – Using PCR assays[1769-1771] and phylogenetic analysis using multi-gene sequences[1772], identification to the species level has been reported in some studies, although the specificity of PCR varies from study to study and has been as low as 70% in some studies[561,1768]. MALDI-TOF MS has been used to identify *Scopulariopsis* isolates in one case report[1773] and the combination of PCR and MALDI-TOF MS in another case report[1701] **(Table 35)**.

Recommendations – The guideline group strongly recommends species identification using ITS2 or D1/D2 or 18S PCR or phylogenetic analysis for species identification from isolates and marginally the use of MALDI-TOF MS for the same purpose.

Microbiology – Susceptibility testing

Evidence – *Scopulariopsis* species typically demonstrate high MICs to many antifungal agents including FCZ, ICZ, 5-FC, and AmB[1774]. Antifungal susceptibility testing using CLSI[561] and EUCAST testing[559] can determine MICs, while having been shown to poorly correlate with E-test[330]. However, there are not enough data documenting a correlation between MICs and clinical outcome nor has a clinically meaningful cutoff value been established[1719,1775] **(Table 35)**.

Recommendations – Given that *Scopulariopsis* spp. typically demonstrate high MICs to many antifungal agents, CLSI or EUCAST testing is strongy recommended to determine antifungal susceptibility, and moderately for guiding antifungal treatment.

Diagnosis - Pathology

Evidence – Histological findings showing dichotomously branched septate hyphae have been reported in multiple studies to be suggestive of *Scopulariopsis* infection, including in those with underlying hematological malignancies[1701,1732,1764], solid organ transplant patients[1776], and a patient with endocarditis[1712]. *Scopulariopsis* spp. can be difficult to distinguish from *Aspergillus* spp., *Fusarium* spp., and *Scesosporium* spp. by morphology alone[1746] **(Table 35)**.

Recommendations – The guideline group strongly recommends histopathological examination of biopsy tissue in cases of suspected *Scopulariopsis* infection.

Diagnosis – Imaging

Evidence – *Scopulariopsis* spp. rarely cause invasive infections in humans and the diagnosis is typically made in immunocompromised individuals. As with other invasive fungal infections, imaging studies have played a crucial role in determining the likely site of *Scopulariopsis* infection[1702,1726,1751] and assisting in the procurement of infected tissue or body fluids[1755] **(Table 35)**.

Table 35. Microbiological, histopathological and imaging diagnostics of *Scopulariopsis* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Microscopy, culture, MIC testing						
Any	To diagnose	Culture and microscopy	A	II	Arroyo JCM 2017[1765]	H&E, GMS, and PAS stainings revealed numerous septate hyphae with dichotomous branching. Cultures confirmed *Scopulariopsis* spp.
					Sattler MMCR 2014[1751]	Allergic sinusitis; Hyaline septate hyphae and globose to pyriform truncate spores at direct examination. Cultures confirmed *Scopulariopsis* spp.
					Salmon CMI 2010[1706]	Biopsy showed numerous septate hyphae by direct observation after Chlorazol-Black staining. Microscopic examination of histological sections stained with lactophenol blue, PAS, H&E and GMS stainings revealed branched septate hyphae and many vesicular swellings of different sizes. Cultures confirmed *Scopulariopsis* spp.
Any	To determine antifungal susceptibility	EUCAST method	A	III	Alastruey-Izquierdo AAC 2018[559]	*S. brevicaulis* had high MICs for all antifungals
Any	To determine antifungal susceptibility	CLSI MIC testing	A	IIu	Sandoval-Denis JCM 2013[561]	N=97, Echinocandins had better *in vitro* activities than azoles
Any	To guide antifungal treatment	CLSI MIC testing	B	IIu	Cawcutt CRM 2015[1709]	N=1, Endocarditis; susceptible to CASPO, MICA and TRB, treatment with CASPO + VCZ, success
					Gavril Infection 2016[1701]	N=1, cutaneous infection; susceptible to MICA, but no correlation between MIC and clinical outcome
					Shaver AJT 2014[1755]	N=1, Lung transplant patient; susceptible to echinocandins, failure
Serology assays						
Any	To diagnose	Aspergillus GM EIA	D	III	Salmon CMI 2010	N=1, disseminated *S. brevicaulis*. Serum *Aspergillus* GM +, thought to be false + due to cross-reactivity with *S. brevicaulis* cell wall components
					Miossec JCM 2011[1766]	N=1, *Scopulariopsis* fungemia. *Aspergillus* GM assays x5 were negative, starting 11 d after transplant
					Iwen MedMycol 2012[1732]	N=1, Invasive *Scopulariopsis* infection. *Aspergillus* GM negative
Aplastic anemia	To diagnose	Aspergillus GM from serum and BAL, BDG from serum and BAL	D	III	Rose JInfect 2014[1711]	N=1. Invasive *Scopulariopsis* infection. BDG and GM (both serum and BAL) negative
Nucleic-acid based assays/MALDI-TOF MS						
Any	To diagnose	PCR directly from sputum/tissue	C	II	Salmon CMI 2010[1706]	N=1. Disseminated *S. brevicaulis* detected by PCR from sputum and tissue and culture
Any	To diagnose	Universal 28S PCR + RFLP directly from sputum	C	II	Bontems BJD 2009[1768]	N=17, *S brevicaulis* isolated in culture from infected nails, 12/17 were correctly identified by PCR-RFLP (spec. 71%)

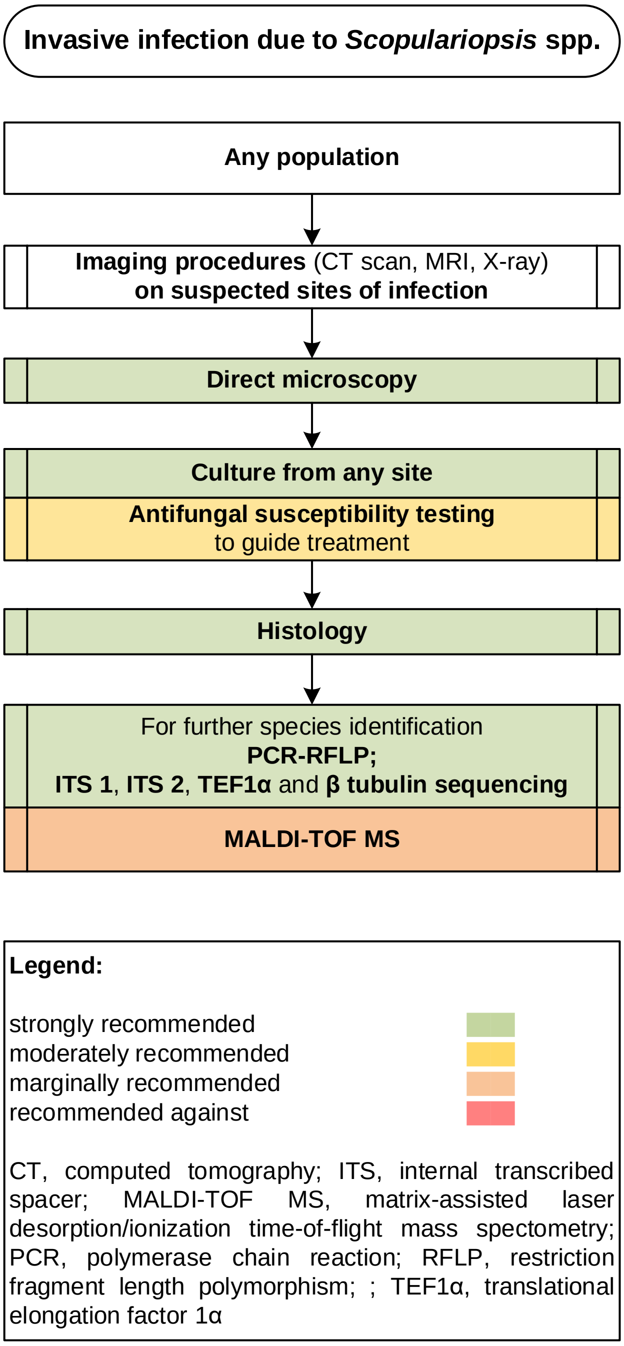
Any with non-dermatophyte onychomycosis	To detect	Development of PCR for detection in nail (keratin)	C	III	Stavrakieva PIPD 2003[1777]	Development of PCR assay for identification of *S. brevicaulis* in clinical samples using species-specific primers. Amplification of 336 bp DNA fragment of ribosomal LSU. Method not applied to nail fragments.
Any	To identify species	PCR-RFLP	A	IIu	Kordalewska PJM 2015[1769]	
					Kordalewska MedMycol 2018[1770]	
					Kordalewska Mycopathol 2016[1767]	N=48, sens 100%, spec 100% on genus level
Any	To identify species	ITS 1/2 and intervening 5.8S nrDNA, *tub2* and *tef1* gene regions	A	IIu	Woudenberg StudMycol 2017[1772]	N=248 isolates: 152 *Microascus*, 88 *Scopulariopsis*, 4 *Yunnania*, 4 out-groups. Multi-gene phylogenies recognized 12 *Scopulariopsis* spp.
Any	To identify *Scopulariopsis*	PCR to 28S rDNA	B	II	Jagielski PJM 2013[1778]	N=40 *Scopulariopsis* spp. and 4 reference strains of *S. brevicaulis* from clinical isolates from hair and nails sequenced. Poor ability to identify the species
					Sandoval-Denis JCM 2013[561]	N= 99 clinical isolates, PCR able to identify 67% to the species level
Onychomycosis	To identify *Scopulariopsis*	PCR to 28S rDNA	B	II	Monod JMM 2006[1771]	N=5 culture-proven isolates of *S. brevicaulis,* PCR was able to correctly identify 4/5 as *S. brevicaulis* and the non-identified isolate was a probably contaminate
					Bontems BJD 2009[1768]	N=17 cases of *S. brevicaulis* isolated in culture from infected nails, 12/17 were correctly identified by PCR-RFLP (spec. 71%)
Any	To identify isolates	ITS2 or D1/D2 or 18S PCR+ sequencing from isolates	A	III	Yang DMID 2012[1764]	N=1, invasive pulmonary *S. brevicaulis* detected by PCR only
					Iwen MedMycol 2012[1732]	N=3, *S S. brevicaulis* detected by PCR and histopathology
					Miossec JCM 2011[1766]	N=1, disseminated *S. brevicaulis* detected by PCR only
					Gluck IJPO 2011[1726]	N=1, *S. brevicaulis* detected by PCR and culture
AML	To identify *Scopulariopsis*	MALDI-TOF MS and PCR	C	III	Gavril Infection 2017[1701]	N=1, invasive *S. brevicaulis* detected by culture, MALDI-TOF MS, and PCR
Any	To identify *Scopulariopsis*	MALDI-TOF MS	C	III	Rath WJOHNS 2019[1773]	N=1, otomycosis, confirmed with MALDI-TOF MS
Tissue-based diagnosis						
Any	To diagnose	Histopathological examination of biopsy tissue	A	II	Iwen MedMycol 2012[1732]	N=33, invasive or disseminated disease. Main features seen in deep cutaneous, pulmonary or sinus areas: hyaline branched septate hyphae, irregularly-shaped hyphae and swollen thick-walled structures, angioinvasion, necrosis. In 12/33, conidia, conidia-like bodies, round or swollen structures or ascospores identified. Comment: swollen structures are unlikely to be conidia that are seen in cases of *Scopulariopsis* onychomycosis
					Yang DMID 2012[1764]	N=1, Bronchial invasion of *S. brevicaulis*
					Taton TID 2017[1776]	N=1, Necrotizing *Microascus* tracheobronchitis. Endobronchial swabs, biopsies, and BAL positive for *Microascus* spp. (teleomorph of *Scopulariopsis* spp.).

					Arroyo MJCM 2017[1765]	N=1, thrombectomy and resection of ascending aortic graft histologically revealed dichotomously branched septate hypha, which grew *S. brevicaulis*
					Gavril Infection 2017[1701]	N=1, invasive cutaneous infection; branched septate hyphae demonstrated at dermal-epidermal junction grew *S. brevicaulis*
Imaging studies						
SOT patients	To assess the cause of pulmonary symptoms	Chest radiograph and CT	B	II	Pate TID 2016[1743]	N=1, pneumonia, *S. brumptii*
		Chest CT	A	II	Shaver AJT 2014[1755]	N=1, pneumonia, pleural effusion, *S. brumptii*
Sinusitis	To assess the clinical manifestations and imaging characteristics of sinusitis caused by *Scopulariopsis*	CT scan of the sinuses	A	II	Gluck IJPO 2011[1726]	Sinus opacification in pediatric patients presenting with sinusitis that was later proven to be caused by *Scopulariopsis*
					Sattler MMCR 2014[1751]	N=1, Mucosal thickening at the floor of the left maxillary sinus that penetrated into the premolar dentition in an apparently immunocompetent patient
					Kammoun JMM 2018[1702]	N=1, Orbital cellulitis resulted from erosion and calcification of the frontal sinus by *Scopulariopsis* spp.

BAL, bronchoalveolar lavage; BDG, Beta-D-Glucan; CASPO, caspofungin; CLSI, Clinical and Laboratory Standards Institute; CT, computed tomography; d, day(s); DNA, deoxyribonucleic acid; EUCAST, European Committee for Antimicrobial Susceptibility Testing; GM, Galactomannan testing; GMS, Grocott-Gomori's methenamine silver; HE, hematoxylin and eosin; ITS, internal transcribed spacer; LSU, large subunit; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; MIC, minimal inhibitory concentration; nrDNA, nuclear ribosomal deoxyribonucleic acid; PAS, periodic acid-Schiff; PCR, polymerase chain reaction; rDNA, ribosomal deoxyribonucleic acid; RFLP restriction fragment length polymorphism; QoE, quality of evidence; SoR, strength of recommendation; SOT, solid organ transplant; TRB, terbinafine; VCZ, voriconazole.

Recommendations – As with other invasive fungal infections, imaging studies such as chest CT for pulmonary symptoms or CT of the sinuses for sinusitis are strongly recommended to assist in diagnosis, when applicable **(Figure 28).**

Figure 28. Optimal diagnostic pathway for *Scopulariopsis* infections when all imaging and assay techniques are available



Treatment approaches to *Scopulariopsis* infections

Treatment in adults

Evidence - *Scopulariopsis* spp. usually exhibit high MICs to all currently available antifungal agents[1719,1775,1779-1781]. ICZ, FCZ and 5-FC have almost no activity against *Scopulariopsis* spp.[561,1719,1775,1781].

Therefore, drugs that should be considered for the treatment of invasive disease include AmB, VCZ, PCZ, echinocandins, and TRB[1719,1775,1779-1781]. Some reports also suggest a high percentage of *in vitro* synergism with antifungal combinations of AmB and ANID (>80%)[1782] or PCZ, CASPO and TRB (~100%)[1779]. However, the relevance of these *in vitro* data is not clear, because there are not enough data documenting a correlation between MICs and the clinical outcome[1719,1775].

Adequate debridement or excision of necrotic tissue and the early start of systemic antifungal treatment appear to be the major means of halting progression of the disease[1702,1712,1747,1776,1783]. In patients with invasive *Scopulariopsis* infection, various combinations of D-AmB, lipid-based AmB formulations[1696,1698,1706,1709,1722,1724,1731,1732,1752,1755,1757,1758,1760,1776,1783,1784], azoles[1706,1743,1750,1752,1755,1758,1776,1785], TRB[1758,1760,1776,1785] and echinocandins[1696,1763,1764,1766] have been reported **(Table 36)**.

