

# THE LANCET

## Infectious Diseases

### Supplementary appendix

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

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## **Global Guideline for the Diagnosis and Management of the Endemic Mycoses**

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## INTRODUCTION

The endemic mycoses are caused by a diverse group of fungi that share several characteristics: each occupies a specific ecological niche in the environment and includes cause disease in healthy hosts..<sup>1</sup> There are numerous species of dimorphic fungi, however *Blastomyces*, *Coccidioides*, *Histoplasma*, *Paracoccidioides*, and *Sporothrix* spp., and *Talaromyces marneffe* (formerly *Penicillium marneffe*) represent the most commonly encountered causes of infections in clinical care. Biosafety is an important consideration when handling these organisms, and laboratories should incorporate national guidance and regulations into their processes and practices to ensure the safety of laboratory staff. There are substantial differences in the geographical distribution, clinical presentation, radiographic manifestations, diagnostic approach, and therapeutic interventions between these mycoses. Management requires recognition of risk factors (e.g. environmental exposure in an endemic region) and appropriate use of diagnostic and therapeutic interventions. Readily available guidance is important to ensure efficient diagnosis and treatment and to optimize patient outcomes.

We issue this comprehensive guidance document to facilitate clinical decision-making and to provide an overview of the areas of uncertainty in the field. We aim to address limitations of previous recommendations, by engaging physicians and scientists involved in various aspects of the endemic mycoses, representing the fields of dermatology, haematology, infectious diseases, intensive care, microbiology, paediatrics, pathology, pharmacology, radiology and surgery. In addition, the guideline group updates current knowledge in the field, and comprises experts from all parts of the world.

Each of the recommendations tabulated can easily be traced back to the source references for maximum transparency. Any new relevant information, published after this document, can be placed in context, and this approach facilitates the writing of future updates. For consistency, a strict methodology was used, consistent with previous guideline documents.<sup>2</sup> As with any other guidance document, this guideline intends to assist in management decisions. Whether specific recommendations are appropriate when managing individual patients needs to be carefully assessed by treating clinicians. Recommendations aim to assist, not to replace, clinical judgment, and management of a patient with an endemic mycosis will always need to be individualized. Moreover, recommendations do not guarantee the availability of specific diagnostics or treatments, or reimbursement by healthcare systems. However, recommendations do reflect the current best available diagnostic and therapeutic management for each of the endemic mycoses.

### **Guideline development**

The general approach applied in the European Confederation of Medical Mycology (ECMM) guideline program has recently been described.<sup>2</sup> We invited experts to participate in this specific guideline in February of 2018. Our selection of experts was determined by their publication activity in the field of the endemic mycoses, their personal involvement in patient management, and their distribution over the world regions defined by the United Nations as previously described.<sup>2</sup>

### **Systematic approach**

The guideline follows the structure and definitions of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines on candidiasis, the ECMM/Mycoses Study Group Education and Research Committee (MSGERC) guidelines on mucormycosis,<sup>2</sup> and the ECMM/ESCMID guidelines on rare invasive fungal infections,<sup>3,4</sup> which are in accordance with

the Grading of Recommendations Assessment, Development and Evaluation (GRADE) and Appraisal of Guidelines for Research & Evaluation (AGREE) systems.<sup>5,6</sup> Few comparative clinical trials have been performed evaluating the endemic mycoses and meta-analyses are therefore not applicable. The PICO (e.g., population, intervention, comparison, outcome) approach was applied, but in this set of guidelines, PICO is displayed within the tables. Treatment strategies and diagnostic assays may both significantly alter patient outcomes, and are thus regarded as interventions. The fixed sequence of seven columns in the tables is pre-defined, and increases transparency. First, a population is defined; then the intention or objective is stated, followed by the intervention. For that logical sequence, strength of recommendation (SoR) and quality of evidence (QoE) are provided, followed by the references on which the recommendation is based, and an index describing the source of level II evidence. In the last column, a comment may be added as appropriate. SoR and QoE are results of independent evaluations, thus allowing a strong recommendation even in the absence of the highest quality evidence (Table 1).

### Authors and contributors

Authors fulfilled the criteria set forth by the International Committee of Medical Journal Editors (IC-232 MJE). For the purposes of this guideline, further requirements reflecting sufficient author contribution were responsiveness throughout the guideline process, receipt of training on the guideline process, and disclosure of conflicts of interest.

**Table 1. Definition of strength of recommendation and quality of evidence**

Strength of recommendation (SoR)	Definition
Grade A	The guideline group <b>strongly</b> supports a recommendation for use
Grade B	The guideline group <b>moderately</b> supports a recommendation for use
Grade C	The guideline group <b>marginally</b> supports a recommendation for use
Grade D	The guideline group moderately supports a recommendation <b>against</b> use

<b>Quality of evidence</b>	<b>Definition</b>
Level I	Evidence from at least 1 properly designed randomized, controlled trial (oriented to the primary endpoint of the trial)  Note: Poor quality of planning, inconsistency of results, indirect evidence would lower the strength of recommendation
Level II	Evidence from at least 1 well-designed clinical trial (including secondary endpoints), without randomization; from cohort or case-controlled analyses; from multiple time series; or from dramatic results of uncontrolled experiments.  Note: Every Level II evidence must have at least one added index
Level III	Evidence from opinions of respected authorities, based on clinical experience, descriptive case studies, or reports of expert committees.
<b>Added Index</b>	<b>Defining the source of level II evidence</b>
r	Meta-analysis of systematic review or randomized controlled trials
t	Transferred evidence: i.e. results from different patient cohorts, or similar clinical circumstances
h	Comparator group: historical control
u	Uncontrolled trials
A	For published abstract presented at international symposia/meeting

## Literature search terms

Authors used the following search strings in PubMed: ("endemic mycoses" OR "endemic fungal infect\*" OR Blastomyc\* OR Coccidioid\* OR Emmonsi\* OR Emergomyc\* OR Histoplasma\* OR Paracoccidioid\* OR Penicilliosis OR "Penicillium marneffeii" OR Sporotrichosis OR Sporothr\* OR Talaromyc\* [All Fields]) AND (epidemiology OR outbreak OR treatment OR therapy OR diagnosis OR diagnostics).

## Work flow

Having members on the guideline group from different time zones is a challenge that we addressed by an initial conference call on the methodology applied, as well as a video tutorial.

Assistance to the group was provided by the coordinators (GRT, TL, AC, ACP). Documents were shared with the authors on a password-protected OneDrive repository, and were updated as needed as previously described.<sup>2</sup> The group individually contributed subsections to a draft, which was circulated to all authors and contributors. At that time any discrepancies in recommendations were resolved by majority vote. Once the authors and contributors agreed on a final draft, a 4-week public consultation phase followed.

A total of 33 scientific societies reviewed and endorsed the guidance document (Supplemental Figure 1).

## **BLASTOMYCOSIS**

Previous guidelines for the diagnosis and treatment of blastomycosis were published by the Infectious Diseases Society of America (IDSA) in 2008 and since this time there has been refinement in our understanding of the taxonomy of *Blastomyces* species, and the epidemiology, diagnosis, and treatment of blastomycosis.<sup>7</sup> Treatment recommendations are based on open label observational studies, retrospective analyses and expert opinion.