Table 36. Therapy for *Scopulariopsis* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
First line treatment						
Any	To cure	L-AmB 3-10 mg/kg qd + VCZ	B	III	Kurata IJH 2018[1698]	N=1, failure
					Isidro JTCS 2006[1731]	N=1, failure
Any	To cure	VCZ iv, step down to therapy po later	B	III	Kammoun JMM 2018[1702]	N=1, success
					Jain CardiovasPathol 2011[1708]	N=1, success
Any	To cure	VCZ + Echinocandin + TRB 250 mg qd	C	III	Taton TID 2017[1776]	N=1, success
					Shaver AJT 2014[1755]	N=1, failure
					Miossec JCM 2011[1766]	N=1, failure
					Wuyts JHLT 2005[1763]	N=1, failure
AML with skin infection	To cure	Echinocandin	D	III	Gavril Infection 2016[1701]	N=1, failure
Any	To cure	AmB lipid formulations +/- other antifungals	B	III	Satyavani SMJ 2010[1752]	N=1, success
					Ellison AOHNS 1998[1722]	N=1, success
					Gentry THIJ 1995[1724]	N=1, success
					Beltrame IJID 2009[1696]	N=1, failure
					Perfect CID 2005[378]	N=3, failure
					Phillips DMID 1989[1746]	N=1, failure
					Neglia AJM 1987[1784]	N=2, failure
					Mohammedi EJCMID 2004[1786]	N=1, failure
					Celard CID 1999[1787]	N=1, failure
					Migrino CID 1995[1738]	N=1, failure
Any	To cure	L-AmB + echinocandin	C	III	Salmon CMI 2010[1706]	N=1, failure
					Iwen MedMycol 2012[1732]	N=1, failure
Any	Cure	ISA	B	III	Cornely Mycoses 2018[381]	N=2, success
Antifungal salvage treatment						
Any	To cure	PCZ	B	III	Cawcutt CMR 2015[1709]	N=1, PCZ salvage after CASPO and VCZ, stable disease
					Pate TID 2016[1743]	N=1, PCZ + TRB salvage after AmB and MICA, success
					Rakita AJT 2015[1750]	N=1, PCZ + TRB salvage after VCZ, success
					Arroyo JCM 2017[1765]	N=1, PCZ + MICA salvage, followed by PCZ mono after AmB and VCZ; success
AML	To cure bronchial infection	VCZ iv + CASPO	D	III	Yang DMID 2012[1764]	N=1, *S. brevicaulis,* salvage after L-AmB and VCZ

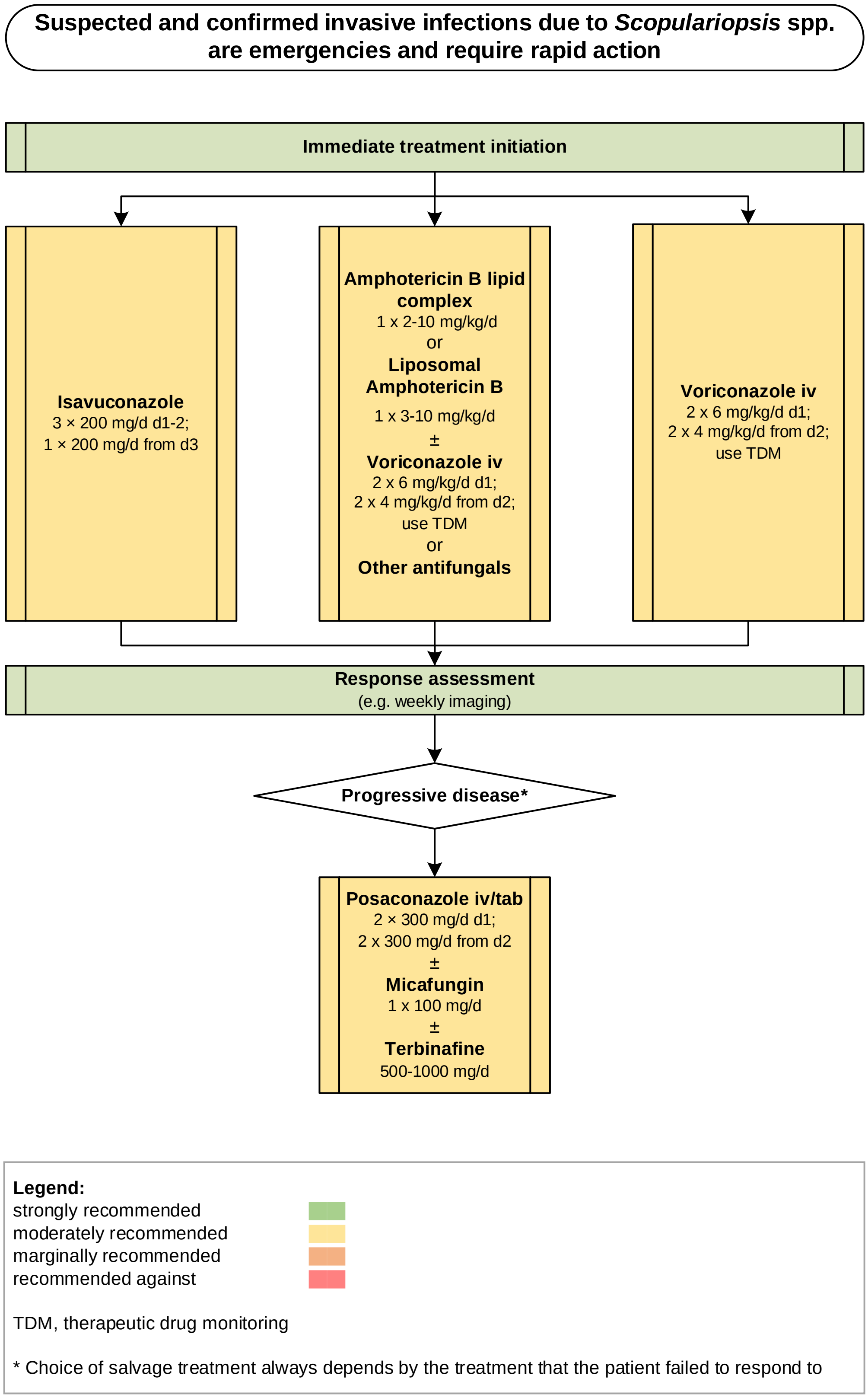
	To cure	ICZ	C	III	Ng MJM 2003[1707]	N=1, *S. brevicaulis*, salvage after AmB
Any	To cure	L-AmB 10 mg/kg qd + VCZ	D	III	Salmon CMI 2010[1706]	N=1, disseminated infection *S. brevicaulis*; L-AmB + CASPO, switch to AmB + VCZ, failed
Other treatment options						
Endogenous fungal endophthalmitis	To cure	Vitrectomy with intravitreal VCZ and 3 wk of oral VCZ	C	III	Raevis CRO 2018[1747]	
Diabetes	To cure	Topical efinaconazole	C	III	Kimura JOD 2018[1788]	N=1, onychomycosis, *S. brevicaulis*. Failure of 13 mo TRB + ICZ, switch to topical treatment
Fungal keratitis	To Cure	AmB topical 0.15%, VCZ 1% + VCZ po, later ICZ po + 2x 5 µg/0.1 ml AmB intracameral	C	III	Wilde IntOphthalmol 2018[1789]	N=1, *Scopulariopsis gracilis*
Treatment duration						
Aortic graft	To cure	PCZ + MICA for 2 mo, then PCZ for 6 mo	C	III	Arroyo JCM 2017[1765]	N=1, success
Lung transplant recipient	To cure	PCZ + TRB for 22 mo	C	III	Rakita AJT 2015[1750]	N=1, success
Prosthetic mitral valve endocarditis with septic emboli	To cure	Combination therapy (L-AmB/VCZ + CASPO) for 8 wk, then chronic suppression with VCZ (shifted to PCZ due to AEs)	C	III	Cawcutt CRM 2015[1709]	N=1, heart surgery and thrombectomy of septic emboli
Liver transplant recipient	To cure	MICA + PCZ + TRB for 47 d, then PCZ and TRB for 2 mo	C	III	Pate TID 2016[1743]	N=1
Endogenous endophthalmitis	To cure	VCZ 4 mg/kg bid for 3 wk	C	III	Raevis CRO 2018[1747]	N=1, + 0.1 mL intravitreal VCZ, vision improvement
Uncontrolled diabetes	To cure	VCZ for 2 mo	C	III	Kammoun JMM 2018[1702]	N=1, sinusitis, surgical debridement, success

Standard dose unless stated otherwise; AE, adverse event; AmB, amphotericin B; AML, acute myeloid leukemia; bid, twice a day; CASPO, caspofungin; d: day(s); DSAEK, Descemet's stripping automated endothelial keratoplasty; ISA, isavuconazole; ICZ, itraconazole; iv, intravenous; L-AmB, liposomal amphotericin B; MICA, micafungin; mo, month(s); PCZ, posaconazole; po, orally; qd, once a day; QoE, quality of evidence; SoR, strength of recommendation; TRB, terbinafine; VCZ, voriconazole; wk, week(s).

Recommendation - According to available data and drug safety profiles[1790], the group moderately recommends L-AmB (monotherapy or combination therapy with VCZ or another antifungal), VCZ monotherapy, or ISA monotherapy as the preferred treatment regimens. Other combination therapy regimens should be considered according to results of *in vitro* studies [1779,1782]. Antifungal regimens that include PCZ delayed release tablet alone or in combination with TRB or MICA are moderately recommended for salvage therapy[1758,1760,1776,1785].

The duration of therapy should be individualized, and based on the site and extent of infection, and on the immune status of the patient **(Figure 29).**

Figure 29. Optimal treatment pathway for *Scopulariopsis* infections in adults when all treatment modalities and antifungal drugs are available



Specific considerations on treatment of Scopulariopsis infection in children

Evidence – Only scarce data exist on invasive infections by *Scopulariopsis* spp. in children[1726,1735,1745,1756,1784,1791]. In these studies, all children had an underlying Hematological malignancy and/or received a HSCT **(Table 37)**.

Table 37. First-line antifungal therapy in children for *Scopulariopsis* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Any	To cure	D-AmB iv	D	III	Krisher JCM 1995[1791]	N=1, 12 yrs, cutaneous infection + lung involvement, *Microascus*; D-AmB, followed by ABCD, failure Review of N=5, 17-40 yrs, AmB +/- ICZ or miconazole; failure 5/5
					Neglia AJM 1987[1784]	N=1, 17 yrs, lung infection, cAmB, + surgery, died
					Krisel CID 1994[1735]	N=1, 12 yrs, sino-nasal infection, cAmB + G-CSF + ICZ for 6 mo, surgery, survived
AML + BMT	To cure	VCZ + CASPO	C	III	Steinbach JInfect 2004[1756]	N=1, 10 yrs, skin and blood infection, survived
AML	To cure	L-AmB iv for 7 d, then switched to CASPO 1.5 mg/kg qd iv (first dose 2 mg/kg) + VCZ 7 mg/kg qd iv for 3 mo; then VCZ 150 mg po bid + CASPO 1.5 mg/kg iv tiw for 3 mo; followed by 6 mo VCZ 150 mg bid	B	III	Petit TLID 2011[1745]	N=1, 11 mo, lung infection, survived
AML	To cure	VCZ 150 mg iv bid initially, after positive culture and susceptibility testing followed by L-AmB, surgery	B	III	Gluck IJPO 2011[1726]	N=1, 17 yrs, sino-nasal infection, survived

Standard pediatric dose unless stated otherwise; ABCD, amphotericin B colloidal dispersion; AmB, amphotericin B; AML, acute myeloid leukemia; bid, twice a day; BMT, bone marrow transplant; CASPO, caspofungin; cAmB, oral encochleated amphotericin B; D-AmB, amphotericin B deoxycholate; G-CSF, granulocyte colonystimulating factor; ICZ, itraconazole; iv, intravenous; L-AmB, liposomal amphotericin B; mo, month(s); po, orally; qd, once a day; QoE, quality of evidence; SoR, strength of recommendation; tiw, three times a week; VCZ, voriconazole; wk, week(s); yrs, years.

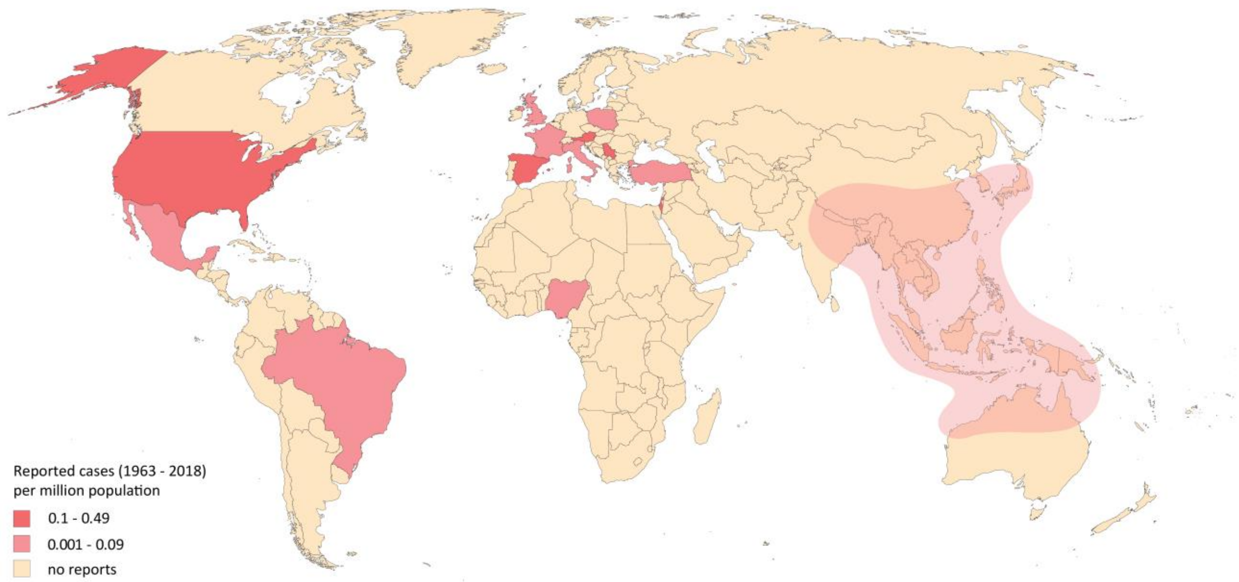
Recommendations – Treatment recommendations are extrapolated from those for adult patients, and L-AmB or VCZ or both in combination are moderately recommended.

8. Penicillium

Epidemiology of infections caused by *Penicillium* spp.

Several *Penicillium* spp. have been redefined as *Talaromyces*; for example, *P. marneffei* and *P. purpurogenum* are now named *Talaromyces marneffei* and *Talaromyces purpurogenus*, respectively, and are therefore not included in this section[1792]. *Penicillium* spp. are ubiquitous in nature and are used in drug and food production industries; *e.g.*, *P. chrysogenum* (formerly *P. notatum*) is used to produce the antibiotic penicillin and *P. camemberti* and *P. roqueforti* are used in cheese making[1793,1794]. *Penicillium* spp. have been recognized as environmental allergens, which are frequently associated with hypersensitivity pneumonitis in exposed workers[1795,1796], with unknown clinical significance. *Penicillium* spp. are rarely pathogenic in humans and are usually considered as laboratory contaminants or non-pathogenic colonizers in clinical material. However it is important to recognize that pathogenic species such as *P. chrysogenum*[1797,1798], *Penicillium citrinum*[1799,1800], *P. decumbens*[1798,1801], *P. commune*[1802], *P. oxalicum*[1803] and *P. purpurogenum* (*T. purpurogenus*)[1798] grow well at 37°C, whereas the majority of common laboratory contaminants do not grow at body temperature. *Penicillium* spp. have been reported as a cause of opportunistic infections leading to mycotic keratitis, endophthalmitis and lung infection[1800,1804-1806]. Disseminated infections such as endocarditis (following valve prosthesis insertion), CNS infection and fungemia also have been reported less frequently[1798,1807,1808]. In addition to immunocompromised humans, fatal *Penicillium* infections in dogs have been described[1809] **(Figure 30)**.

Figure 30. Worldwide distribution of infections caused by *Penicillium* spp. (reported cases between 1963 and 2018 per million population). The red cloud marks regions where penicilliosis (caused by various *Penicillium* spp.) is endemic.

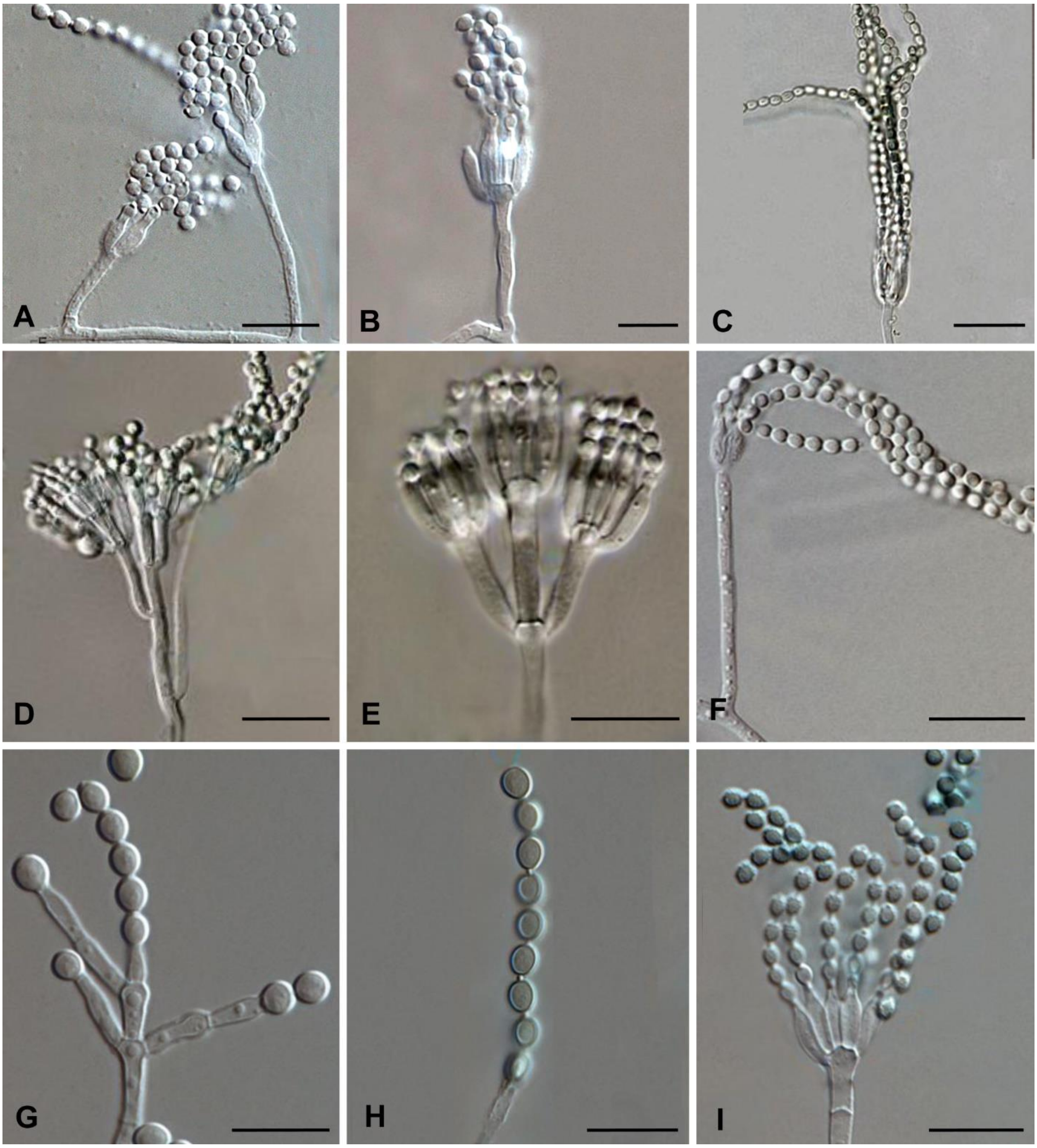


Cases of severe *Penicillium*-related infections reported in the medical literature were identified in a PubMed search on September 12, 2019. The search string included all *Penicillium* spp. listed in the Index Fungorum database (accessed September 12, 2019) and "(infection OR invasive OR disseminated OR patient [Title/Abstract] OR case [Title/Abstract] OR cases [Title/Abstract] OR report [Title/Abstract] OR isolate [Title/Abstract]) NOT marneffei [Title]" that yielded 834 publications. In total, 75 cases from 13 countries were identified, 34 since the year 2000 [49,173,606,1116,1740,1798,1800,1804-1808,1810-1848]. Most cases were reported from the United States (n=40), Nigeria (n=8), Spain (n=6), and United Kingdom (n=5). Few cases of invasive *Penicillium*-related infections were reported from endemic countries[1799,1803,1849,1850]. The number of cases reported between 1963 and 2018 is presented as cases per million population per country. The resident population per country was obtained from www.worldometers.info[321]. The grey cloud marks regions where penicilliosis is endemic and from where travel-related cases are possible (South East Asia, North India, Bhutan and Nepal, North Korea, Papua-New Guinea, and North Australia [1851-1853].

Diagnosis of *Penicillium* infections

Evidence - Conventional diagnosis using microscopy and culture is essential for identification of *Penicillium* spp. A positive culture from deep sterile tissues confirms the diagnosis[1798,1799,1824]. Definitive diagnosis of *Penicillium*-related infections needs to recognize invasive fungal elements by histological examination of tissue sections [1798,1799]. Infections caused by *Penicillium* spp. may be overlooked or misdiagnosed as aspergillosis due to nonspecific clinical and radiological findings[1803]. In addition, direct microscopic examination of both genera shows similar hyaline septate hyphae (hyalohyphomycosis) (**Figure 31)**.

Figure 31. Microscopic morphology of *Penicillium* spp.[19]



Panel A, *P. canis*, simple conidiophores, arising from creeping and aerial hyphae; **Panel B-C**, *P. capsulatum*, short conidiophores and smooth-walled, monoverticillate. Phialides densely aggregated in small whorls; **Panel D**, *P. chrysogenum*, conidiophores smooth-walled, penicilli usually terverticillate, flask-shaped phialides; **Panel E**, *P. citrinum* conidiophores smooth-walled, penicilli biverticillate; **Panel F**, *P. decumbens*, conidiophores rough-walled, penicilli monoverticillate, flask-shaped phialides; **Panel G-H**, *P. roqueforti*, conidiophores arising from submerged hyphae, relatively wide, with tuberculate walls, stage-branched penicillin; **Panel I**, *P. spinulosum,* conidiophores, smooth- to rough-walled; penicilli monoverticillate, flask-shaped phialides. Scale bars = 10 µm.

In routine laboratory evaluation, identification does not usually go beyond the genus level due to a huge number of species and the lack of expertise in identification at species level. Morphological identification to the species level is very difficult therefore molecular identification using ITS and β-tubulin sequencing is the gold standard, with MALDI-TOF MS being an alternative[559,1604,1854-1856]. Serological cross-reaction in the GM assay or *Aspergillus*-specific lateral flow device test has been observed[335,1857-1859]. Clinical diagnosis using imaging techniques[1854] or non-culture-based tests such as BDG have been used to diagnose *Penicillium* infections[1860]. Antifungal susceptibility testing results are variable and species-specific[559,1803,1861]. Terbinafine and echinocandins showed the best *in vitro* activity against *Penicillium* spp., AmB showed intermediate antifungal activity, while the azole MICs differed between isolates[1604], with higher MICs especially in *P. citrinum*, *P. oxalicum* and *Penicillium rubens*[559,1604,1803] **(Table 38)**.

Table 38. Microbiological, histopathological and imaging diagnostics of *Penicillium* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Microscopy, culture, MIC testing						
Any	To diagnose	Tissue biopsy	A	III	Mok JCM 1997[1799] Lyratzopoulous JInfect 2002[1798] Hesse MMCR 2017[1824] Chowdhary OFID 2014[1803]	
Any	To diagnose	Culture	A	III	Mok JCM 1997[1799] Lyratzopoulous JInfect 2002[1798] Keceli IUN 2005[1830] Iwasaki JJO 2008[1862] Oshikata BMCPM 2013[1850] Hesse MMCR 2017[1824] Chowdhary OFID 2014[1803]	
Any	To diagnose	Microscopy	A	III	Geltner Transpl 2013[1861] Chowdhary OFID 2014[1803]	
Any	To identify species	Microscopy	B	IIr	Visagie StudMycol 2014[1863]	*Hamigera*, *Paecilomyces*, *Rasamsonia*, *Sagenomella*, *Talaromyces*, and *Trichocoma* also show *Penicillium*-like "brush" structures
Any	To guide treatment and correlate MIC with outcome	Antifungal susceptibility testing, EUCAST method	B	III	Alastruey-Izquierdo AAC 2018[559]	
Any	To guide treatment and correlate MIC with outcome	Antifungal susceptibility testing, CLSI method	B	III	Chowdhary OFID 2014[1803] Geltner Transplantation 2013[1861] Barcus ACMA 2005[1864] Lyratzopoulos JInfect 2002[1798] Kantarcioğlu Mycoses 2004[1829]	

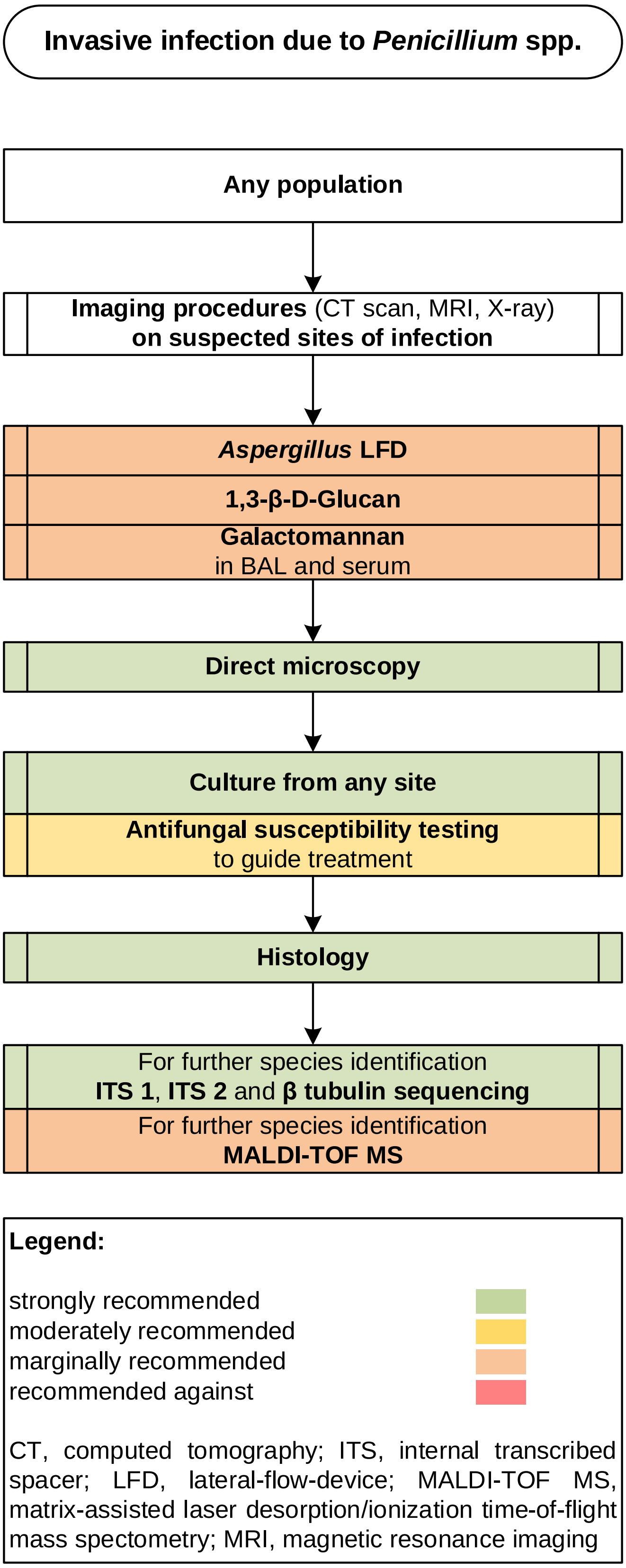
					Lyratzopoulos JInfect 2002[1798]	
Any	To determine *in vitro* activity by generating MIC	Antifungal susceptibility testing, CLSI method	C	IIu	Guevara-Suarez JCM 2016[1604]	
Serology assays						
Any	To detect *Penicillium* spp. other than T. marneffei	Galactomannan EIA	C	III	Mennink-Kersten LID 2004[1865]	May cross-react with *Penicillium* spp.
Any	To detect *Penicillium* spp. other than T. marneffei	*Aspergillus*-specific Lateral Flow Device	C	III	Thornton CVI 2008[335]	Cross-reacts with *Penicillium* spp. (but not with *T. marneffei*)
					Prattes Mycoses 2015[1857]	
					Willinger Transpl 2014[1858]	
Any	To detect *Penicillium* spp. other than T. marneffei in blood	BDG in serum	C	III	Odabasi MedMycol 2006[1860]	Cell wall of *Penicillium* spp. contains BDG
Nucleic-acid based assays						
Any with sinusitis	To diagnose	Beta-tubulin sequencing (from sinus content)	B	III	Radulesco Mycopathol 2018[1854]	N=1, fungus ball, *P. roqueforti*
Any	To diagnose	28S rRNA broad-range real-time PCR + sequencing in various clinical specimen	C	II	Vollmer JCM 2008[1855]	Panfungal, *Penicillium* just to the genus level
Any	To identify species	MALDI-TOFF MS	C	III	Lau BMCM 2016[1866]	
Any	To identify species	Culture + ITS/beta-tubulin sequencing	A	IIu	Guevara-Suarez JCM 2016[1604]	N=77 clinical isolates
					Mohammadi JRMS 2017[1867]	
					Oshikata BMCPM 2013[1868]	N=1
					Chen BMCID 2013[1869]	N=1
					Reboux JMM 2019[1856]	
Tissue-based diagnosis						
Any	To diagnose	Histopathology of biopsy tissue	A	III	Barcus ACMA 2005[1864]	
					Lyratzopoulos JInfect 2002[1798]	
					Lyratzopoulos JInfect 2002[1798]	
					Hall AHJ 1974[1823]	
					Yoshida Chest 1992[1870]	
					Gelfand SMJ 1990[1820]	
					D'Antonio JCM 1997[1871]	
Imaging studies						
Any	To detect sinusitis	Cranial CT	A	III	Radulesco Mycopathol 2018[1854]	N=1
Any	To detect dissemination	PET/CT	B	III	Qiu BMCID 2015[1872]	N=2
Any	To detect and assess brain lesions / abscesses	Cranial CT	B	III	Beh MJM 2009[1873]	N=1
					Noritomi RIMT 2005[1874]	N=1
					Zhang MCO 2016[1875]	N=1
Any	To detect and assess brain lesions / abscesses	MRI	A	III	Ye IJMM 2015[1876]	N=1
					Beh MJM 2009[1873]	N=1
					Noritomi RIMT 2005[1874]	N=1
					Zhang MCO 2016[1875]	N=1
Any	To detect and assess pneumonia	Chest radiography	C	III	Cheng MedMycol 1998[1877]	N=3
					Hung AJRCCM 2013[1878]	N=1
					Lu CMJ 2005[1879]	N=6
					Yadav IJPM 2019[1880]	N=1
					Ye IJMM 2015[1876]	N=1
					Sun CMI 2006[1881]	N=24
					McShane Thorax 1998[1882]	N=1
					Chen BMCID 2013[1869]	N=1
					Zhang MCO 2016[1875]	N=1
Any	To detect and assess pneumonia	Chest CT	A	III	Cheng MedMycol 1998[1877]	N=3
					Geltner Transpl 2013[1861]	N=1
					Hung AJRCCM 2013[1878]	N=1
					Lu CMJ 2005[1879]	N=3
					Yadav IJPM 2019[1880]	N=1
					Ye IJMM 2015[1876]	N=2
					Jung JKMS 2012[1883]	N=1
					Santos MedMycol 2006[1884]	N=1, 8 yo, CGD
					Lin HIVM 2009[1885]	N=19

					McShane Thorax 1998[1882]	N=1
					Qiu BMCID 2015[1872]	N=14
					Chen BMCID 2013[1869]	N=1
					Aviles-Robles IJID 2016[1804]	N=1
					Beh MJM 2009[1873]	N=1
					Wang Mycopathol 2018[1886]	N=4
					Zhang MCO 2016[1875] Chowdhary OFID 2014[1803]	N=1
Any	To detect and assess abdominal/lymph node infections	Abdominal CT	A	III	George IJMM 2008[1887]	N=1
					Lu CMJ 2005[1879]	N=1
					Yadav IJPM 2019[1880]	N=1
					Mancao PedRadiol 2003[1835]	N=1
					Othman ATP 2006[1888]	N=1
					Aviles-Robles IJID 2016[1804]	N=1
					Beh MJM 2009[1873]	N=1
Any	To detect and assess bone infections	Bone radiography	B	III	Chan JBJSB 1990[1889]	N=1
					Louthrenoo BJR 1994[1890]	N=8
					Sudjaritruk BMCID 2012[1891]	N=1
Any	To detect and assess bone infections	CT spine / bones	A	III	Louthrenoo BJR 1994[1890]	N=1
					Qiu BMCID 2015[1872]	N=14

BDG, Beta-D-Glucan; CGD, chronic granulomatous disease; CLSI, Clinical and Laboratory Standards Institute; CT, computed tomography; EIA, enzyme-linked immunoassay; EUCAST, European Committee for Antimicrobial Susceptibility Testing; ITS, internal transcribed spacer; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; PET, positron emission tomography; pt, patient; QoE, quality of evidence; rRNA, ribosomal ribonucleic acid; SoR, strength of recommendation; yo, years old

Recommendations – The guideline group strongly recommends conventional diagnostic techniques such as microscopy and culture as well as histopathological analysis of tissue sections. Molecular diagnosis in clinical specimens is moderately supported, while ITS and β-tubulin sequencing of isolates is strongly recommended for species identification. The group marginally supports GM, BDG and the *Aspergillus* LFD for diagnosis of *Penicillium*-related infections. MIC determination is moderately recommended to guide treatment. Imaging is variably recommended depending on the case and patient condition, but strongly recommended to clinically diagnose invasive infections **(Figure 32).**

Figure 32. Optimal diagnostic pathway for *Penicillium* infections, when all imaging and assay techniques are available



Treatment approaches for infections caused by *Penicillium* spp.

<u>Treatment in adults</u>

Evidence – Data guiding the treatment of *Penicillium* infections is scarce and mainly obtained from case reports. For first-line antifungal therapy, L-AmB has been used in many instances with variable results for invasive infections[1798]. Failure has been reported with both AmB alone or in combination with other antifungal agents[1798,1800], while successful treatment with AmB has been reported in other case series[1845,1892]. Clinical failure of VCZ treatment may be related to high VCZ MICs in infections caused by *P. oxalicum*[1803], a finding not uncommon in other *Penicillium* spp. such as *P. citrinum* and *P. rubens*[559,1604]. PCZ has been successfully used to treat infections caused by *P. oxalicum* with high MICs against VCZ[1803]. Treatment durations of 6 weeks have been reported in patients with successful outcome[1803]. Salvage treatment with parenteral VCZ resulted in satisfactory global response in 9 of 10 patients in one study[376]. In invasive infections, surgical resection of pulmonary nodules resulted in a successful outcome in most reported cases[1854,1869] **(Table 39)**.