### **Epidemiology**

*Blastomyces* spp. have been isolated in soils near freshwater drainage systems although it is clear that wind is likely to play a role in dispersal.<sup>8</sup> Traditionally, blastomycosis has been attributed to *Blastomyces dermatitidis*, although molecular evaluation of primarily North American clinical and environmental isolates identified the presence of the cryptic species *B. gilchristii*.<sup>9</sup> These species are indistinguishable clinically and by the clinical microbiology laboratory. While these remain the most important causes of blastomycosis in North America, additional species have recently been moved to or described in *Blastomyces*. The phenotypically distinct species *B. helicus*, and *B. parvus*, initially described in *Emmonsia* (as *E. helica* and *E. parva*) have now been placed in the *Blastomyces* genus,<sup>10,11</sup> although only the former causes blastomycosis while the later causes adiaspiromycosis, a granulomatous lung disease primarily of animals. There are two additional distinct species recently described that cause blastomycosis (to date only in Africa and the Middle East): *B. percursus* and *B. emzantsi*.<sup>12,13</sup> Finally, *B. silveriae* is unusual among the members of the genus in that it is not known to be a pathogen.

*Blastomyces dermatitidis/gilchristii* are seen primarily in the southeastern and southcentral United States bordering the Mississippi and Ohio River basins, the northcentral states bordering the Great Lakes, areas surrounding the St. Lawrence Seaway, and extending from Quebec into Saskatchewan in Canada (Appendix Figure 1). *B. helicus* is rare and has been found in western parts of Canada and the United States.<sup>14</sup> Blastomycosis has also been reported from 25 countries in Africa, 5 countries in the Middle East, as well as in India, and occasionally other areas throughout the world. In Africa and the Middle East, blastomycosis is caused primarily by *B. percursus* (which has a range extending from Israel to South Africa) and *B. emzantsi* (to date known only from South Africa).<sup>10,12</sup> Outbreaks of blastomycosis have primarily been reported near waterways and have coincided with major construction projects within the endemic region.<sup>15</sup> The range of blastomycosis may be expanding, with cases now reported regularly from the state of New York, and other areas previously considered outside the traditional region of endemicity.<sup>16</sup>

### **Clinical presentation**

Blastomycosis develops following inhalation of airborne conidia, and the clinical manifestations vary upon the extent of exposure and the immune status of the host. Asymptomatic infection, acute or chronic pneumonia, and extrapulmonary disease are all well described manifestations of the disease process.<sup>17</sup> Host genetic factors may be important for determining risk of disease development. A non-rural outbreak disproportionately affected Hmong persons, suggesting increased susceptibility.<sup>18,19</sup> A genome wide association study later identified an IL-6 related locus that may be involved in susceptibility.<sup>20</sup>

Pulmonary symptoms are common with acute infection and include non-productive cough, fever, shortness of breath, and weight loss. Chronic pneumonia is present in some patients, and symptoms include cough, sputum production, sometimes with haemoptysis, chest pain, and weight

loss.<sup>21,22</sup> In as many as 8-15% of patients requiring hospitalization, acute respiratory distress syndrome (ARDS) may develop and mortality rates vary from 40-89%.<sup>17,23-25</sup>

Blastomycosis primarily involves the lung (91% of cases) although other organs may be involved, especially the skin (~18%) (Appendix Figure 2A) and less often bone (4%), genitourinary system (2%) and central nervous system (CNS) (1%).<sup>26</sup> Dissemination with multiorgan involvement has been reported frequently but perhaps was more common before oral azole antifungal agents became available.<sup>27</sup>

## **Diagnosis**

### *Culture and microscopy*

**Evidence** - Microscopic examination of samples of involved tissues, including skin, purulent material, bronchoalveolar lavage (BAL) fluid, and cerebrospinal fluid (CSF) should be assessed using an optical brightener, such as calcofluor white or blankophor. For histopathological examination of tissues, the most helpful stains are Periodic acid-Schiff (PAS) and Gomori methenamine silver (GMS). The typical appearance of *Blastomyces* species is a round to oval, multinucleate yeast cell, 8-15  $\mu\text{m}$  in size, with a single broad-based bud (Appendix Figure 2D). An exception is *B. helicus* which tends to form multiple buds that can appear as branching chains.<sup>14</sup> In *B. percursus*, short hyphal-like fragments can accompany yeast cells; these were observed in ~20% of cases in Africa and the Middle East. When the classic form is not observed, the differential diagnosis should include *Coccidioides*, *Cryptococcus*, and *Histoplasma* spp.<sup>17</sup> Prior reports have estimated the sensitivity of histopathology as ~80%.<sup>28</sup>

Culture positivity provides a definitive diagnosis but in some cases may fail to grow. Samples should be cultured on Sabouraud dextrose or potato dextrose agar. *B. dermatitidis* will grow

on cycloheximide-containing media. All plates should be incubated for up to 6 weeks at 25-30°C although most isolates are detected within 5-10 days (Table 2).<sup>17</sup> The use of matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) has reduced the time to identification of isolates and may be helpful depending upon availability and if converted to the mould form appropriate precautions should be adhered to.<sup>29</sup> Identification to the species level is infrequently needed during clinical practice.

**Table 2. Conventional methods in the diagnosis of blastomycosis.**

Population	Intention	Intervention	SoR	QoE	References and comment
Any	Diagnosis	Microscopy Histopathology, PAS, GMS	A	Iiu	Saccante <sup>17</sup> (multinucleate yeast cells with a single broad-based bud); Patel <sup>28</sup> (sensitivity 80%)
Any	Diagnosis	Culture	A	Iiu	Saccante <sup>17</sup> (Sabouraud dextrose agar, potato dextrose agar, inhibitory mould agar, 25-30°C up to 6 weeks) Martynowicz <sup>30</sup> (BAL positive in 67%, sputum positive in 86%, bronchial secretion positive 100%) Padhye <sup>31</sup> Stockman <sup>32</sup> (DNA probe and exo-antigen) commercially available

**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence, BAL, Bronchoalveolar lavage; GMS, Gomori-Methenamine Silver; PAS, Periodic acid-Schiff.

Colonies may be white to gray to brown. Microscopically, septate hyphae with a diameter of 1-2 µm and oval single-cell conidia at the tip of conidiophores (“lollipop”) are characteristic but not specific (Appendix Figure 2E). For definitive identification a DNA probe (AccuProbe, Hologic,

Sunnyvale, CA, USA) was previously available.<sup>31</sup> However, cross-reactivity with *Blastomyces parvus* (formerly *Emmonsia parva*), *Emergomyces canadensis*, *Gymnascella hyalinospora*, and *Paracoccidioides brasiliensis* has been described.<sup>32-34</sup>

### *Serology, antigen detection and molecular methods*

#### *Serology*

**Evidence** - For antibody detection, complement-fixation assays are available, but these tests exhibit a low sensitivity (57%) and specificity (30%).<sup>35</sup> Immunodiffusion assays are more sensitive (65%) and specific (80%).<sup>36</sup> Enzyme immunoassays (EIA) have shown a similar sensitivity of 77% with a specificity of more than 90%.<sup>37</sup> A newer EIA using a cell wall adhesion antigen, BAD-1 may exhibit improved sensitivity and specificity than prior EIA methods.<sup>38</sup>

#### *Antigen detection*

**Evidence** - An antigen detection assay is available in the US with a reported sensitivity of 85- 93% and a specificity of 79-99% although this has not been validated for less common *Blastomyces* species (MiraVista, Indianapolis, IN, USA).<sup>39-43</sup> Urine testing seems to be more sensitive than serum or BAL.<sup>39-42</sup> However, cross reactivity is seen in patients with histoplasmosis, paracoccidioidomycosis, and talaromycosis (formerly penicilliosis).<sup>43</sup> The value of (1-3)- $\beta$ -D-glucan (BDG) has been investigated in only a few patients, and does not appear to have clinical utility.<sup>44</sup> Van der Veer et al. described two patients with blastomycosis who had positive *Aspergillus* galactomannan in BAL fluid<sup>45</sup> showing the possibility of cross-reactivity.