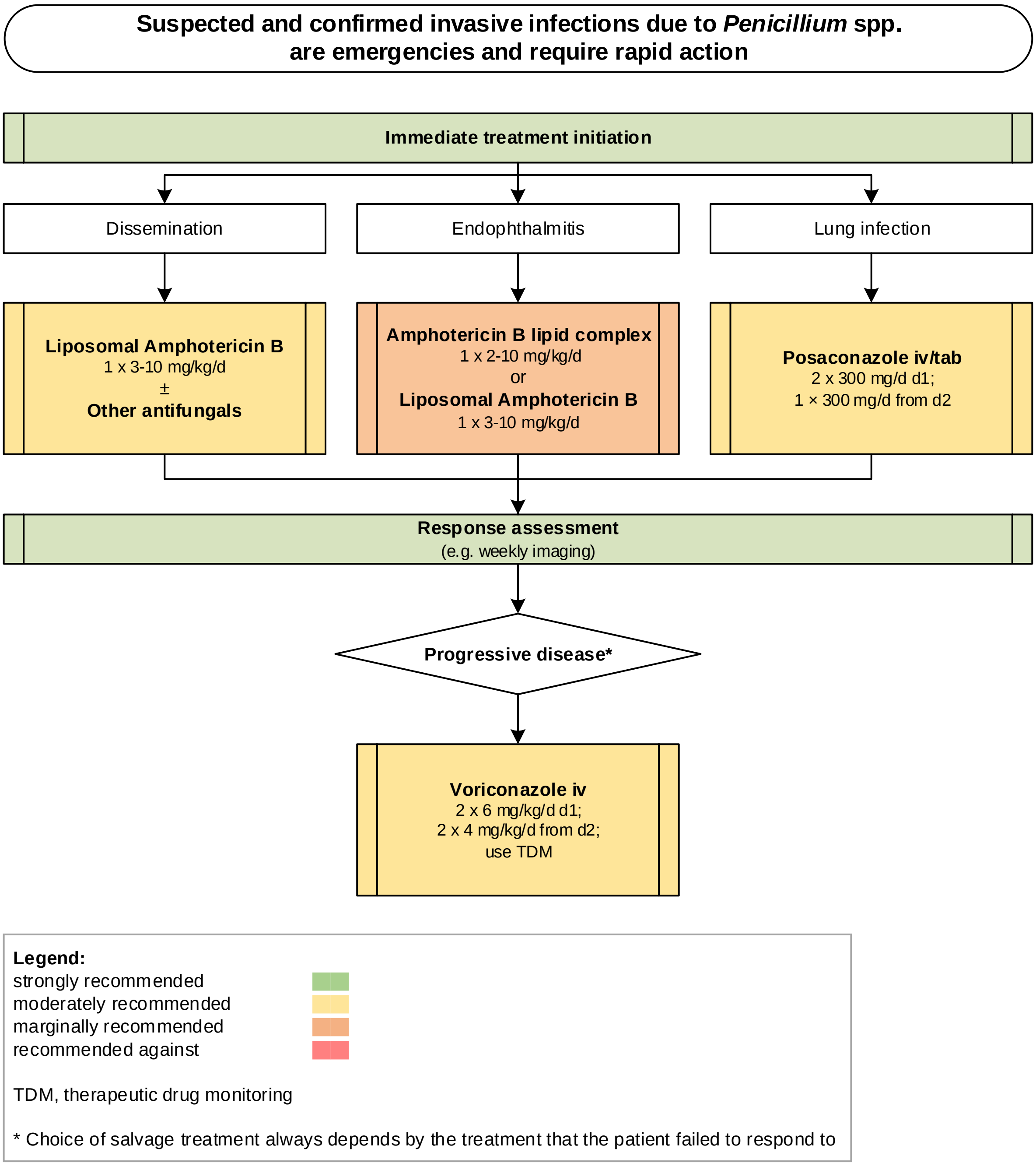
Table 39. Therapy for *Penicillium* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
First-line antifungal therapy						
Exogenous endophthalmitis	To cure	FCZ po; AmB intravitreal	D	III	Garg JCRS 2016[1800]	N=1, *P. citrinum*, failure
Exogenous endophthalmitis	To cure	AmB iv, topical, intracameral	C	III	Iwasaki JJO 2008[1862]	N=1, *Penicillium* spp., success
Exogenous endophthalmitis	To cure	MICA 100 mg qd for 9 d, VCZ 200 mg qd po for 9 d + 5-FC 6000 mg qd for 9 d, + VCZ eye drop qds	C	III	Kanda IMCRJ 2018[1892]	N=1, *Penicillium* spp., success
Endogenous endophthalmitis	To cure	AmB iv, 5-FC	C	III	Swan AJO 1985[1845]	N=1, *Penicillium* spp., success
Any with pulmonary infection due to *P. oxalicum*	To cure	PCZ	B	III	Chowdhary OFID [1803]	N=2, Posaconazole oral solution for 6 weeks, both cured
Any with disseminated infection	To cure	L-AmB +/- other drugs *e.g.* ICZ, 5-FC	B	III	Lyratzopoulos JInfect 2002[1798]	N=1, *P. chrysogenum*, L-AmB + 5-FC, failure
					Lyratzopoulos JInfect 2002[1798]	N=1, *P. decumbens*, L-AmB + ICZ, outcome unclear
Antifungal salvage treatment						
Any	To cure	VCZ iv	B	IIu	Perfect CID 2003[376]	N=10, response 9/10
Other Treatment Options						
Any	To cure	Surgery	B	IIIu	Chen BMCID 2013[1869]	N=1, *P. capsulatum*, success
					Aviles-Robles IJID 2016[1804]	N=1, *P. chrysogenum*, success
					Radulesco Mycopathol 2018[1854]	N=1, *P. roqueforti*, success
Treatment duration						
Any	To cure	≥6 wk antifungal treatment	C	III	Chowdhary OFID 2014[1803]	N=3, *P. oxalicum*, response to PCZ 2/3, failure 1/3

Standard dose unless stated otherwise; 5-FC, 5-Fluorocytosine; AmB, amphotericin B; d, day(s); FCZ, fluconazole; iv, intravenous; ICZ, itraconazole; L-AmB, liposomal amphotericin B; MICA, micafungin; PCZ, posaconazole; po, orally; qd, once a day; qds, four times a day; QoE, quality of evidence; SoR, strength of recommendation; VCZ, voriconazole; wk, week(s).

Recommendations – The guideline group moderately supports L-AmB alone or in combination with other antifungals for invasive infections caused by *Penicillium* spp. The guideline group marginally supports systemic treatment with lipid-based formulations of AmB for *Penicillium*-related endophthalmitis. The group moderately recommends salvage therapy with parenteral VCZ. Surgery is moderately recommended when feasible **(Figure 33).**

Figure 33. Optimal treatment pathway for *Penicillium* infections <u>in adults</u> when all treatment modalities and antifungal drugs are available



Specific considerations on treatment of* infections caused by *Penicillium* spp. *in children

Evidence – Pulmonary infections caused by *Penicillium* spp. have been described in children following lung transplantation and in those with CGD[1811] **(Table 40)**.

Table 40. First-line antifungal therapy in children for *Penicillium* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Lung trans-plant	To cure	VCZ + ANID	C	III	Ammermann ClinTransplant 2017[1811]	N=3, *Penicillium* spp., 3/3 survived
Standard pediatric dose unless stated otherwise; ANID, anidulafungin; QoE, quality of evidence; SoR, strength of recommendation; VCZ, voriconazole.						

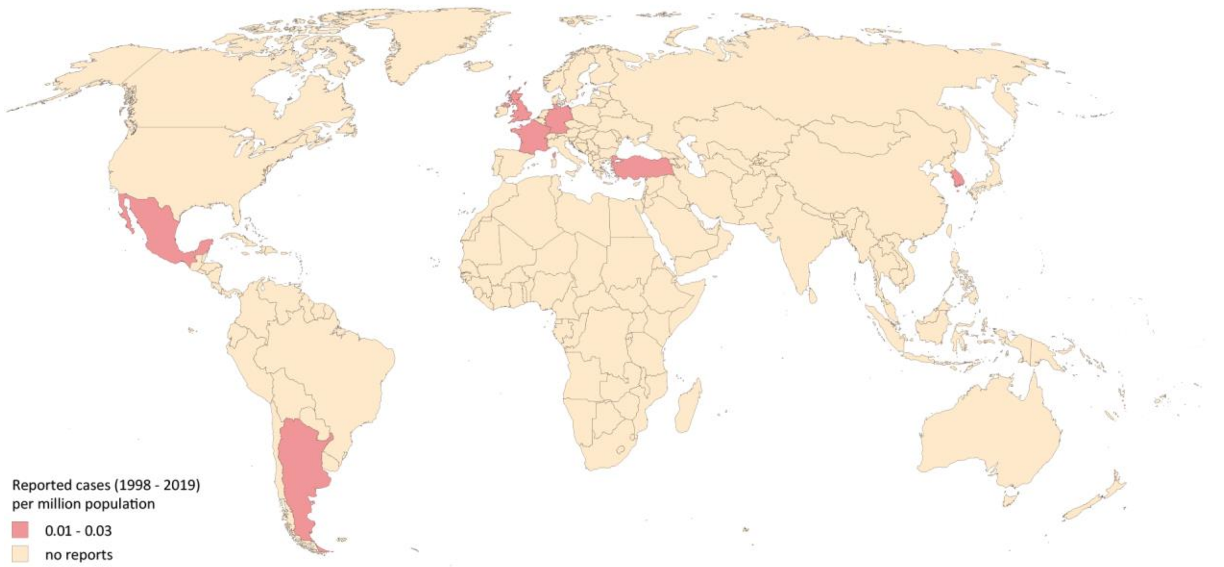
Recommendations – VCZ is moderately recommended as a first-line treatment option, although quality of evidence is weak[1811].

9. Non-*marneffei Talaromyces*

Epidemiology of infections caused by non-*marneffei Talaromyces* spp.

Talaromyces spp. belong to the order Eurotiales and are ubiquitously found in air, soil, and indoor environments. The vast majority of reported infections due to *Talaromyces* spp. are related to *T. marneffei* (formerly *P. marneffei*), which is endemic in China and in tropical regions of South East Asia, and are mostly associated with advanced HIV infection[1851]. Very few cases of talaromycosis caused by species other than *marneffei* have been reported from non-endemic regions *e.g.*, Europe and America over the past 20 years[1798,1884,1893-1898]. Infections have occurred in immunocompromised patients with CGD, malignancy, or long-term corticosteroid treatment for other chronic diseases. Infections are caused by *T. purpureogenus*, *T. stollii*, *T. piceae*[1884,1897], and *T. amestolkiae*, and mainly affect the lung and rarely other organs[1798,1898]. Hematogenous spread is common in *T. marneffei*-related infections; conversely, dissemination has rarely been reported for other *Talaromyces* spp.[1897] (**Figure 34)**.

Figure 34. Worldwide distribution of infections caused by non-*marneffei Talaromyces* spp. (reported cases between 1998 and 2019 per million population)



Cases of severe *Talaromyces*-related infections reported in the medical literature were identified in a PubMed search on July 29, 2019. The search string included all *Talaromyces* spp. that were identified in the Index Fungorum database (accessed 27. July 2019): (*Talaromyces* and *T.* each plus the following: *albobiverticillius*, *amelstokiae*, *apiculatus*, *assiutensis*, *atroroseus*, *aurantiacus*, *austrocalifornicus*, *bacillisporus*, *barcinensis*, *boninensis*, *brunneus*, *calidicanius*, *cecidicola*, *coalescens*, *convolutus*, *dendriticus, derxii, duclauxii, echinosporus, emodensis, erythromellis, euchlorocarpius, flavus, funiculosus, galapagensis, hachijoensis, helicus, indigoticus, intermedius, islandicus*, *lagunensis*, *leycettanus*, *loliensis*, *luteus*, *macrosporus*, *malagensis*, *mimosinus*, *minioluteus*, *muroii*, *palmae*, *panamensis*, *paucisporus*, *phialosporus*, *piceus*, *pinophilus*, *pittii*, *primulinus*, *proteolyticus*, *pseudostromaticus*, *purpureus*, *purpureogenus*, *rademirici*, *radicus*, *ramulosus*, *retardatus*, *rotundus*, *ruber*, *rubicundus*, *rugulosus*, *ryukyuensis*, *sabulosus*, *siamensis*, *stipitatus*, *stollii*, *subinflatus*, *sublevisporus*, *tardifaciens*, *thermocitrinus*, *trachyspermus*, *ucrainicus*, *udagawae*, *unicus*, *variabilis*, *varians*, *vermiculatus*, *flavus*, *vermiculatus*, *verruculosus*, *viridis*, *viridulus*, *wortmannii*) NOT *marneffei* [title]) that yielded 608 publications. In total, 10 cases were identified from 7 countries[1116,1798,1884,1893-1898]. Number of cases reported between 1998 and 2019 is presented as cases per million population per country. The resident population per country was obtained from www.worldometers.info[321].

Diagnosis of non-*marneffei Talaromyces* infections

Evidence - Non-*marneffei Talaromyces* spp. are rarely encountered in clinical specimens submitted to the diagnostic laboratory. Conventional diagnosis by microscopy and culture is essential to see the *Penicillium*-like structures[1893,1895]. Histopathological examination is crucial to demonstrate invasiveness with septate hyphae[1884]. Morphological identification is very challenging, therefore sequencing of the ITS and β-tubulin-encoding genes has been applied for species identification[1856]. Imaging with CT scan helps with the localisation of fungal infections[1894]. To guide treatment, antifungal susceptibility testing is important to correlate MIC with treatment outcome. In one study echinocandins seem to have the best *in vitro* activity[1604] **(Table 41)**.

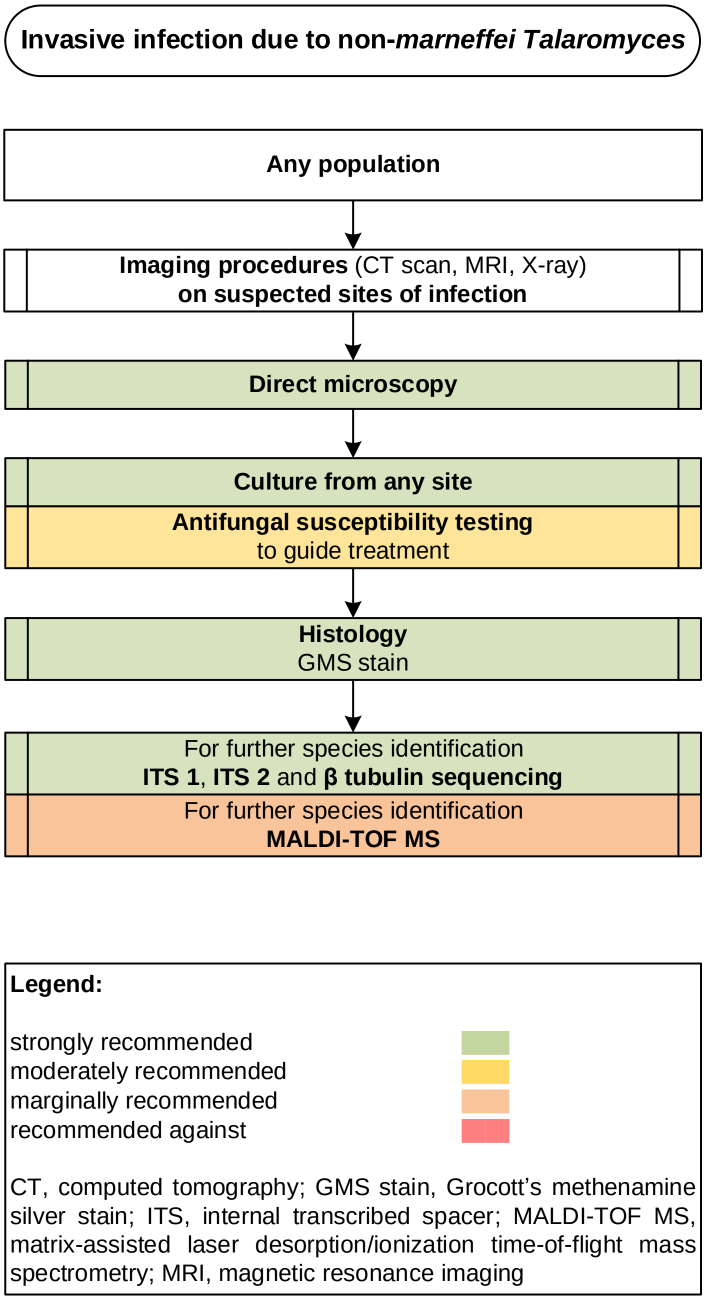
Table 41. Microbiological, histopathological and imaging diagnostics for non-*marneffei Talaromyces* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Microscopy, culture, MIC testing						
Any	To diagnose	Tissue biopsy stained by GMS	A	III	Sili MMCR 2015[1895]	By GMS staining, hyphal structures were shown and by PCR investigation *Talaromyces* sp. was identified
Any	To diagnose	Culture	A	IIIu	Villanueva-Lozano JIC 2017[1893]	N=1, BAL culture on PDA
Any	To diagnose	Microscopy	A	IIIu	Zainudin PSHC 2018[1899]	Microscopy revealed *Penicillium*-like structure, identified as *P. chrysogenum* by PCR
					Villanueva-Lozano JIC 2017[1893]	N=1, Microscopy showed *Penicillium*-like structure proved by PCR amplification as *T. amestolkiae*
Any	To guide treatment and correlate MIC with outcome	Broth microdilution (M-38A2 CLSI)	B	IIIu	Guevara-Suarez JCM 2016[1604]	Susceptibility testing of *T. amestolkiae*, *Talaromyces purpurpurogenus.* Best *in vitro* effect had echinocandins, however, only a few *Talaromyces* isolates were tested in this study
Nucleic-acid based assays/MALDI-TOF MS						
Any	To identify clinically important *T. marneffei* and non-*marneffei* spp.	Bruker MALDI-TOF MS system	C	III	Lau BMCM 2016[1866]	N=59 isolates with documented penicilliosis. Database is suboptimal. Among four species phylogenetically closely related to *T. marneffei*, only *Penicillium brevicompactum* and *P. chrysogenum* were identified, while *Talaromyces aurantiacus* and *Talaromyces stipitatus* strains were not identified
Any	To identify species	ITS1/ITS2/ITC sequencing for molecular species identification of *Talaromyces-/Penicillium*-like fungi (25-30°C) yeast-like (35-37°C) forming red pigment	A	III	Ryu LMO 2017[1900]	Sanger sequencing of the ITS regions covering ITS1, 5.8S, and ITS2, and the β-tubulin gene from the genomic DNA revealed *Talaromyces albobiverticillius*