#### *Molecular methods*

**Evidence** – Nucleic acid amplification using polymerase chain reaction (PCR) tests for detection of DNA of *B. dermatitidis* in clinical specimens is not commercially available. In-house assays have shown a moderate sensitivity (60%-86%).<sup>46-48</sup> Identification of *Blastomyces* isolates to the species level is also feasible, although not routinely necessary.<sup>14</sup>

### *Susceptibility testing*

**Evidence** - Guidelines for the *in vitro* susceptibility testing of *Blastomyces* spp. do not exist. No breakpoints are available, and the clinical relevance of susceptibility testing results is currently uncertain. *In vitro* susceptibility testing has shown low mean inhibitory concentrations (MICs) for itraconazole, posaconazole, voriconazole, isavuconazole and amphotericin B.<sup>49-52</sup> Notably, fluconazole exhibits diminished activity compared with other triazoles.

**Table 3. Role of non-invasive diagnostics in the diagnosis of blastomycosis.**

Population	Intention	Intervention	SoR	QoE	References and comment
Any	Diagnosis	Serology	D	IIIu	Turner <sup>35</sup> Klein <sup>36</sup> Klein <sup>37</sup> Richer <sup>38</sup> (Serology is generally unhelpful in the diagnosis of blastomycosis due to the high degree of cross-reactivity with other endemic mycoses)
Any	Diagnosis	Antigen detection in urine, CSF, BAL	A	IIu	Durkin <sup>39</sup> Bariola <sup>40</sup> Connolly <sup>41</sup> Frost <sup>42</sup> Wheat <sup>43</sup> (Sensitivity 85-93%, Specificity 79-99% - also cross-reactivity to <i>Histoplasma</i> , <i>Paracoccidioides</i> , <i>Talaromyces</i> )
Any	Diagnosis	Molecular based tests on clinical material	B	IIu	Bialek <sup>46</sup> Sidamonidze <sup>47</sup> Babady <sup>48</sup> (in-house real time PCR performance characteristics differ by specimen type: sensitivity 86% [12/14]; specificity 99% [773/778])

**Legend:** , SoR, Strength of Recommendation; QoE, Quality of Evidence; BAL, Bronchoalveolar lavage; CSF, Cerebrospinal fluid; PCR, Polymerase chain reaction

## **Blastomycosis Diagnostic Recommendations**

Specimens such as skin scrapings, biopsies, purulent material, BAL, urine, or CSF should be examined microscopically by using an optical brightener and/or different stains (haematoxylin-eosin (H&E), PAS, GMS) (SoR A, QoE IIu). Fungal culture on universal media and Sabouraud dextrose agar/potato dextrose agar with and without cycloheximide should be incubated for up to 6 weeks at 25-30°C (SoR A, QoE IIu). The macroscopic and microscopic appearance of *Blastomyces* is characteristic but not specific. Laboratories with a low level of experience with this pathogen should send isolates to a reference lab for definitive identification using a DNA probe, MALDI-TOF, or DNA sequencing (SoR A, QoE IIu).

In the U.S. and Canada a *Blastomyces* antigen assay showing an acceptable sensitivity and specificity (>80%) is available. Testing of the urine is preferred over other sample types (SoR A, QoE IIu). For antibody detection, enzyme immunoassays are preferred although there is almost no utility in testing for blastomycosis by this method due to the cross-reactivity with other endemic fungi (SoR B, QoE IIu). Guidelines for susceptibility testing of *Blastomyces* spp. do not exist, no breakpoints are available, and the clinical relevance of susceptibility testing results remains uncertain. Available data indicates low MICs for amphotericin B and most triazoles (SoR C, QoE IIu).

### ***Imaging***

**Evidence** – Chest radiographs typically shows scattered or lobar infiltrates.<sup>23</sup> Two forms may be distinguished: localized lung disease, including consolidation, masses, and cavitary lesions; and diffuse lung disease with a nodular or interstitial pattern. Pleural effusion or calcification may also be seen.<sup>53</sup> Further studies have identified air-space consolidation with an alveolar pattern, most

commonly in the upper lobe. Also mass-like infiltrates, pleural effusions, cavitation and lymphadenopathy may be seen.<sup>54</sup>

Chest imaging (CT scan) typically reveals air bronchograms, consolidation and nodules. Nodules are more commonly <3 cm, but may be larger. There is infrequently a miliary pattern. In one series, lymphadenopathy was seen in one third and pleural effusions in one quarter of patients.<sup>55</sup> ARDS may be seen as well.

Descriptions of the radiographic manifestations in children are limited; however, one study found that in the paediatric population, imaging most commonly reveals single lobe involvement. Cavitation and pleural effusion may occur.<sup>56</sup>

For CNS disease, magnetic resonance imaging (MRI) is more sensitive than CT. Key findings are enhancing lesions that may be single or multiple and that mimic brain abscess or tumour with associated oedema and meningeal enhancement when meningitis is present; infarct and vasculitis may be seen although are uncommon.<sup>57</sup>

### **Blastomycosis Imaging Recommendations**

All patients with blastomycosis should have a chest radiograph performed (SoR B, QoE III). Additional imaging is based on symptoms, to ascertain if complications of disease have occurred, and to determine a response to therapy (SoR B, QoE III). All patients with suspected CNS involvement should undergo brain MRI (SoR B, QoE III).

### ***Treatment rationale and recommendations***

Antifungal therapy is recommended for all forms of blastomycosis. There is an absence of randomized, comparative treatment trials. Therefore, non-randomized treatment studies, case series,

and expert experience provide the rationale for antifungal therapy and duration recommendations. The severity of patient illness and the underlying level of immunosuppression guide these treatment choices (Table 4). For patients with severe disease, liposomal amphotericin B (L-AmB) or an alternative amphotericin B formulation is recommended in most cases. Prior to the availability of L-AmB, most clinical experience was with amphotericin B deoxycholate (AmB-d).<sup>58</sup> Case series reporting AmB-d outcomes for treatment durations that allowed administration of 1-2 grams resulted in success rates in over 90% of patients.<sup>58</sup> A growing number of smaller case series report similar success with L-AmB therapy using doses of 3-5 mg/kg/d.<sup>57,59-63</sup> Use of the lipid formulations have largely supplanted use of AmB-d due to the significant safety advantage of lipid formulations.<sup>64</sup> Additionally, the availability of the triazole agents has shortened the required duration of AmB therapy to 1-2 weeks for many patients. Following clinical improvement with AmB, stepdown to a triazole is recommended. The triazole component is most often continued for 6-12 months depending upon the site of disease, with more prolonged courses recommended for CNS or bone involvement based upon relapses in case series.

Most triazole experience is with itraconazole (200-400 mg/day), which is recommended as the first line triazole based upon success rates of 90-95% in a prospective phase 2 study.<sup>65</sup> A small trial with fluconazole was less successful than prior reports of itraconazole,<sup>66</sup> but efficacy with higher dose fluconazole (400-800 mg/day) therapy was moderately successful (87%) and can be used in patients intolerant to other triazoles.<sup>67</sup> Case series with voriconazole have demonstrated outcomes comparable to itraconazole, including favourable efficacy in disease of the CNS.<sup>68-70</sup> Case reports with posaconazole and more recently isavuconazole suggest efficacy although there is limited experience with these agents to date.<sup>71-73</sup> Echinocandins should not be used in the treatment of blastomycosis due to the lack of proven *in vivo* efficacy.

Effective treatment of patients with less severe disease, including isolated pulmonary infection in an immunocompetent host, is achieved with triazole therapy alone for 3-6 months. The variable pharmacokinetics of triazoles in the treatment of blastomycosis, including itraconazole, voriconazole, and posaconazole, require therapeutic drug monitoring (TDM) for dose optimization. Recent improvements in triazole formulations have resulted in higher serum levels than those observed with conventional formulations (SUBA-itraconazole and posaconazole DR tablets).<sup>74,75</sup> A threshold trough concentration linked to treatment efficacy for blastomycosis has not been defined. However, serum itraconazole levels of  $>1 \mu\text{g/mL}$  have been associated with favourable outcomes for other systemic fungal infections.<sup>76</sup>

The management of blastomycosis associated ARDS is difficult and guided by case reports and small series. L-AmB is the mainstay of management. Corticosteroids have been used regularly for this indication in some centres but the evidence for efficacy is limited and expert consensus is lacking.<sup>23</sup> Venous-venous extracorporeal membrane oxygenation (ECMO) has been used, often with success although this is likely affected by reporting bias.<sup>77,78</sup> Some (but not all) reports have suggested L-AmB may become sequestered in ECMO tubing,<sup>79</sup> which may require higher dosing (6-7.5 mg/kg/day).<sup>79,80</sup>

### **Blastomycosis Treatment Recommendations**

All patients with blastomycosis should be treated. Patients with severe disease should receive L-AmB induction therapy in most cases (SoR A, QoE IIIu). Alternative amphotericin B formulations are acceptable if L-AmB is not available. Following clinical improvement with AmB stepdown to itraconazole is recommended for 6-12 months (SoR A, QoE IIu). Longer courses of therapy are recommended for those with CNS or bone involvement (SoR A, QoE IIIu). TDM is generally recommended during treatment (SoR A, QoE IIIu). Alternative triazoles can be used in cases of

itraconazole intolerance although higher doses of fluconazole are required (SoR A, QoE Iiu) and evidence with other triazoles is limited (SoR A, QoE IIIu).

#### Acute Respiratory Distress Syndrome

Patients with blastomycosis-associated ARDS should be treated with L-AmB (or alternative amphotericin B formulations) at a dose of 5 mg/kg/day (SoR A, QoE IIIu). Corticosteroids should be considered (SoR C, QoE IIIu). Rescue ECMO should be considered when possible (SoR B, QoE IIIu). We recommend L-AmB at higher doses during ECMO (6-7.5 mg/kg/day) (SoR C, QoE IIIu)

**Table 4. Recommendations for the treatment of blastomycosis**

Population	Intention	Intervention	SoR	QoE	References and comment
Severe disease	To cure	L-AmB or AmB-d	A	Iiu	Parker <sup>58</sup> (N=93, 79% success with dose >1.5g [total dose]). AmB-d is only recommended when L-AmB is not available
SOT	To cure	L-AmB followed by triazole	A	Iiu	Gauthier <sup>60</sup> (N=11), Kauffman <sup>61</sup> (N=9), Grim <sup>62</sup> (N=8)
CNS	CNS treatment	L-AmB followed by triazoles	A	IIIu	Bariola <sup>57</sup> (N=9), Bush <sup>59</sup>
Initial therapy in mild-moderate disease or as continuation following response to AmB (except for CNS disease)	To cure	Itraconazole 200-400 mg/day tablet	A	Iiu	Dismukes <sup>65</sup> (N=45; MSG itraconazole study, >90% success)
In patients intolerant of other azoles who have mild disease or as stepdown therapy after AmB	To cure	Fluconazole 200-400 mg/d	B	Iiu	Pappas <sup>66</sup> (N=23, MSG study)
In patients intolerant of other azoles who have mild disease	To cure	Fluconazole high dose 400-800 mg/day	B	Iiu	Pappas <sup>67</sup> (N=39, MSG high dose fluconazole, 85-89% success)

or as stepdown therapy after AmB					
Any	To cure	Voriconazole	B	IIIu	Freifeld <sup>70</sup> (N=8)
CNS relapse	CNS treatment	Voriconazole	B	IIIu	Borgia <sup>68</sup> (N=3, Successful voriconazole salvage for CNS)
CNS	CNS treatment	Voriconazole	B	IIIr	Ta <sup>81</sup> (N=7, Retrospective literature review suggesting voriconazole effectiveness for CNS blastomycosis)
Any	To cure	Posaconazole	B	IIIu	Proia <sup>71</sup> Day <sup>72</sup> (case reports)
Any	To cure	Isavuconazole	B	IIfu	Thompson <sup>73</sup> (N=3, VITAL study, open label, non-randomized, phase 3)
ARDS	To improve survival	Corticosteroids	C	IIIu	Schwartz <sup>23</sup> (n=22)
ARDS	To improve survival	Rescue ECMO	B	IIIu	Bednarczyk <sup>77</sup> (n=4), Al-Fares <sup>78</sup> (n=5)
ARDS on ECMO	To improve survival	L-AmB 6-7.5 mg/kg/day	C	IIIu	Zhao <sup>80</sup>

**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence; AmB-d, Amphotericin B deoxycholate; CNS, Central nervous system; ECMO, extracorporeal membrane oxygenation, Itra, Itraconazole; L-AmB, Liposomal amphotericin B; MSG, Mycoses Study Group

## COCCIDIOIDOMYCOSIS

Coccidioidomycosis is caused by the endemic fungi *Coccidioides immitis* and *C. posadasii*. These fungi survive well in areas of low precipitation (12-50 cm per year), with few winter freezes and alkaline soil.<sup>82</sup> The inoculum needed for infection is small and may be as low as a single arthroconidium.

Previous guidelines for the treatment of coccidioidomycosis have been published by the IDSA in 2005 and were recently updated in 2016.<sup>83</sup> So far, only a single comparative clinical trial has been performed in the study of coccidioidomycosis<sup>84</sup> and the majority of our understanding of the disease comes from open label observational studies, retrospective data collections, case series and expert opinion.

### Epidemiology

There are currently two species of *Coccidioides*, although recent phylogenetic evaluation has suggested additional genetic species may be present. However these have not yet received general acceptance as distinct species.<sup>85</sup> *Coccidioides immitis* is primarily found in California, Washington, and northwest Mexico, while *Coccidioides posadasii* is found in the southwestern United States, Mexico, and arid regions of Argentina, Brazil, Columbia, Guatemala, Honduras, Paraguay, and Venezuela.<sup>86</sup> *C. posadasii* isolated from central and northern South America (Guatemala and Venezuela) may represent a novel distinct species (Appendix Figure 3).<sup>87</sup> Within the U.S., the number of coccidioidomycosis cases continues to increase yearly. The majority of cases are reported from Arizona and California and the incidence can vary greatly by geographic region and season.<sup>88</sup> Epidemic outbreaks have been observed after dust storms, earthquakes, and wildfires.