Tissue-based diagnosis						
Any	To diagnose	Histopathology of biopsy tissue	A	III	Santos MedMycol 2006[1884]	Multinucleated giant phagocytic cell with budding fungal elements in patient with pulmonary nodule
Imaging studies						
Any	To assess the clinical manifestations and imaging characteristics of pneumonia caused by *Talaromyces* spp.	Chest CT	A	III	Atalay LIM 2016[1894]	N=1, bilobular infiltrates and *T. purpurogenus* in sputum
BAL, brochoalveolar lavage; DNA, deoxyribonucleic acid; CLSI, Clinical and Laboratory Standards Institute; CT, computed tomography; GMS, Grocott-Gomori's methenamine silver; ITS, internal transcribed spacer; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; MIC, minimal inhibitory concentration; PCR, polymerase chain reaction; PDA, ; PDA, potato dextrose agar; QoE, quality of evidence; SoR, strength of recommendation; *T.*, *Talaromyces*						

Recommendations – The guideline group strongly recommends microscopy and culture to diagnose *Talaromyces*-related infections, as well as histopathological evaluation (GMS staining) of tissue biopsies to distinguish between true infection and colonization. Molecular identification of isolates by sequencing the ITS regions is strongly recommended, while MALDI-TOF MS is marginally recommended by the group for species identification. Antifungal susceptibility testing is moderately recommended to guide treatment and correlation between MIC and outcome. CT scan is strongly recommended to clinically diagnose the infections **(Figure 35)**.

Figure 35. Optimal diagnostic pathway for non-*marneffei Talaromyces* infections , when all imaging and assay techniques are available



Treatment approaches to non-*marneffei Talaromyces* infection

<u>Treatment in adults</u>

Evidence – In view of the infrequency/rarity of infections caused by non-*marneffei Talaromyces* spp., information on antifungal treatment is available from a few published case reports. L-AmB has been successfully used as a first-line treatment[1798]. Salvage treatment of non-*marneffei Talaromyces*-related infections with a combination of AmB plus ICZ/TRB has been unsuccessful[1798]. In invasive infections, surgical resection of pulmonary nodules resulted in a successful outcome[1884] **(Table 42)**.

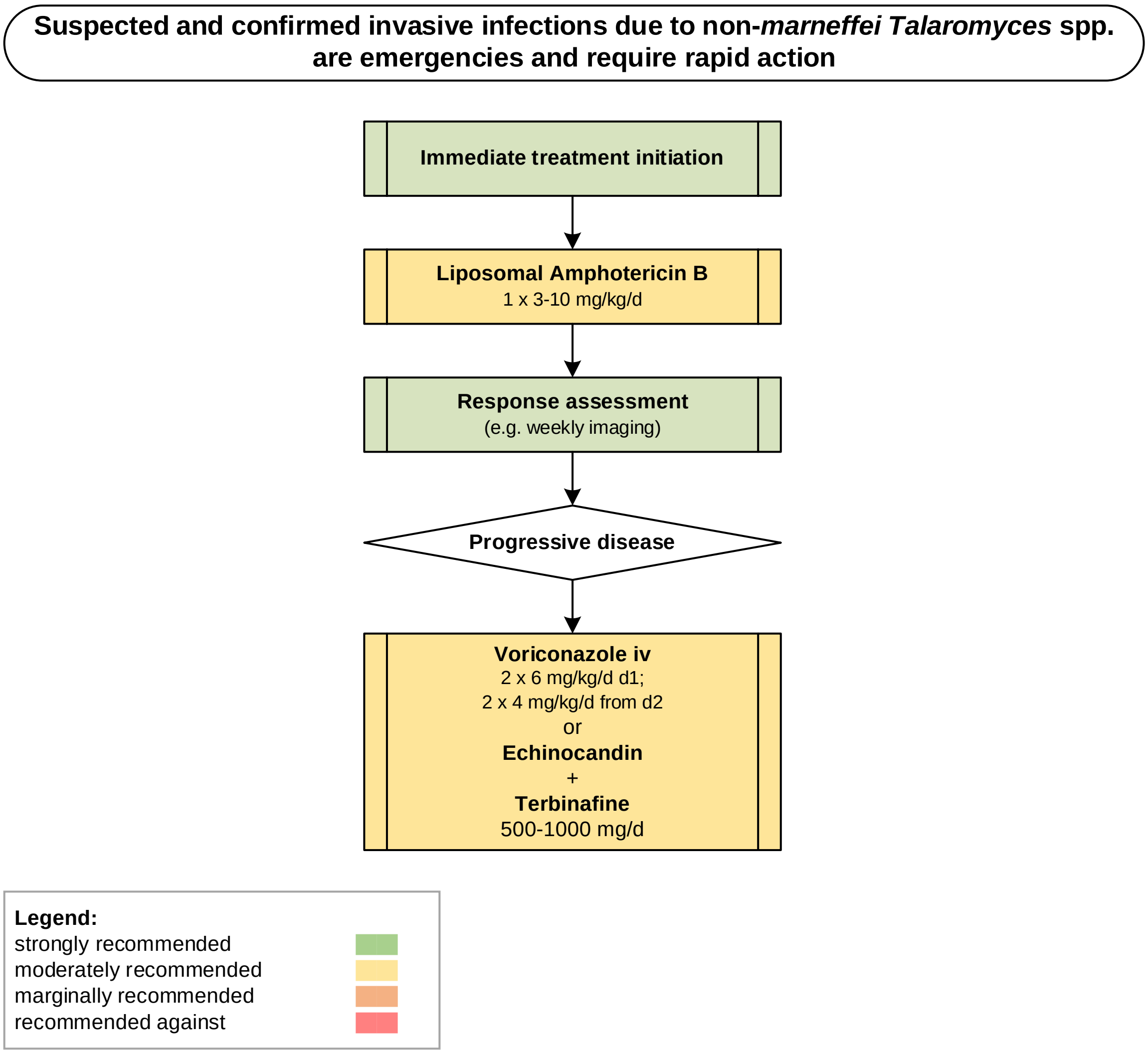
Table 42. Therapy for non-*marneffei Talaromyces* infections

Population	Intention	Intervention	SoR	QoE	Reference	
First-line antifungal therapy						
Any	To cure	L-AmB	B	IIIu	Lyratzopoulos JInfect 2002[1798]	Higher MICs for azoles compared to AmB and echinocandins
					Guevera-Suarez JCM 2016[1604]	
					Lyratzopoulos JInfect 2002[1798]	N=1, CGD, *Talaromyces purpureogenus*, success
Antifungal salvage treatment						
Any	To cure	Lipid formulations AmB + ICZ + /- TRB	C	III	Lyratzopoulos JInfect 2002[1798]	N=2, 2/2 died
Any	To cure	VCZ iv	C	III	Santos MedMycol 2006[1884]	N=1, pulmonary nodule and adjacent rib osteomyelitis, *Talaromyces picea,* + surgery, cured
Any	To cure	TRB and echinocandins	C	III	Guevera-Suarez JCM 2016[1604]	TRB and echinocandins had higher *in vitro* activity than AmB
Other treatment options						
Any	To cure	Surgery	B	IIIu	Santos MedMycol 2006[1884]	N=1, *Talaromyces picea*, success
Treatment duration						
Any	To cure	12 wk of therapy	C	III	Lyratzopoulos JInfect 2002[1798]	N=1, survived

Standard dose unless otherwise stated AmB, amphotericin B; CGD, chronic granulomatous disease; ICZ, itraconazole; L-AmB, liposomal amphotericin B; MIC, minimal inhibitory concentration; PCZ, posaconazole; QoE, quality of evidence; SoR, strength of recommendation; wk, week(s)

Recommendations – Treatment with L-AmB is moderately recommended by the group. The guideline group moderately recommends surgical resection, and marginally salvage therapy with VCZ or an echinocandin plus TRB **(Figure 36).**

Figure 36. Optimal treatment pathway for non-*marneffei Talaromyces* infections in adults when all treatment modalities and antifungal drugs are available



Specific considerations on treatment of non-marneffei Talaromyces infections in children

Evidence – Specific pediatric data and case reports are lacking.

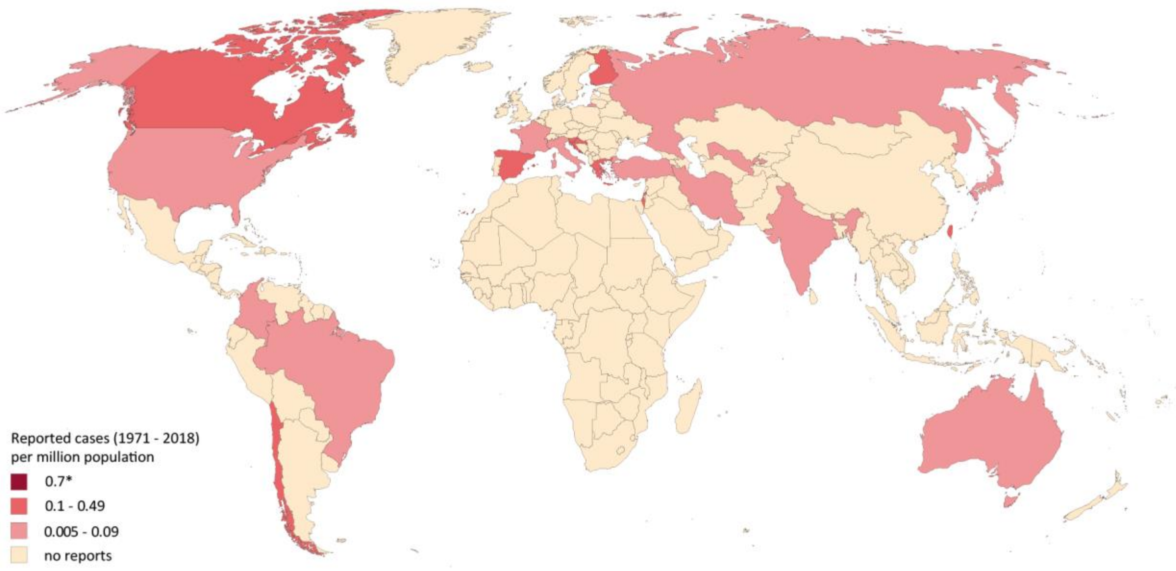
Recommendations – Treatment recommendations follow those in adults.

10. *Paecilomyces*

Epidemiology of *Paecilomyces* infections

Paecilomyces spp. are members of the order Eurotiales. They are filamentous, saprophytic, and thermo-tolerant fungi that are ubiquitously found in soil, food products, decaying organic material, and house dust[1901]. In the past, *Paecilomyces* spp. were often considered as contaminants when isolated clinically, but recently they are becoming recognized worldwide as important cause of infections primarily in immunocompromised patients or patients with indwelling catheters[1902]. However, immunocompetent individuals can also be affected, *e.g.*, by direct inoculation of fungus following trauma[1903,1904]. The majority of these infections is caused by *P. variotii* spp. complex that is composed of *P. variotii sensu stricto*, *P. formosus*, *P. divaricatus*, *P. brunneolus*, and *P. dactylethromorphus*[1905]*. P. variotii* is the asexual state of *Byssochlamys spectabilis*. Microbiological identification of *Paecilomyces* spp. is challenging due to its morphological similarity to some *Rasamsonia* and *Hamigera* spp.[1906]. Phylogenetic analyses showed that *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) belongs to the order Hypocreales and thus shall not be considered together with *Paecilomyces* spp.[1905]. Species identification is crucial for patient management since both species appear to have different susceptibility profiles and clinical response to antifungal agents[1903,1907]. *P. variotii* infection can affect many different organ systems and presents with various manifestations including pneumonia, skin and soft tissue infections, endophthalmitis, peritonitis, osteomyelitis, and bloodstream infections, especially in immunocompromised patients[1908-1914] **(Figure 37)**.

Figure 37. Worldwide distribution of infections caused by *Paecilomyces* spp. (reported cases between 1971 and 2018 per million population)



Cases of severe *Paecilomyces*-related infections reported in the medical literature were identified in a PubMed search on October 31, 2019 including all *Paecilomyces* spp. identified in the Index Fungorum database (accessed 27. July 2019) in the PubMed search string (*Paecilomyces* plus the following: *antarcticus*, *aspergilloides*, *atrovirens*, *baarnensis*, *borysthenicus*, *breviramosus*, *brunneolus*, *brunneolus*, *burcii*, *byssochlamydoides*, *canadensis*, *cinnamomeus*, *clavisporus*, *cossus*, *cremeoroseus*, *cylindricosporus*, *divaricatus*, *echinosporus*, *erectus*, *fimetarius*, *fulvus*, *fuscatus*, *gunnii*, *hawkesii*, *heliothis*, *hepiali*, *huaxiensis*, *indicus*, *isarioides*, *laeensis*, *longipes*, *loushanensis*, *mandshuricus*, *maximus*, *maximus*, *maximus*, *militaris*, *musicola*, *niphetodes*, *odonatae*, *parvisporus*, *pascuus*, *penicillatus*, *persimplex*, *puntonii*, *purpureus*, *ramosus*, *rariramus*, *saturatus*, *simplex*, *smilanensis*, *stipitatus*, *subglobosus*, *suffultus*, *tabacinus*, *taitungiacus*, *tenuis*, *variotii*, *verrucosus*, *verticillatus*, *victoriae*, *vinaceus*, *wawuensis*, *xylariiformis*, *zollerniae*) AND (infection OR invasive OR fungal infection OR fungemia OR blood OR disseminated OR subcutaneous OR case [Title/Abstract] OR report [Title/Abstract] OR case series [Title/Abstract] OR patient OR isolate) that yielded 662 publications. In total, 93 cases were identified from 23 countries, 67 since the year 2000[572,1116,1903,1907,1914-1969]. Most cases were reported from the United States (n=16), Spain (n=13) and Taiwan (n=14). The number of cases reported between 1971 and 2018 is presented as cases per million population per country. The resident population per country was obtained from www.worldometers.info[321]. *Five cases were reported from Hong Kong SAR (0.7 cases per million population between 1971 and 2018)[1925,1943,1944,1957].

Diagnosis of *Paecilomyces* infections

A species distinction is achieved in the course of diagnosis on the basis of various microbiological and molecular criteria.

Evidence – The initial step in laboratory diagnosis is histopathological examination and microscopy, which reveal non-specific branched septate hyphae[1965]. Culture is essential for species identification. Based on the culture morphology on different growth media, species determination can be achieved[1597,1915,1965,1970-

[1973]. Histology is also required for identification of fungi, classification and evaluation of irregular hyphae[1913].

Molecular-based methods can be used for species identification from DNA extracted from clinical isolates with subsequent Sanger sequencing, but not for the detection of fungal DNA directly from clinical material. Differentiation occurs via PCR-based DNA amplification of the rRNA gene regions ITS1 and ITS2 and of the 28S D1 and D2 regions. Additionally the amplification of the β-tubulin-encoding gene for species identification has been mentioned. Genbank analysis and sequence alignment are used for exact species assignment[1597,1938,1965,1970,1971,1974-1976]. Occasionally MALDI-TOF MS technology is used for species identification[1926].