Additionally, the true range of this endemic mycosis has been called into question following the recent identification of *Coccidioides immitis* in the soil in Washington state, as well as clinical cases acquired within this region.<sup>16,89,90</sup>

### **Clinical Presentation**

Coccidioidomycosis is a highly variable disease. Following exposure and inhalation of the infectious form (arthroconidia), ~60% of people develop asymptomatic infection or a mild self-resolving respiratory illness. The majority of those who present with clinically relevant infection exhibit respiratory symptoms within 1-3 weeks of exposure with fever, cough, chills, night sweats, weight loss and/or joint pain (“Valley fever”). Up to 50% of symptomatic patients develop a mild, diffuse erythematous or maculopapular rash covering the trunk and/or limbs within the first few days of the onset of symptoms – a clinical sign that may be useful to differentiate the patient’s symptoms from those caused by bacterial pneumonia.<sup>91</sup> Erythema multiforme, erythema nodosum (Appendix Figure 4A), and Sweet’s syndrome have each been described with infection.<sup>92</sup> Coccidioidomycosis causes 17-29% of all community acquired pneumonia (CAP) within the endemic regions.<sup>93,94</sup> These CAP cases are commonly misdiagnosed and receive unneeded antibacterial treatment.<sup>95</sup> The majority of acute pulmonary disease resolves over weeks to months with or without treatment, and antifungal therapy has not been proven to hasten clinical resolution of acute disease.<sup>96</sup>

Following the acquisition of coccidioidomycosis, a small percentage of patients (1-3%) will develop a persistent form of the disease ranging from chronic pulmonary coccidioidomycosis (including nodules [Appendix Figure 4B], cavities and fibrocavitary pneumonia) to extrathoracic disseminated infection. Dissemination is more commonly observed in those of African or Oceanic ancestry, those receiving immunosuppressive therapy, and in pregnant women who acquire infection during and after the second trimester.<sup>97</sup> Dissemination to the skin, bone/joint, and central

nervous system are the most common extrapulmonary manifestations, and careful evaluation of symptoms and physical examination is integral to accurate diagnosis and treatment. CNS symptoms are non-specific and include headache, nausea, vomiting and vision changes.

## **Diagnosis**

### *Culture and microscopy*

**Evidence** - The diagnosis of coccidioidomycosis is proven by culture of *Coccidioides* spp. from any clinical site, including sputum, BAL, CSF, or tissue although cultures may be negative, particularly in the CSF. Blood cultures are positive only in highly immunocompromised patients and even in these circumstances are typically negative.

*Coccidioides* spp. grow on routine mycologic media including Sabouraud dextrose agar incubated at 25-30°C, but may also grow at higher temperatures on routine bacteriological media, such as blood agar. Mycelial growth may be seen as early as 4-5 days following incubation on solid media. *Coccidioides* growing on culture media can be a significant biohazard to laboratory personnel. If *Coccidioides* is suspected, culture plates should be destroyed or taped closed and moved to appropriate facilities to handle these cultures. Macroscopically colonies are typically white to gray although they may produce variable coloured pigment (yellow/brown to purple) over time (Appendix Figure 4C). Microscopically, as arthroconidia mature (Appendix Figure 4D), the alternating disjunct cells undergo lytic degradation, releasing barrel-shaped arthroconidia (~2-5 µm in length), which are the infectious particles. Inside the host (37°C) the arthroconidia transform into spherules (up to 120 µm) (Appendix Figure 4E), which are thick-walled spherical structures containing hundreds of endospores, each approximately 2-4 µm, which are released if the spherule ruptures. Each endospore can develop into a spherule as well, continuing the process within the

host. *Prototheca wickerhamii* may resemble small spherules, and *Rhinosporidium seeberi* may simulate larger ones. The *Coccidioides* AccuProbe assay (Hologic, Sunnyvale, CA, USA) is useful for confirmation of unknown isolates as *Coccidioides* species although it does not distinguish between the two species of *Coccidioides*.<sup>98</sup>

**Table 5. Conventional methods in the diagnosis of coccidioidomycosis.**

Popula- tion	Intention	Intervention	SoR	QoE	References and comment
Any	Diagnosis	Direct micro- copy of culture or biospecimen, ob- servation of spherules or ar- throconidia	B	III	Saubolle <sup>99</sup> (Potential of close relatives of <i>Coccidioides</i> having similar spherule morphology ( <i>Emmonsia</i> spp.); hyphae may have similar arthroconidia)
Any	Diagnosis	Culture of fungus from biospeci- mens	B	III	Saubolle <sup>99</sup> (Growth at room temperature re- sults in hyphae with arthroconidia at 1-2 weeks)

**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence

### Serology

**Evidence** - The majority of patients are diagnosed with coccidioidomycosis using serologic testing. Enzyme immunoassays (EIA), immunodiffusion (ID) and complement fixation (CF) testing are commercially available and exhibit differing sensitivity and specificity. A typical coccidioidal infection results in serum IgM production within 1-3 weeks of symptoms onset followed shortly (4-8 weeks) thereafter by IgG production.

A qualitative latex agglutination (LA) test using heat-treated coccidioidin as antigen is available from several commercial sources (LA-Cocci antibody system [IMMY, Norman, OK, USA]; and *Coccidioides* latex agglutination [Meridian Bioscience, Cincinnati, OH, USA]). This test is simple and rapid to perform, however the false positive rate is higher than that observed with the ID and/or CF methods. It is not recommended for screening CSF specimens because false-positive reactions can occur.<sup>100</sup>

The Premier *Coccidioides* EIA (Meridian) is a qualitative enzyme immunoassay (EIA) test for detection of IgM and IgG antibodies in serum or CSF specimens. False-positive reactions have been obtained with sera from some patients with blastomycosis and other endemic mycoses, and this test is now often used for “screening” with positive EIA results evaluated by ID and CF tests for confirmation.<sup>101</sup> A lateral flow assay has also been recently developed and may have the benefit of rapid screening for the presence of coccidioidal antibodies and confirmatory studies are ongoing. Other EIA tests have been recently developed (MiraVista, Indianapolis, In, USA) with a favourable sensitivity, however EIA tests appear to be less specific than immunodiffusion.<sup>102</sup> The specificity of immunodiffusion, which can be used to detect both IgM and IgG antibodies, is >95%. The CF test is less sensitive than immunodiffusion, but is helpful for disease prognosis and temporal evaluation of a therapeutic response to antifungal treatment when quantitative titres are obtained.<sup>103</sup>

### *Skin testing*

**Evidence** - A reformulated skin test for coccidioidomycosis (Spherusol, Nielsen BioSciences, San Diego, CA, USA) has been recently approved for use in the detection of delayed-type hypersensitivity responses to patients previously diagnosed with coccidioidomycosis. The reformulation was approved for patients recovering from acute pneumonia but it may be useful to stratify patients

who have previously had coccidioidomycosis from those who have not.<sup>104</sup> Recent studies have questioned the overall sensitivity and specificity compared to the previously available skin test(s) (Spherulin and Coccidioidin).<sup>105,106</sup>

### *Antigen detection*

**Evidence** - Coccidioidal antigen testing is also available in commercial laboratories using an EIA based method and can be used on blood, urine or other clinical specimens. In the highly immunocompromised, antigenuria has been detected in up to 70% of patients.<sup>107</sup> The majority of patients in this study with positive antigen testing had evidence of extrathoracic disseminated coccidioidomycosis and/or HIV infection. Antigenuria testing may also be useful in the solid organ transplant population. Positive urine antigen test results in other populations, including patients with acute or chronic pulmonary disease and non-immunosuppressed patients with disseminated coccidioidomycosis, are infrequently observed, therefore the test is generally unhelpful in these patient populations. Cross-reactivity with other endemic fungi has been observed, thus interpretation in the appropriate clinical context is needed.<sup>108,109</sup> Detection of antigen in CSF has also been reported and may assist in the diagnosis in selected cases of coccidioidal meningoencephalitis.<sup>110</sup>

*Coccidioides* spp. produce significant amounts of BDG, and the performance characteristics of this test in sera are similar to those observed for other fungal infections. However, given its sensitivity in sera of 44% and lack of specificity, BDG testing has a limited role in the evaluation of coccidioidomycosis in general.<sup>111</sup> BDG testing of CSF samples in patients with coccidioidal meningitis has shown high sensitivity (96%) and serial samples are positive after years of treatment, suggesting persistence of positivity with chronic infection.<sup>112</sup>

### *Molecular methods*

**Evidence** - PCR assays have been used for the detection of *Coccidioides* spp. from clinical samples (GeneSTAT.MDx DxNA LLC, St. George, UT, USA). Few clinical studies have been published to date. While results are available more rapidly, the sensitivity of PCR is similar to that of culture (~50%).<sup>113,114</sup> Other PCR tests have been investigated but are not commercially available.<sup>115</sup>

#### *Susceptibility testing*

**Evidence** - Guidelines for the *in vitro* susceptibility testing of *Coccidioides* spp. do not exist. Although *in vitro* susceptibility testing has found elevated fluconazole MIC<sub>50</sub> values for *Coccidioides* isolates,<sup>116</sup> the clinical significance of this remains unclear. No breakpoints are available, and the clinical relevance of susceptibility testing results merits further study.<sup>117</sup>

### **Coccidioidomycosis Diagnostic Recommendations**

We recommend that specimens, including skin or tissue biopsies, sputum, BAL, or CSF samples should be examined microscopically for the presence of *Coccidioides*. Standard fungal stains such as GMS (SoR B, QoE III) may increase the yield. Identification of spherules in a clinical specimen is considered evidence of proven disease even in the absence of positive culture results. Cultures should be held for up to 6 weeks (SoR B, QoE III).

We recommend serologic testing of blood in patients with suspected coccidioidomycosis (SoR A, QoE II). The performance characteristics of commercially available serologic tests vary, and an understanding of these differences is essential for proper interpretation. Those with positive results should undergo repeat quantitative serologic testing (CF) approximately every 12 weeks during their care to evaluate a response to therapy (SoR A, QoE II). A CSF sample should be obtained from all patients suspected of meningitis. We recommend only immunodiffusion or CF testing when evaluating CSF (SoR A, QoE II). We suggest coccidioidal antigen testing only when

the diagnosis is in question (e.g. negative serologic results or immunocompromised patient) (SoR C, QoE IIu).

**Table 6. Role of non-invasive diagnostics in coccidioidomycosis.**

Population	Intention	Intervention	SoR	QoE	References and comment
<i>Serology testing</i>					
Any	Diagnosis	Immunodiffusion	A	IIt	Wieden <sup>118</sup> Kaufman <sup>119</sup> Pappagianis <sup>120</sup> Kaufman <sup>121</sup>
Any	Diagnosis and response to therapy	Complement fixation	A	IIt	Mchardy <sup>103</sup> Pappagianis <sup>120</sup> (Considered gold standard, high specificity – significant differences between labs)
Any	Diagnosis	EIA	A	IIt	Kaufman <sup>121</sup> Grys <sup>122</sup> Blair <sup>123</sup> (EIA tests are appropriate screening tests although due to rate of false-positivity they should be confirmed by ID or CF testing)
Any	Diagnosis	Lateral flow assay (serology)	C	IIu	May be useful as a screening test similar to EIA <sup>124</sup>
Meningitis	Diagnosis and response to therapy	Immunodiffusion or CF	A	IIt	Pappagianis <sup>120</sup> (High sensitivity and specificity in CNS)
<i>Antigen testing</i>					
Immunocompromised	Diagnosis	EIA	C	IIu	Durkin <sup>107</sup> (false-negatives common in blood, more helpful in CSF, of uncertain benefit when used longitudinally in CSF)
Any	Diagnosis	EIA	D	IIIu	Bamberger <sup>110</sup> (antigen testing is not helpful in majority of immunocompetent coccidioidomycosis patients)

Any	Diagnosis	(1-3)- $\beta$ -D-glucan	D	Iiu	Thompson <sup>111</sup> (N=228, negative predictive value=64%); Stevens <sup>112</sup> (N=37, high sensitivity in CSF)
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**Legend:** , SoR, Strength of Recommendation; QoE, Quality of Evidence; EIA, Enzyme immunoassay, CF, Complement Fixation; ID, immunodiffusion

### *Imaging*

**Evidence** – Radiographic features of coccidioidomycosis are nonspecific. The most common presentation is a focal infiltrate indistinguishable from CAP.<sup>125</sup> Patients may have a normal chest X-ray, or a consolidation or interstitial findings may be observed. Pleural effusions may occur in a small proportion of patients and range in size from very small and clinically irrelevant to empyema requiring surgical intervention. The size of the effusion does not correlate with risk of dissemination.<sup>126,127</sup> Intrathoracic lymphadenopathy is observed in ~20% of patients undergoing CT imaging<sup>128</sup> – and may also be seen on plain imaging. Lymphadenopathy was thought to demonstrate regional spread from the pulmonary parenchyma to the lymphatic system and represent early disseminated disease; however, further studies have shown that patients with mediastinal lymphadenopathy are not an increased risk of dissemination, disproving this presumed association.<sup>129</sup>

Severe manifestations of acute pulmonary coccidioidomycosis are uncommon and most frequently observed following a high inoculum exposure or significant underlying immunodeficiency. Respiratory failure and ARDS may be seen in these groups. A miliary pattern on chest imaging has been reported in acute pneumonia and indicates haematogenous or lymphatic spread. Imaging findings show multiple, small, millet-seed nodules throughout the lung parenchyma that are radiographically indistinguishable from those seen with tuberculosis.<sup>130</sup>

Pulmonary infiltrates from *Coccidioides* should be followed to resolution with repeat imaging after the initial infection. The initial infiltrate may “heal” with a resultant pulmonary nodule or persistent small cavity (typically at 6 months). If an antecedent history of coccidioidomycosis is not known by subsequent clinicians, or if patients are not adequately educated about the sequelae from their initial infection, these nodules can be indistinguishable from malignancy and in many cases, unfortunately, patients undergo surgical biopsy or resection for diagnosis.<sup>130-132</sup>

Primary infection may become complicated over time with cavitation resulting in thick or thin walled cavities. Furthermore, cavities near the pleural space can rupture producing bronchopleural fistula or hydropneumothorax. Chronic fibrocavitary disease may be observed occasionally.<sup>101</sup> Extrapulmonary dissemination can involve any organ system and patient symptoms will direct imaging of potentially affected regions. CNS disease typically presents as basilar meningitis, and vasculitic infarcts are not uncommon.<sup>133,134</sup> Hydrocephalus is a frequently observed complication. Spinal disease, including psoas abscesses, may also be seen in those with dissemination. Monoarticular disease, most prevalent in the knee, can be noted on MRI.

### **Imaging Recommendations**

All patients with coccidioidomycosis should have a chest radiograph (SoR A, QoE IIu). If an infiltrate is present on initial evaluation we recommend repeat imaging of the chest in 6 months to determine if a nodule has developed to avoid a later evaluation for cancer (SoR A, QoE III). For patients slow to respond to antifungal therapy we recommend additional imaging, such as CT of the thorax, to determine if complications of primary infection have occurred (SoR B, QoE IIu). Repeated radiographic imaging in cases of chronic pulmonary infection or disseminated infection is indicated to assess a successful response to therapy (SoR A, QoE IIu). All patients with coccidioid meningitis should undergo an MRI of the head at baseline to assess for possible vasculitic

infarcts or hydrocephalus (SoR A, QoE III). We do not recommend routine PET/CT or bone scans in patients with coccidioidomycosis unless a specific clinical concern exists (SoR D, QoE III).