For susceptibility testing, the EUCAST[1974] and CLSI M38-A2 microdilution methodologies[1965] have shown AmB and echinocandins to be active against clinical *P. variotii* isolates*.* Among the triazoles, ICZ and PCZ showed clinically relevant activity against *P. variotii*. PCZ and TRB showed good *in vitro* activity with ICZ the second most active and VCZ the less active triazole[1970].

Imaging technologies (chest CT) are mainly used for detection of suspected pulmonary infections[1915,1977] **(Table 43)**.

Table 43. Microbiological, histopathological and imaging diagnostics of *Paecilomyces* infections

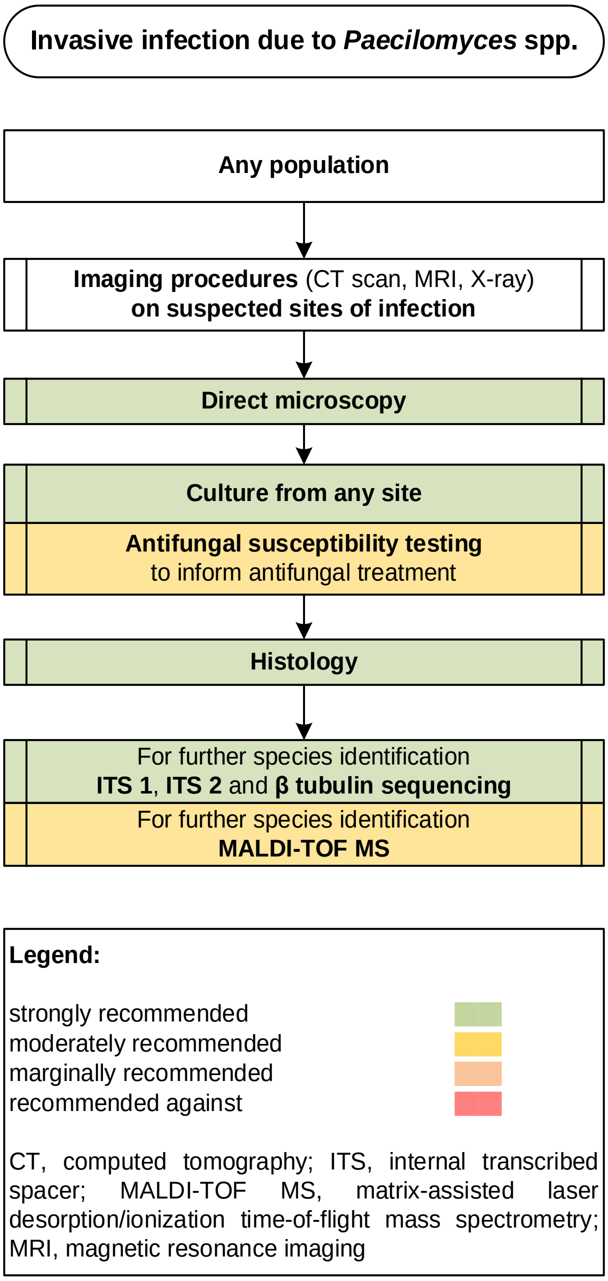
Population	Intention	Intervention	SoR	QoE	Reference	Comment
Microscopy, culture, MIC testing						
Any	To diagnose	Culture	A	III	Samson StudMycol 1974[1978]	Differentiate between *P. lilacinum* and *P. variotii*
					Houbraken JCM 2010[1970]	
					Barker MedMycol 2014[1597]	Sabouraud glucose brain heart infusion agar at 30°C
					Abolghasemi Tanaffos 2015[1915]	Sabouraud dextrose agar
					Eren TID 2018[1913]	Potato dextrose agar
Any	To diagnose	Microscopy	A	III	Eren TID 2018[1913]	Gram and Giemsa staining
					Uzunoglu JMM 2017[1965]	Lactophenol cotton blue
Any	To guide treatment	EUCAST microdilution protocol	B	III	Castelli AAC 2008[1974]	
					Feldman Mycoses 2016[1903]	
Any	To guide treatment	Broth microdilution method according to CLSI guidelines (M38-A)	B	III	Aguilar AAC 1999[1774]	
					Houbraken JCM 2010[1970]	
Nucleic acid-based assays/MALDI-TOF MS						
Any	To identify species	PCR: ITS1, ITS2, and β-tubulin gene +/- 5.8S rDNA	A	IIu	Houbraken JCM 2010[1970]	
					Uzunoglu JMM 2017[1965]	
					Kantarcioglu Mycoses 2003[1938]	
					Barker MedMycol 2014[1597]	

					Castelli AAC 2008[1974]	
Any	To identify species	MALDI-TOF MS	B	III	Chen FMicrobiol 2015[1926]	
					Barker MedMycol 2014[1597]	
Tissue-based diagnosis						
Any	To diagnose	Histopathological examination of biopsy tissue	A	III	Eren TID 2018[1979]	
Imaging studies						
Any	To identify CNS infection	Cranial MRI	B	III	Kantarcioglu Mycoses 2003[1938]	
Any	To identify pulmonary infection	Chest (HR) CT	A	III	Marques EFIM 2019[1904]	
					Abolghasemi Tanaffos 2015[1915]	

CLSI, Clinical and Laboratory Standards Institute; CT, computed tomography; EUCAST, European Committee for Antimicrobial Susceptibility Testing; HR, high-resolution; ITS, internal transcribed spacer; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; PCR polymerase chain reaction; QoE, quality of evidence; rRNA, ribosomal ribonucleic acid; SoR, strength of recommendation;

Recommendations - Direct microscopy and culture followed by PCR sequencing of the ITS and D1/D2 regions for species identification are strongly recommended, as is histopathological examination of tissue. Antifungal susceptibility testing is moderately recommended to guide treatment. Imaging modalities such as chest CT are strongly recommended if applicable **(Figure 38).**

Figure 38. Optimal diagnostic pathway for *Paecilomyces* infections, when all imaging and assay techniques are available



Treatment approaches to *Paecilomyces* infections

Treatment in adults

Evidence – L-AmB generally demonstrates good *in vitro* activity[1980] and different AmB formulations have been reported with varying but usually good responses[1907,1914,1924,1929,1943,1944,1947,1953,1964]. Antifungal combination therapies have only been described in individual cases (*e.g.*, L-AmB in combination with ICZ[1907,1965] or AmB in combination with ANID[1907]), and were associated with favourable outcomes. ICZ and

PCZ show good activity against *P. variotii*[1980] and have been successfully used for salvage therapy[1915,1966,1964]. The appropriate length of treatment for *P. variotii* infections is unclear. Successful case reports cite treatment duration ranges from 4 to 12 weeks[1907,1915,1964,1965,1979]. For dialysis-associated peritonitis, shorter treatment duration of 10 days is reported in two cases in combination with peritoneal catheter removal[1947].

Various authors have reported good treatment responses after surgical interventions[1921,1943,1944,1962,1967] and removal of venous or intraperitoneal catheter systems[1924,1947,1964,1965] **(Table 44)**.

Table 44. Therapy for *Paecilomyces* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
First-line antifungal therapy						
Any	To cure	L-AmB	B	IIIt	Salle JInfect 2005[1911]	N=1, fungemia, response
					Chamilos JInfect 2005[1924]	N=1, *P. variotii*, + CVC removal, response, MICs VCZ 8 µg/ml, AmB 0.25 µg/ml, PCZ 0.06 µg/ml
					Steiner CRID 2013[1910]	N=1, pneumonia, *P. variotii*, failure
					Bellanger Mycopathol 2017[1907]	N=1, fungemia, + ANID, success
					Dharmasena BMJ 1985[1929]	N=1, pneumonia, success
					Kantarcioglu Mycoses 2003[1938]	N=1, CNS infection, failure
					Cohen-Abbo Infection 1995[1914]	N=1, response
					Lam Eye 1999[1943]	N=1, endophthalmitis + fungemia, + surgery, success
Any	To cure	VCZ	D	III	Eren TID 2018[1979]	N=1, skin infection, + surgery, success
Any	To cure	PCZ tablet	C	III	Marques EJCRIM 2019[1904]	N=1, pulmonary mycetoma, died
Any	To cure	ICZ po	C	III	Abolghasemi Tanaffos 2015[1915]	N=1, pneumonia, success
					Vasudevan IJD 2013[1966]	N=1, subcutaneous hyalohypomycosis, success
Peritoneal dialysis patients	To cure peritonitis	L-AmB iv +/- ICZ po	B	IIIt	Torres PDI 2014[1908]	N=3, + catheter removal N=3, + laparotomy N=1, success 2/3
					Uzunoglu JMM 2017[1965]	N=1, success
					Marzec JCM 1993[1947]	N=4, + catheter removal N=4, success 4/4
Antifungal salvage treatment						
Any	To cure	PCZ	B	III	Feldmann Mycoses 2016[1903]	N=1, pneumonia, response
					Bellanger Mycopathol 2017[1907]	N=1, fungemia, success
Any	To cure	ICZ po	B	III	Lee JHJT 2002[1944]	N=1, sternotomy wound infection, + surgery, success
Other treatment options						
Peritoneal dialysis patients	To cure	Catheter removal	A	III	Torres PDI 2014[1908]	N=3, initial response 3/3, success 2/3
					Uzunoglu JMM 2017[1965]	N=1, success
					Marzec JCM 1993[1947]	N=4, success 4/4
Endophthalmitis	To cure	Vitrectomy and AmB 5 µg intravitreal	C	III	Tarkkanen AOS 2004[1962]	N=1, initial response but several relapses
					Lam Eye 1999[1943]	N=1, endophthalmitis + fungemia, success
Any	To cure	Surgical debridement	B	III	Eren TID 2018[1979]	N=1, skin infection, + surgery, success
					Lee JHJT 2002[1944]	N=1, sternotomy wound infection, success
Treatment duration						

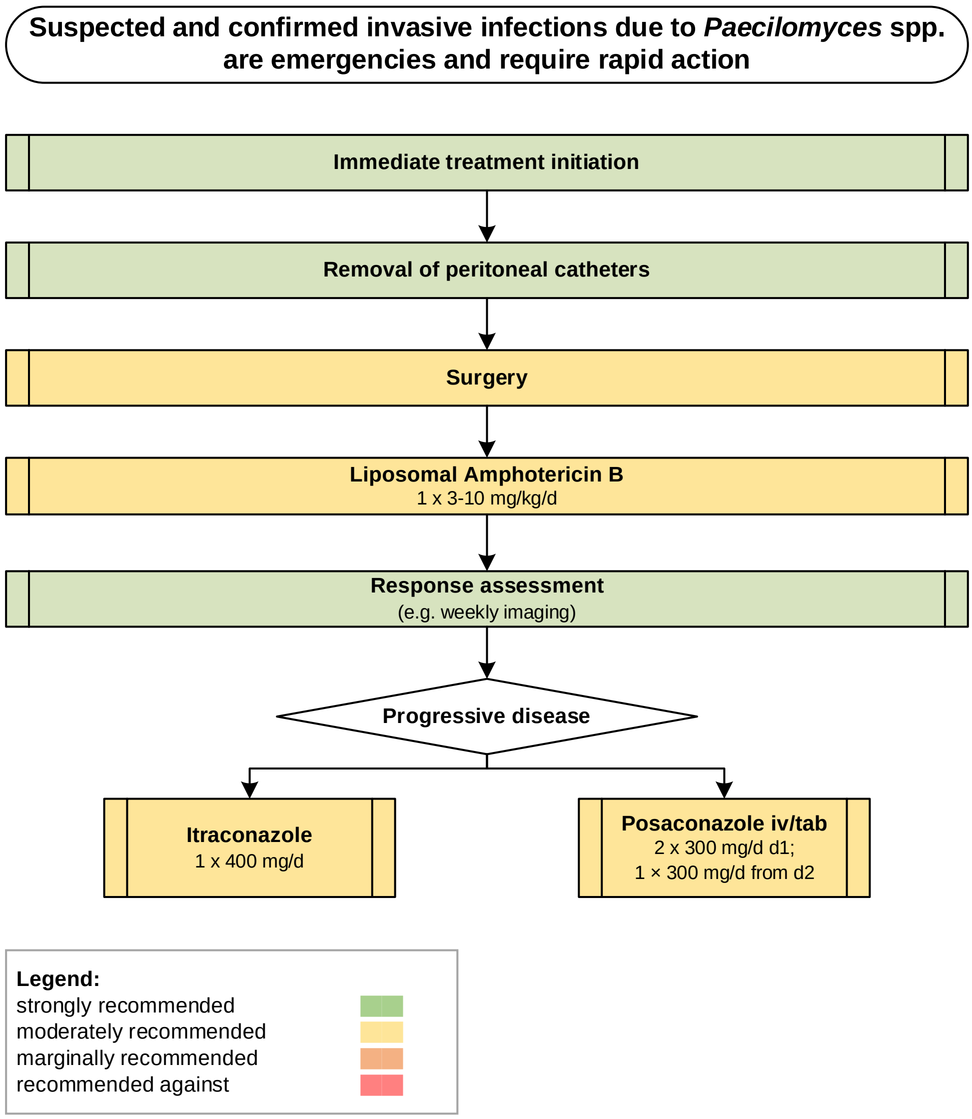
Diabetic	To cure pneumonia	4 wk ICZ	C	III	Abolghasemi Tanaffos 2015[1915]	N=1, success
Solid organ transplant recipients	To cure skin infection	6 wk VCZ po	C	III	Eren TID 2018[1944]	N=1, success
Any	To cure pulmonary mycetoma	6 wk PCZ	C	III	Marques EJCRIM 2019[1904]	N=1, died
Peritoneal dialysis patients	To cure peritonitis	4-8 wk L-AmB iv + ICZ po	C	III	Torres PDI 2014[1908]	N=3, initial response 3/3, success 2/3
					Uzunoglu JMM 2017[1965]	N=1, success
					Marzec JCM 1993[1947]	N=4, >10 d AmB deoxycholate, success 4/4
Hematological malignancy	To cure fungemia	6-12 wk L-AmB, step down to PCZ po possible	C	III	Bellanger Mycopathol 2017[1907]	N=1, success
					Salle JInfect 2005[1911]	N=1, response

Standard dose unless stated otherwise; ANID, anidulafungin; AmB, amphotericin B; CNS, central nervous system; CVC, central venous catheter; d, day(s); ICZ, itraconazole; iv, intravenous; L-AmB, liposomal amphotericin B; MIC, minimal inhibitory concentration; PCZ, posaconazole; po, orally; qd, once a day; QoE, quality of evidence; SoR, strength of recommendation; VCZ, voriconazole; wk, week(s).

Recommendation – We moderately support the use of L-AmB (3-10 mg/kg qd) as a first-line antifungal monotherapy. We moderately recommend PCZ tablet (300 mg/d maintenance) or ICZ oral (400 mg/d) for salvage treatment. Treatment duration is a personalized decision and should be tailored to clinical signs. In general, weeks to months of therapy are given and we marginally support 4 to 12 weeks of treatment.

We moderately support a recommendation of surgical debridement of infected tissues and strongly support removal of peritoneal catheters in peritoneal dialysis patients with peritonitis **(Figure 39)**.

Figure 39. Optimal treatment pathway for *Paecilomyces* infections in adults when all treatment modalities and antifungal drugs are available



Specific considerations on treatment of Paecilomyces infections in children

Evidence – Only a few cases of *P. variotii*-related infection in children are described. In individual cases, successful treatment was achieved with AmB or VCZ[1924,1928,1951,1952,1967] **(Table 45)**.

Table 45. Therapy in children for *Paecilomyces* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
First-line antifungal therapy						
Solid organ transplant recipient	Infectious source control/pneumonia	L-AmB qd or 10 mg/kg tiw +/- ICZ po OR VCZ 7 mg/kg bid	B	III	Das PedTransplant 2000[1928]	N=1, 9 ys, Lung TX, pneumonia, several fungi in BAL, including *Aspergillus versicolor, Aspergillus fumigatus, Aspergillus niger*, *Penicillium* spp., *P. variotii*, questionable response, died from complications of bronchopneumonia
					Polat Mycopathol 2015[1951]	N=1, 16 yrs, Live TX, peritonitis, success
Antifungal salvage treatment						
Hematological malignancy	To cure fungemia	L-AmB	C	III	Chamilos JInfect 2005[1924]	N=1, 14 yrs, CVC infection, + catheter removal, success
Treatment duration						
Any	To cure peritonitis	4-6 wk of therapy	C	III	Rinaldi PedNephrol 2000[1952]	N=1, success
					Polat Mycopathol 2015[1951]	N=1, success
Any	To cure	6-8 wk of therapy with L-AmB, followed by ICZ	C	III	Chamilos JInfect 2005[1924]	N=1, fungemia, success
					Das PedTransplant 2000[1928]	N=1, pneumonia, questionable response
Any	To cure splenic abscess	14 mo of therapy with FCZ + 5-FC	C	III	Wang DMID 2005[1967]	N=1, success

Standard pediatric dose unless otherwise stated; 5-FC, 5-fluorocytosine; A *Aspergillus*; BAL, bronchoalveolar lavage; bid, twice a day; CVC, central venous catheter; FCZ, fluconazole; ICZ, itraconazole; L-AmB, liposomal amphotericin B; mo, month(s); po, orally; qd, once a day; QoE, quality of evidence; SoR, strength of recommendation; tiw, three times a week; VCZ, voriconazole; wk, week(s); yrs, years.