**Table 7. Radiographic imaging of coccidioidomycosis**

Population	Intention	Intervention	SoR	QoE	References and comment
Any	Diagnosis	Chest X-ray	A	Iu	Ampel <sup>135</sup> (N=31), Catanzaro <sup>125</sup> (Cases of confirmed coccidioidomycosis may have negative chest X-ray)
People with slow resolution of respiratory symptoms and extreme fatigue	Diagnosis	Chest X-ray	B	Iu	Blair <sup>96</sup> (N=36, Limits: including only primary pulmonary coccidioidomycosis)
Suspected complication of infection	Diagnosis	Chest CT	B	Iu	Smith <sup>136</sup> (Nodules or cavities develop in 4-8%)
CNS	Assess for complications of infection and prior to shunt placement for hydrocephalus if present	Brain MRI	A	III	Imaging at baseline. Patients with CNS coccidioidomycosis with increased intracranial pressure will not resolve this problem without placement of shunt, early imaging and neurosurgical consultation recommended
Patient with unexplained high CF titres	To provide whole-body metabolic and anatomic assessment of disease	PET/CT	D	III	Rarely indicated and false positives and negatives may occur

**Legend:** , SoR, Strength of Recommendation; QoE, Quality of Evidence; CF, Complement fixation; CT, Computed tomography; MRI, Magnetic resonance imaging; PET, Positron emission tomography.

### ***Treatment rationale and recommendations***

**Evidence** – Significant differences in treatment practices of primary pulmonary coccidioidomycosis exist among physicians. Many clinicians support a period of observation rather than anti-fungal therapy as most patients will clear their infection without long-term sequelae. Others

advocate for treatment of all symptomatic patients in the hopes of hastening symptom resolution. No placebo-controlled study has been successfully completed to compare the rapidity of symptom resolution in those treated vs those untreated. Two observational studies have shown antifungal therapy does not appear to impact the risk of extrapulmonary dissemination.<sup>96,137</sup> Significant fatigue often persists after initial infection and patient may benefit from physical therapy as they recover.<sup>138,139</sup>

Treatment should be given to all patients with underlying immunosuppression, significant cardiopulmonary comorbidities, or those with prolonged infection. Treatment is generally offered to ethnicities at high risk for complications (African and Oceanic ethnicities). Patients exhibiting weight loss >10%, night sweats for >3 weeks, and infiltrates exceeding 50% of one lung or bilateral disease should be treated as well, particularly in those with CF titres  $\geq 1:32$ .<sup>83</sup> Primary infection should be treated with fluconazole or itraconazole.

Severe pulmonary disease should be treated with an amphotericin B formulation followed by a triazole (fluconazole/itraconazole). For those with ARDS some physicians would start corticosteroids in a 21-day taper similar to the treatment of *Pneumocystis* pneumonia although not all advocate for this approach. The duration of therapy varies by site and severity of disease. Typically, 3-6 months is preferred for primary pulmonary disease, and many recommend clinical follow-up for a year or longer after initial infection.<sup>140</sup> Coccidioidal serology should not be used as the sole criterion to continue antifungal therapy. In particular, antifungal therapy can be discontinued in most patients prior to the serological tests becoming negative.<sup>141</sup> In contrast, chronic pulmonary disease (chronic pneumonia and chronic cavitary disease) often requires prolonged if not life-long therapy for disease control. Although there are no studies evaluating the recommended

interval of serological testing, typically testing is performed every 2-4 months, depending on disease severity and the clinical response to treatment.<sup>142</sup>

Disease refractory to fluconazole is treated with a mould-active triazole (itraconazole, voriconazole, posaconazole) or amphotericin B depending on disease severity<sup>83,117</sup>. Itraconazole has been found to be superior to fluconazole after 12 months of therapy in those with progressive non-meningeal coccidioidomycosis.<sup>84</sup> Experience with posaconazole and voriconazole is limited to observational open-label and retrospective studies,<sup>143,144</sup> and there is limited experience in the treatment of coccidioidomycosis with isavuconazole.<sup>73,145</sup>

Meningitis remains a particularly morbid form of the disease and should be considered in patients with sustained headache or other CNS symptoms.<sup>146</sup> Vasculitis and hydrocephalus develop in many cases and significantly complicate management. Treatment with fluconazole is recommended in those with early or mild disease, although typically high doses are needed (>800 mg daily).<sup>147,148</sup> Those intolerant or refractory to fluconazole may benefit from an alternative triazole.<sup>144,145,149,150</sup> Refractory disease can also be treated with the combination of a triazole and intravenous lipid amphotericin B formulation, although data regarding this approach are scant. Intrathecal therapy for recalcitrant disease is required in severe cases. Consultation with a clinician experienced with this approach is strongly recommended to avoid toxicity and maximize efficacy.<sup>151,152</sup> The utility of adjunctive corticosteroid therapy has been shown in a retrospective study of patients with coccidioidal vasculitis and can significantly decrease the risk of a second cerebrovascular accident.<sup>134</sup>

Pregnancy is a unique risk factor for coccidioidomycosis and represents a significant challenge given the teratogenicity of triazoles during the first trimester, particularly the high doses and prolonged therapy often necessary in the treatment of coccidioidomycosis. If therapy is required

during pregnancy, amphotericin B is recommended with lipid formulations preferred. Some experts would treat with amphotericin B for the duration of pregnancy, while others would recommend a transition to a triazole during the 2<sup>nd</sup> or 3<sup>rd</sup> trimester. Discussion with a physician experienced in the treatment of coccidioidomycosis in pregnancy is paramount in these cases.<sup>97</sup>

The course of infection and treatment in immunosuppressed population is frequently difficult. Patients with HIV are unlikely to benefit from primary prophylaxis,<sup>153</sup> however those with confirmed infection should be started on antiretroviral therapy as soon as possible. Antifungal therapy can be discontinued in patients who have had 6 months of therapy and their CD4 count is >250 cells/ $\mu$ L.<sup>154</sup>

Patients undergoing solid organ transplantation or hematopoietic stem cell transplantation within the endemic region should undergo clinical and serologic testing for evidence of infection. Patients with evidence of past infection are treated with fluconazole 400mg for at least 12 months after transplant to prevent reactivation of disease and are frequently given therapy indefinitely.<sup>155-</sup>

158

Those without evidence of infection living within the endemic region should receive antifungal prophylaxis (fluconazole 200-400mg daily) at the time of transplant; this should continue for 6-12 months.<sup>83</sup>

**Coccidioidomycosis Treatment Recommendations** - We recommend patients with primary pulmonary coccidioidomycosis be individually assessed to determine if they may potentially benefit from antifungal therapy (SoR C, QoE III). We recommend physical therapy be considered in those patients with profound fatigue associated with acute pulmonary coccidioidomycosis (SoR B, QoE III). Chronic pulmonary coccidioidomycosis should be treated with fluconazole or itraconazole (SoR A, QoE I). We recommend fluconazole or itraconazole as first-line therapy for patients with

extrapulmonary manifestations of dissemination, with itraconazole preferred if there is bone or joint involvement (SoR A, QoE I). Severe manifestations or refractory disease should be treated with L-AMB (SoR B, QoE IIu). During pregnancy, L-AmB is recommended during the first trimester and possibly after (SoR A, QoE III). Fluconazole failure should be treated with itraconazole, voriconazole or posaconazole (SoR A, QoE III).

We recommend fluconazole (800 mg/day adjusted for renal function) as initial therapy in the treatment of mild coccidioidal meningitis (SoR A, QoE IIu) although some clinicians would recommend L-AmB as initial therapy for all patients with meningitis (SoR A, QoE III). Patients with CNS infection unresponsive to fluconazole should receive itraconazole (SoR A, QoE IIu), posaconazole or voriconazole (SoR B, QoE IIu). There is limited experience with isavuconazole in the treatment of coccidioidomycosis at this time (SoR C, QoE III). Intravenous L-AmB is recommended as an adjunct in cases without a complete response to triazole antifungals or in those with severe disease (SoR C, QoE III), and intrathecal AmB-d is recommended in patients who fail available triazole therapy (SoR, B, QoEIII). We recommend the use of systemic corticosteroids in cases of vasculitis associated with coccidioidal meningitis (SoR B, QoE IIu).