Recommendation – Although there is limited evidence to support a specific regimen, we moderately support the use of L-AmB (3 mg/kg qd or 10 mg/kg tiw), or VCZ as first-line treatment for *Paecilomyces*-related infections in children.

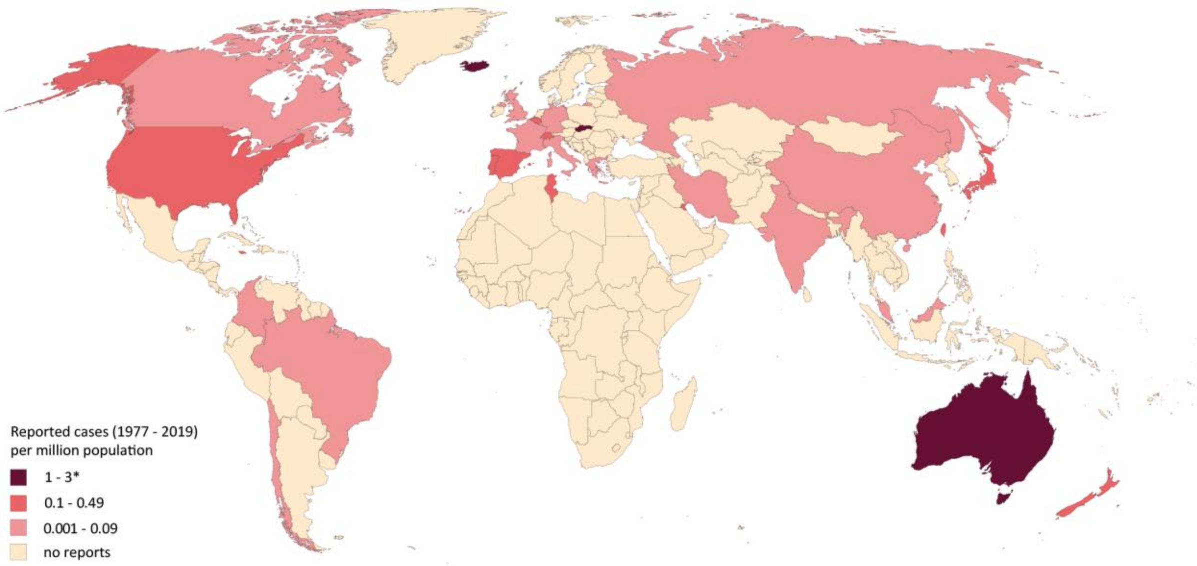
11. *Purpureocillium*

Epidemiology of *Purpureocillium* infections

P. lilacinum (formerly *Paecilomyces lilacinus*) is a saprobic, non-dermatophyte mold with a worldwide distribution and can be commonly found in soil. Due to its nematophagous potential it is widely used as a bio-control agent in agriculture and has been isolated from water streams in the Middle East, possibly as a run-off from agricultural use[1981,1982].

P. lilacinum has a tropism for ocular structures, thus, the most frequent clinical manifestations in humans are keratitis and endophthalmitis, followed by cutaneous and subcutaneous infections[1983]. The most common route of infection is via external invasion but endogenous infections also have been reported, mainly in immunocompromised patients. In a US study in 2 referral centers, ~4% of cases of fungal keratitis were caused by *P. lilacinum*[1984]. Frequently, eye infections are associated with intra-ocular lens implantation, trauma and the use of soft contact lenses[1918,1983-1985]. Up to one third of reported *Purpureocillium*-associated keratitis cases required enucleation[1983]. Poor outcome is possibly related to the limited efficacy of topical natamycin, which is the treatment of choice for *Fusarium*-associated fungal keratitis, the main causative pathogen[1983]. The use of topical VCZ has demonstrated good effects in advanced cases of *Purpureocillium* keratitis[1935,1986]. Cutaneous and sub-cutaneous infections mainly occur in transplant or other immunosuppressed patients, especially those with underlying malignancy[1983]. Cases of non-ocular, non-cutaneous infections caused by *P. lilacinum* have been described but these are in general rare[1987-1992]. *P. lilacinum* presents moderate virulence[1993]. Infections are rarely disseminated, mainly in severely immunocompromised patients[1991,1994,1995]. In Spain, an incidence of 1% has been reported in patients following lung transplantation[606] **(Figure 40).**

Figure 40. Worldwide distribution of infections caused by *Purpureocillium* spp. (reported cases between 1977 and 2019 per million population)



Cases of severe *Purpureocillium*-related infections reported in the medical literature were identified in a PubMed search on October 30, 2019 using the search string "*Purpureocillium atypicola* OR *Purpureocillium lavendulum* OR *Purpureocillium lilacinum* OR *Paecilomyces lilacinus* OR *Purpureocillium sodanum* OR *Purpureocillium takamizusanense*" that yielded 395 publications. Cases were identified in four additional publications in the *Paecilomyces* search. Overall, 250 cases from 28 countries have been selected, 171 cases reported since the year 2000 [572,606,655,881,1116,1344,1346,1918,1926,1930,1945,1973,1975,1976,1984,1986-1992,1994-2097]. Most cases were reported from the USA (n=113), Australia (n=26), Spain (n=17), India and Japan (each n=13). Nine patients with *Purpureocillium*-related infections that were reported during an outbreak due to contaminated skin lotion in Switzerland were excluded [2062]. Number of cases reported between 1977 and 2019 is presented as cases per million population per country. The resident population per country was obtained from www.worldometers.info[321]. *One case of infection caused by *P. lilacinum* was reported from Iceland (3 cases per million population between 1977 and 2019)[2025].

Diagnosis of *Purpureocillium* infections

Evidence – Distinction between species is achieved in the course of diagnosis on the basis of various microbiological and molecular criteria. Direct microscopy of infected tissues is used for the characterization and identification of numerous hyaline and septate hyphae of molds [2073]. Rarely conidiophores and phialides can be observed[1972,1975]. Culture is crucial for species identification. Based on culture morphology on different growth media the species determination can be performed[2009,2073,2085]. Histology is also required for identification of fungi and visualization of numerous separate hyphae and arthroconidia within granulomas[2009,2085]. For susceptibility testing the use of E-test strips has been described for *Purpureocillium* spp.[2073]. MICs were high for AmB, ICZ, PCZ, CASPO and MICA , while VCZ had a relatively low MIC.

Molecular methods are used for species identification, but not for detection of fungal DNA from clinical material. Differentiation occurs via PCR-based DNA amplification of rRNA gene regions, mainly 28S D1 and D2 regions, but also ITS1 and ITS2[2009,2085]. PCR is performed from cultured clinical isolates. Genbank analysis and sequence alignment are used for the exact species assignment[2009,2019,2073,2085] **(Table 46)**.

Table 46. Microbiological and histopathological diagnostics of *Purpureocillium* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
Microscopy, culture, MIC testing						
Any	To diagnose	Culture	A	III	Demitsu JDermatol 2017[2009]	Biopsy on SDA
					Saghrouni MedMycol 2013[2073]	Biopsy on SDA

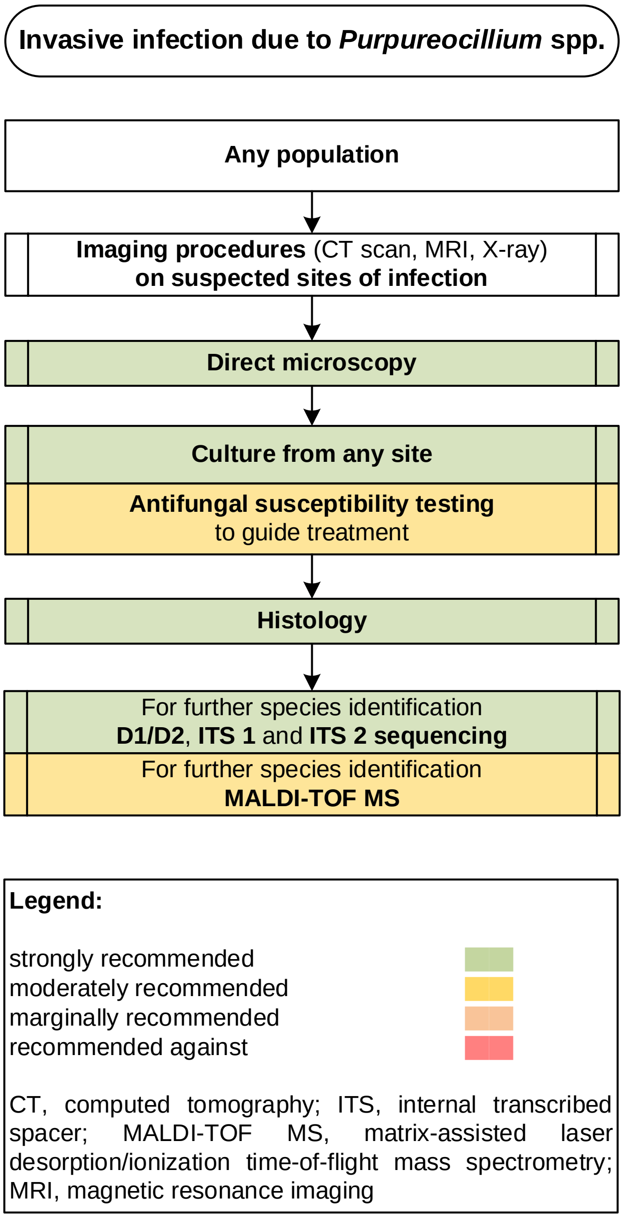
					Trinh TID 2017[2085]	Biopsy on brain heart infusion agar
					Narita AMO 2015[1973]	
					Antas MicrobesInfect 2012[1972]	Malt extract agar
					Nagamoto MMJ 2014[1975]	SDA
Any	To diagnose	Microscopy	A	III	Saghrouni MedMycol 2013[2073]	KOH direct examination
					Antas MicrobesInfect 2012[1972]	
					Todokoro IntOphthalmol 2014[1976]	Gram and Fungiflora Y stainings
					Nagamoto MedMycol 2014[1975]	Koh-Parker ink-direct microscopy
Any	To test suscepti-bility	E-test strips (bioMérieux, France)	B	III	Saghrouni MedMycol 2013[2073]	
Nucleic-acid based assays/MALDI-TOF MS						
Any	To diagnose in bi-opsy	In-house 18S rRNA PCR	C	III	Trinh TID 2017[2085]	
Any	To identify species	PCR rRNA D1/D2 or ITS region	A	III	Demitsu JDermatol 2016[2009]	
					Saghrouni MedMycol 2013[2073]	
					Todokoro IntOphthalmol 2014[1976]	
					Nagamoto MedMycol 2014[1975]	
					Innocenti Mycoses 2011[1971]	
					Guo JMII 2019[2019]	
Immunocompetent	To identify cause of facial skin le-sion	MALDI-TOF MS using a micro-flex LT instrument (Bruker Dal-tonics Germany)	B	III	Saghrouni MedMycol 2013[2073]	
Tissue-based diagnosis						
Any	To diagnose	Histopathology of biopsy tis-sue	A	III	Trinh TID 2017[2085]	
					Demitsu JDermatol 2017[2009]	
					Saghrouni MedMycol 2013[2073]	
					Antas MicrobesInfect 2012[1972]	

AmB, amphotericin B; ITS, internal transcribed spacer; KOH, potassium hydroxide; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; PCR, polymerase chain reaction; QoE, quality of evidence; rRNA, ribosomal ribonucleic acid; SDA, Sabouraud Dextrose Agar; SoR, strength of recommendation

Recommendations - Direct microscopy, and culture followed by sequencing of the 28S D1 and D2 regions and of ITS1 and ITS2 for species identification are strongly recommended, as is histopathological examination of tissue. Identification of isolates with MALDI-TOF MS are moderately recommended. Antifungal susceptibility testing is moderately recommended to guide antifungal treatment.

If all diagnostic options are available, one should follow the management pathway **(Figure 41)**.

Figure 41. Optimal diagnostic pathway for *Purpureocillium* infections, when all imaging and assay techniques are available



Treatment approaches to *Purpureocillium* infections

Treatment in adults

Evidence - VCZ demonstrates generally good *in vitro* activity against *P. lilacinum*[1970,1974] and several successful therapy approaches with VCZ have been reported[1988,2007,2069,2085,2086]. Furthermore, some reports of successful treatment with VCZ in combination with TRB have been published[1344,2009,2086,2092]. For salvage therapy, the results for AmB have been mixed (not effective[1999,2008,2024], effective[1975,1989,2083]). AmB generally shows poor activity against *P. lilacinum in vitro* [1970,1974]. PCZ and ICZ have been reported to be effective in individual cases[1346,1973,1999,2012] but usually do not show *in vitro* activity against *Purpureocillium lilacinum*[1974].

Appropriate length of treatment for *P. lilacinum* infections is unclear. Successful cases cover ranges from a few weeks to 7 months[1344,1918,1973,1975,1988,2007,2009,2012,2046,2069,2085,2086].

Surgical debridement of infected tissue appears to be a major means of resolution of the infection if the lesions are localized[1346,1987,2001,2007,2019,2022,2069,2074,2086] **(Table 47)**.

Table 47. Therapy for *Purpureocillium* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
First-line antifungal therapy						
Hematological malignancy	To cure	PCZ	C	III	Hobson ChestInfect 2013[2098]	N=1, pneumonia, success
Any	To cure	VCZ +/- surgical intervention	B	III	Ciecko ENTJ 2010[2007]	N=1, invasive fungal rhinitis, + surgery, success
					Huang Mycopathol 2011[2099]	N=1, cutaneous hyalohyphomycosis, response
					Keshtkar-Jahromi Mycopathol 2012[1988]	N=1, cutaneous + synovial infection, success
					Rimawi Mycopathol 2013[1475]	N=1, subcutaneous infection, + surgery, success
					Trinh TID 2017[2085]	N=1, kidney infection, success
					Pastor CMI 2006[1983]	N=119*, P. lilacinum,* oculomycosis and (sub)cutaneous infection, VCZ mono +/- TRB, success
					Martin CID 2002[1994]	N=1, disseminated infection, response
					Schweitzer AJO 2012[2074]	N=1, bursitis, + surgical debridement, success
Any with cutaneous / subcutaneous infection	To cure	VCZ +/- TRB 250 mg qd +/- surgical intervention	B	III	Hilmarsdottier SJID 2000[2100]	N=1, cutaneous infection, success
					Lavergne TID 2012[1344]	N=1, cutaneous hyphomycosis, *P. lilacinum* and *A. alternata*, success
					Demitsu JDermatol 2016[2009]	N=1, subcutaneous hyalohyphomycosis, success
					Ounissi TransProceed 2009[2101]	N=1, cutaneous hyalohyphomycosis, response
					Van Schoonevelt TID 2008[2087]	N=1, cutaneous infection, success
Any	To cure	ICZ 400 mg +/- surgical intervention	C	III	Hall IJD 2004[2022]	N=1, cutaneous hyalohyphomycosis, success
					Hecker JAAD 1997[2024]	N=2, response 2/2
					Clark CID 1999[2008]	N=1, soft tissue infection, ICZ no response, switch to TRB, success
Any	To cure	L-AmB 5 mg/kg qd	C	IIIt	Walsh TID 1999[2102]	
					Clark CID 1999[2008]	N=1, soft tissue infection, L-AmB no response, switch to TRB, success
Any with keratitis	To cure	VCZ topical and VCZ iv/po +/- VCZ 1 mg/0.1 ml intravitreal +/- surgical interventions	B	IIu	Chew CJO 2016[2103]	N=3, 1/3 no visual improvement, 2/3 enucleation
					Ali SeminOphthalmol 2015[1918]	N=28, 7/28 therapeutic keratoplasties, 1/28 enucleation

					Turner BMCRN 2015[2086]	N=21, VCZ 17/21 (81%) for a minimum of 3 mo, topical 11/21 (52%), intracameral 6/21 (29%), intravitreal 3/21 (14%), surgical intervention 18/21 (86%), penetrating keratoplasty 8/21 (38%), 5/21 second penetrating keratoplasty (24%), pars plana vitrectomy 9/21 (43%), second pars plana vitrectomy 4/21 (19%), enucleation 4/21 (19%), multiple protracted and poor outcomes
					McLintock CEO 2013[2046]	N=1, scleritis, keratitis, + several surgeries, success but poor outcome
					Oliveira BMJCR 2019[2104]	N=1, + surgery, success
					Todokoro IntOphthalmol 2014[1976]	N=2, success 2/2
					Wu Cornea 2010[2105]	N=1, success but poor outcome
					Juyal IJPM 2018[2106]	N=1, trauma, treatment response, + keratoplasty, success
Any with endophthalmitis	To cure	VCZ topical + VCZ iv/po +/- VCZ intravitreal 1 mg/0.1 ml +/- surgical interventions	C	III	Trachsler KMA 2012[2107]	N=1, treatment response
					Yoshida IntOphthalmol 2018[2108]	N=1, endophthalmitis, + surgery, response but poor outcome
					Garbino SJID 2002[2109]	N=1, + surgery, response
Antifungal salvage treatment						
Any with keratitis	To cure	PCZ	C	III	Arnoldner Cornea 2014[1999]	N=1, keratitis, + surgery, success
Any with endophthalmitis	To cure	ICZ 100 mg + miconazole topical + natamycin topical	C	III	Narita AMO 2015[1973]	N=1, endophthalmitis, + surgery, success
Any with cutaneous / subcutaneous infection	To cure	TRB po 250 mg qd or bid +/- surgical intervention	C	III	Clark CID 1999[2008]	N=1, success
					Blackwell BJD 2000[2001]	N=1, + surgery, outcome not reported
Any with cutaneous / subcutaneous infection	To cure	PCZ	C	III	Ezzedine ADV 2012[2012]	N=1, good response, lost to follow-up
					Saegeman ACB 2012[1346]	N=1, *P. lilacinum* and *Alternaria infectoria*, response
Any with cutaneous infection	To cure	L-AmB 150 mg qd	C	III	Nagamoto MedMycol 2014[1975]	N=1, success
Immunocompetent patients with onychomycosis	To cure	Amorolfine 5% nail lacquer + TRB systemic 250 mg qd + ICZ 200 mg qd	D	III	Innocenti Mycoses 2011[1971]	N=1, failure
					Pontini GIDV 2016[2110]	N=1, failure
Peritoneal dialysis patients with Peritonitis	To cure	TRB po 250 mg + VCZ	C	III	Wolley PDI 2012[2092]	N=1, + cathether removal, success
Other treatment options						
Any with cutaneous or subcutaneous infection	To cure	Surgical debridement	A	III	Blackwell BJD 2000[2001]	N=1, outcome not reported
					Saegeman ACB 2012[1346]	N=1, *P. lilacinum* and *Alternaria infectoria*, response
					Ciecko ENTJ 2010[2007]	N=1, invasive fungal rhinitis, success
					Rimawi Mycopathol 2013[1475]	N=1, success
					Hall IJD 2004[2022]	N=2, + surgery, 1/2 lost to follow up, 1/2 died of other causes
Endophthalmitis	To cure	Vitrectomy	B	III	Guo JMII 2019[2019]	N=1, no antifungal drugs, success
Immunocompetent patients with lung abscess	To cure	Surgical lobectomy	C	III	Ono Respiration 1999[1987]	N=1, no further antifungal treatment, success
Immunocompetent patients with septic bursitis	To cure	Surgical debridement	B	III	Schweitzer AJO 2012[2074]	N=1, success
Peritoneal dialysis patients with peritonitis	To cure	Catheter removal	B	III	Wolley PDI 2012[2092]	N=1, success
Treatment duration						
Any	To cure keratitis	Average therapy length 24.7 wk	B	IIu	Ali SeminOphthalmol 2015[1918]	N=28, longer duration for cases in later disease course, outcome variable
					McLintock CEO 2013[2046]	N=1, immunocompromised patient, 6 mo of therapy with topical, oral and

						intravitreal VCZ, topical VCZ continued for 10 mo, success but poor outcome
Any	To cure ocular mycoses	Minimum of 3 mo VCZ po +/- VCZ topical, intracameral or intravitreal	B	IIu	Turner BMCRN 2015[2086]	N=21, multiple protracted and poor outcomes
Any	To cure endophthalmitis	7 mo of therapy with topical miconazole + pimacine ointment + 3 mo of therapy with ICZ	C	III	Narita AMO 2015[1973]	N=1, success
Solid organ transplant recipients	To cure invasive fungal rhinitis	5 wk of therapy with VCZ	C	III	Ciecko ENTJ 2010[2007]	N=1, success
Immunocompetent patients	To cure septic bursitis	6 wk of overall therapy with VCZ po	C	III	Schweitzer AJO 2012[2074]	N=1, success
Peritoneal dialysis patients	To cure peritonitis	13 mo of overall therapy with VCZ, 12 mo with TRB	C	III	Wolley PDI 2012[2092]	N=1, success
Any	To cure cutaneous / subcutaneous infections	3-7 mo of antifungal therapy	B	III	Lavergne TID 2012[1344]	N=1, cutaneous infection, 7 mo TRB, success
					Nagamoto MedMycol 2014[1975]	N=1, skin infection, 2.5 mo L-AmB, success
					Rimawi Mycopathol 2013[1475]	N=1, subcutaneous infection, 12 wk VCZ, success
					Trinh TID 2017[2085]	N=1, cutaneous infection, 12 wk VCZ, success
					Keshtkar-Jahromi Myopathol 2012[1988]	N=1, cutaneous and synovial infection, 12 wk VCZ, success
					Demitsu JDermatol 2016[2009]	N=1, subcutaneous hyalohyphomycosis, 3 mo VCZ + TRB, success
					Ezzedine ADV 2012[2012]	N=1, cutaneous infection, 4 wk PCZ, good response
					Huang Mycopathol 2011[2099]	N=1, cutaneous infection, 3 wk VCZ oral + nystatin topical, good response
Standard dose unless stated otherwise; bid, twice a day; d, day(s); ICZ, itraconazole; iv, intravenous; L-AmB, liposomal amphotericin B; mo, month(s); PCZ, posaconazole; po, orally; qd, once a day; QoE, quality of evidence; SoR, strength of recommendation; TRB, terbinafine; VCZ, voriconazole; wk, week(s).						

Recommendations – The guideline group moderately supports first-line monotherapy with VCZ in all patients. ICZ (SUBA formulation preferred), PCZ and L-AmB are marginally recommmeded alternatives. For cutaneous and subcutaneous infections, combination therapy with VCZ plus TRB is moderately supported. For salvage therapy we marginally recommend L-AmB, PCZ or ICZ monotherapy. We moderately recommend a treatment duration of at least 3 months for ocular and cutaneous/subcutaneous infections. The guideline group strongly supports a recommendation for surgical debridement **(Figure 42).**

Figure 42. Optimal treatment pathway for *Purpureocillium* infections <u>in adults</u> when all treatment modalities and antifungal drugs are available

Specific considerations on treatment of Purpureocillium infections in children

Evidence - Only a few cases are reported with good treatment response to AmB formulations[1989,2083,2111].

In an individual case, successful treatment is described with VCZ[2073] **(Table 48)**.

Table 48. Therapy in children for *Purpureocillium* infections

Population	Intention	Intervention	SoR	QoE	Reference	Comment
First-line antifugal therapy						
Immunocompromised patients	To cure	AmB lipid formulations	C	IIIt	Sillevis Smitt ADC 1997[2111]	N=1, 12 yrs, lung infection, response
					Tan JCM 1992[2083]	N=1, 18 mo, fungemia, D-AmB, success
					Silliman JInfect 1992[1989]	N=1, 4 yrs, two abdominal wall abscesses, D-AmB, response
Immunocompetent patients with cutaneous infection	To cure	VCZ 400 mg	B	III	Saghrouni MedMycol 2013[2073]	N=1, success
Treatment duration						
Immunocompetent patients	To cure cutaneous hyalohyphomycosis	3 mo VCZ	C	III	Saghrouni MedMycol 2013[2073]	N=1, success
Immunocompromised patients	To cure lung infection	4 wk AmB	C	III	Sillevis Smitt ADC 1997[2111]	N=1, 12 yrs, response

Standard pediatric dose unless otherwise stated; L-AmB, liposomal amphotericin B; mo, month(s); QoE, quality of evidence; SoR, strength of recommendation; VCZ, voriconazole; wk, week(s); yrs, years.

Recommendations – In line with recommendations in adults, the guideline group moderately supports the use of VCZ and marginally supports the use of L-AmB for treatment in children.

Summary of Treatment Recommendations

The most important treatment recommendations of this guideline are summarized in Table 49.

Table 49. Recommended systemic antifungal treatment in adults with other rare mold infections.#

Mold infections caused by / Antifungal Treatment	First line	First Line Alternative	Second Line	Avoid	Salvage*
Fusariosis	VCZ or VCZ + L-AmB/ABLC	L-AmB/ABLC	ISA or PCZ	D-AmB	PCZ
Lomentosporosis	VCZ + TRB	VCZ	ISA or PCZ	L-AmB	VCZ
Scedosporiosis	VCZ	VCZ + L-AmB or VCZ + ABLC or VCZ + Echinocandins or VCZ + TRB	ISA or PCZ or ICZ	L-AmB	VCZ + Echinocandins or PCZ
Phaeohyphomycosis	VCZ	L-AmB +/- Echinocandin or Triazole	ISA	D-AmB	ISA or PCZ or VCZ
Phaeohyphomycosis: Cutaneous/Subcutaneous infection	ICZ or VCZ				
Phaeohyphomycosis: Disseminated infection	PCZ or VCZ + Echinocandin OR TRB				

Phaeohyphomycosis: *Exserohilium rostratum*	VCZ +/- L-AmB		L-AmB + triazoles other than VCZ		
***Rasamsonia* spp.**	CASPO or MICA	CASPO + L-AmB or PCZ, or MICA + L-AmB or PCZ		Azole monotherapy	
***Schizophyllum* spp. and other basidiomycetes:** *Schizophyllum commune*	L-AmB; Stepdown to PCZ		VCZ		
***Schizophyllum* spp. and other basidiomycetes:** *Coprinopsis cinereal/Hormographiella aspergillata*	L-AmB +/- inhaled L-AmB or L-AmB +/- VCZ			Echinocandins	L-AmB or VCZ
***Scopulariopsis* spp.**	ISA or VCZ	L-AmB +/- VCZ			PCZ +/- MICA +/- TRB
***Penicillium* spp.:** dissemination	L-AmB +/- other antifungals				VCZ
***Penicillium* spp.:** lung infection	PCZ				
non-*marneffei Talaromyces* spp.	L-AmB				VCZ or Echinocandine + TRB
***Paecilomyces* spp.**	L-AmB				ICZ or PCZ
***Purpureocillium* spp.**	VCZ		ICZ or L-AmB or PCZ		ICZ or L-AmB or PCZ
***Purpureocillium* spp.:** cutaneous/subcutaneous infection	VCZ + TRB				

Detailed recommendations regarding dosages can be found in Table 2.
ABLC, Amphotericin B lipid complex; AmB, Amphotericin B; D-AmB, Amphotericin B deoxy-cholate; ICZ, Itraconazole; ISA, ISA; iv, intravenous; L-AmB, liposomal amphotericin B; MICA, micafungin; PCZ, posaconazole; TRB, terbinafine; VCZ, VCZ

Legend:
strongly recommended
moderately recommended
marginally recommended
recommended against

TDM, therapeutic drug monitoring

* Choice of salvage treatment always depends by the treatment that the patient failed to respond to

Future directions

Unmet needs

Despite recent advances in diagnostic testing and antifungal therapies, significant challenges remain in the management of rare mold infections. Diagnosis is based on conventional identification methods and culture, with molecular-based identification and testing often requiring referral to specialist laboratories with an expertise in phenotypic identification, including ECMM Excellence Centers[17]. Even when available, molecular identification using standardized sequencing techniques is mostly restricted to identification of isolates, while more rapid tests that can be applied directly to clinical samples are needed[2112]. A promising method for faster identification of fungal isolates is MALDI-TOF MS, though current CE certified databases need to be substantially enhanced to become clinically useful and additional research is needed to reliably identify molds. MALDI-TOF MS requires a large number of strains to generate reliable reference data for identification. In order to improve molecular identification methods and MALDI-TOF MS libraries or develop new diagnostic tools, comprehensive well-curated and publicly accesible collections of clinical isolates need to be maintained at central repositories for research purposes. Since randomized trials are impractical due to the rare occurrence of these infections, prospective and detailed clinical registries such as the FungiScope registry[10,367], are important to refine treatment strategies, which may be specifically tailored for a particular pathogen and clinical syndrome. Furthermore, development of unique animal models for some of these fungi is important for validation of some of the current therapeutic recommendations and for development of novel therapies, including immunomodulatory treatment. Finally, establishment of an online, searchable database of infections caused by rare molds, their clinical presentations and antifungal susceptibilities would assist in the management of difficult cases. In the digital era, we are now able to connect data sources globally to help optimize therapy of these often refractory infections.

Priority research questions

The immediate research questions are similar for the individual rare molds. Common research themes for the rare molds are the need to develop better diagnostic tools and antifungal agents, as well as to identify unique biomarkers, understand pathogenesis and elucidate host defense mechanisms.

1. Improved diagnostics: Culture-based diagnostics are slow or may be falsely negative due to various factors including ongoing antifungal treatment or prophylaxis[2120,2121]. Biopsies are not always possible due to associated risks for the patients. Although non-culture-based diagnostics including point-of-care tests[2122,2123] and molecular diagnostics[2124] have been developed for aspergillosis and to some extent also mucormycosis, the rare molds remain difficult to diagnose in a timely manner due to lack of rapid diagnostic tests. Research should focus on rapid diagnostic tests that may involve PCR testing. Although pan-fungal PCR targeting the ITS1 region of the ribosomal RNA gene can identify rare molds, this test is most accurate on fresh tissue in which hyphae are visible and less useful for samples containing low amounts of mold[341]. Furthermore, multiplex PCR testing using the ITS1 and ITS2 regions as well as beta-tubulin on blood cultures, which have flagged positive has identified molds such as *Fusarium* spp. and *L. prolificans*[338]. However, more sensitive targeted tests which could predict or identify development of invasive infection earlier than traditional methods and could impact on management of these infections, which are often only diagnosed with advanced infection, are needed.

   Other advances include metabolite mass spectrometry[2125] of breath samples to identify volatile organic compound signatures specific for fungi, which has been successfully applied for diagnosis of invasive pulmonary aspergillosis[2126] and could be extended to invasive pulmonary infections with other molds. Metagenomic sequencing of blood or body fluids is now possible[2127] but while there is some experience in using this to detect bacteria and viruses, fungi have not been evaluated. The same applies for high resolution melting techniques[2128]. Inexpensive and portable sequencing machines will make these technologies widely available and may be a solution in countries without laboratory infrastructure[2129]. Innovative technologies, such as clustered regularly interspaced short palindromic repeats (CRISPR)-based diagnostic tools, may lead to point-of-care assays[2129]. PET CT or MRI scans with *Aspergillus* antibodies or siderophores labelled to nuclear medicine isotopes have been used to diagnose aspergillosis[2130,2131], but rely on sensitive and specific fungal biomarkers that will need to be developed for these rare conditions[2132]. Finally, future studies are needed to search for laboratory markers for treatment response assessment, including immunologic markers[2121,2133].

2. Improved treatments: With a limited number of antifungals currently available, there is an urgent need for studies designed to establish a correlation between *in vitro* susceptibility results and *in vivo* response, to better target treatment. Due to a number of new antifungal agents in the development pipeline, options for treating these difficult infections may improve in the near future[2134]. Olorofim is currently being evaluated in human studies and has activity against *L. prolificans*, *Scedosporium* spp.[560,2135], and some *Fusarium* spp.[2136]. Evaluation of activity against the other rare molds is urgently required. Drugs in an earlier phase of development that have shown activity against rare molds include auranofin with *in vitro* activity against *Lomentospora* spp. and *Scedosporium* spp., although the mechanism of action is yet unclear[2137]. The glycosylphosphatidylinositol (GPI) synthesis inhibitor fosmanogepix, which weakens the cell wall and impairs fungal growth has *in vitro* activity against *Fusarium* spp., black molds, *Lomentospora* spp., *Scedosporium* spp., and *P. lilacinum*[2138]. This drug also was successful in a neutropenic mouse model of disseminated fusariosis[2139]. To bring these promising new agents to clinical practice requires a concerted collaborative effort and partnerships between industry, regulators, and clinicians, which will be critical. Mechanisms of antifungal resistance in these rare molds should be further studied in order to develop new antifungal agents to overcome intrinsic resistance, an example being *L. prolificans* and triazoles[2140].

Beyond drug therapy, adoptive T-cell therapy[2141], chimeric antigen receptor (CAR) T cells (artificially designed receptors that are introduced into T cells) [2142] and neutrophils engineered with bifunctional small molecules that bind the antifungal targets and have immunostimulatory compounds to enhance the immune response[2143] are possibly feasible approaches, which should be assessed.

3. The mycobiome: The third research question is understanding the mycobiome of sites such as the respiratory tract, the gastrointestinal tract, and skin in healthy subjects and during immunosuppression and if this is a factor in allowing rare molds to become invasive[2144-2147].

Acknowledgment

We thank Christina Toebben and Dr. Nathalie Bauer for review and valuable contributions to the manuscript. The authors are indebted to Susann Blossfeld and Dr. Caroline Bruns for technical support with this manuscript.

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