In the HIV population we do not recommend primary prophylaxis regardless of the CD4 cell count. In HIV patients with confirmed coccidioidomycosis we recommend continuation of antifungal therapy until the infection is well controlled and the CD4 cell count exceeds 250 cells/ $\mu$ L for at least 6 months (SoR A, QoE IIu). In solid-organ transplant patients with a clinical history of coccidioidomycosis we recommend fluconazole (200-400mg daily) for the first six months following transplantation (SoR A, QoE III); thereafter we recommend lifelong triazole antifungal prophylaxis, preferably with fluconazole (200 mg daily adjusted for renal function). In solid organ transplant recipients with positive coccidioidal serology or who receive an organ from

a donor with evidence of coccidioidomycosis we recommend indefinite triazole prophylaxis (SoR A, QoE III). Solid organ transplant recipients who reside in a hyper-endemic region for coccidioidomycosis should receive triazole prophylaxis for the first year following transplantation (SoR A, QoE IIu). No definitive recommendations can be made regarding screening nor duration of therapy in patients with coccidioidomycosis and who are receiving tumour necrosis factor (TNF)- $\alpha$  inhibitors.

**Table 8. Treatment of coccidioidomycosis**

Popula- tion	Intention	Intervention	SoR	QoE	References and comment
Any	Treatment of Primary pneumonia	Majority of patients do not need antifungal treatment and should be individually assessed	C	III	Dickson <sup>159</sup> (N=354, 92% recovered without complication)
Any	Primary pneumonia with profound fatigue	Education, physical therapy	B	III	Blair <sup>96</sup> (fatigue is often last symptom to resolve)
Any	To treat	Fluconazole or itraconazole	A	I	Galgiani <sup>84</sup> (N=198, By 12 months 57% responded to fluconazole and 72% to itraconazole; P=0.05)
Any	Extrapulmonary	Fluconazole or Itraconazole	A	I	Catanzaro <sup>160</sup> (N=75, 86% efficacy in fluconazole study); Galgiani <sup>84</sup> (Itraconazole efficacy as above)
Any	Extrapulmonary	Voriconazole, Posaconazole	B	IIu	Anstead <sup>161</sup> Stevens <sup>162</sup> Kim <sup>144</sup> (Voriconazole and posaconazole demonstrated efficacy in salvage setting)
Any	Extrapulmonary	L-AMB and ABLC	B	III	Sidhu <sup>163</sup> (N=69, efficacy in 73% of ABLC, 69% of L-AmB)
Pregnancy	Pneumonia	L-AMB	A	III	Bercovitch <sup>97</sup> (Expert opinion best practices)

CNS	To treat	Fluconazole or Itraconazole	A	Iiu	Galgiani <sup>147</sup> (N=47, Fluconazole - 79% response rate); Tucker <sup>149</sup> (N=8, Itraconazole, improvement in 7/8)
CNS	To treat	Intrathecal AMB-d	B	Iiu	Winn <sup>164</sup> Einstein <sup>165</sup> Ho <sup>151</sup> (Significant provider expertise is required in the use of this therapy and it should be used in refractory disease only)
CNS	To treat	Voriconazole, Posaconazole or isavuconazole	B	Iiu	Cortez <sup>166</sup> Proia <sup>167</sup> Schein <sup>168</sup> Heidari <sup>145</sup> (Voriconazole, posaconazole, and isavuconazole hav demonstrated efficacy in salvage setting)
CNS	To treat	L-AmB	C	III	Stewart <sup>150</sup> (Single case report, monotherapy with L-AmB should only be used in absence of other options)
CNS-vasculitis	To treat	Corticosteroids	B	Iiu	Thompson <sup>134</sup> (N=221, adjunctive corticosteroids in those with coccidioidal vasculitis decreased secondary cerebrovascular events)
SOT with moderate to severe disease	To treat	L-AmB	A	III	Galgiani <sup>83</sup> (Expert opinion based on provider experience with those with severe or rapidly progressing infection)
SOT with mild disease	To treat	Fluconazole or itraconazole	A	III	Galgiani <sup>83</sup> (Triazoles should be continued indefinitely. Caution triazole coadministration with cyclosporine, tacrolimus or sirolimus)
SOT	To prevent relapse	Fluconazole indefinitely	A	III	Galgiani <sup>83</sup> (Triazoles should be continued indefinitely. Caution triazole coadministration with cyclosporine, tacrolimus or sirolimus)

**Legend:** , SoR, Strength of Recommendation; QoE, Quality of Evidence; AMB-d, Amphotericin B deoxycholate;

CNS, Central nervous system; L-AmB, Liposomal amphotericin B; ABLC, amphotericin B lipid complex; SOT, Solid organ transplantation.

## **EMERGOMYCOSIS**

Emergomycosis is a fungal disease caused by thermally dimorphic fungi in the recently described Onygenalean genus, *Emergomyces*.<sup>169</sup> Some reports were previously published referring to the disease as disseminated emmonsiosis and the pathogens as *Emmonsia* species,<sup>170-183</sup> prior to a taxonomic overhaul involving the Onygenales.<sup>10,184</sup> There have been no published guidelines for the diagnosis or management of emergomycosis. No clinical trial has been performed, and thus

knowledge about this disease and its management is gleaned from small observational studies, case reports and limited *in vitro* analyses.

## **Epidemiology**

There are currently five described species of *Emergomyces* reported to cause human disease. The geographic distribution of reported cases is shown in Appendix Figure 5. To date, nearly all cases of emergomycosis have involved immunocompromised patients. Disease caused by *E. pasteurianus* has been reported in patients from Europe (including France<sup>178</sup>, Italy<sup>182,185</sup>, Spain<sup>176</sup>, and the Netherlands<sup>186</sup>); Asia (China<sup>171,180</sup> and India<sup>173</sup>); and Africa (Lesotho, South Africa<sup>10</sup> and Uganda<sup>187</sup>). Emergomycosis caused by *E. africanus* has been reported only from South Africa, Lesotho and Zimbabwe in immunocompromised patients with advanced HIV infection in all but one, who had received prior kidney transplantation.<sup>172,174,179,183,188</sup> Since the introduction of molecular-based identification methods of dimorphic fungi in some South African laboratories, emergomycosis has been recognized as the most frequently diagnosed endemic mycosis in South Africa.<sup>189</sup> Emergomycosis caused by *E. canadensis* has been infrequently reported in central and western regions of North America, including cases from Saskatchewan, Colorado and New Mexico.<sup>190</sup> All reported patients have been immunocompromised, including with advanced HIV infection and solid organ transplantation.<sup>190</sup> Emergomycosis caused by *E. orientalis* has been reported only once, in a patient with diabetes mellitus from China.<sup>191</sup> *E. europaeus* infection has also been only reported once, from Germany, in a patient on chronic steroids for rheumatoid arthritis.<sup>175</sup>

## **Clinical Presentation**

Emergomycosis appears to be primarily a disseminated fungal disease. The most commonly reported clinical manifestation is the appearance of widespread skin lesions, present in 95% of

patients at diagnosis. Generally these appear as papules and plaques (Appendix Figure 6A).<sup>172</sup> Pulmonary disease is also common, with abnormal chest imaging observed in 86% of patients in whom the diagnosis was confirmed (Appendix Figure 6B).<sup>172</sup> Radiographic abnormalities include consolidation, reticulonodularity, and lymphadenopathy.<sup>172</sup> However, isolation of *E. africanus* from respiratory samples is rare and co-infection with *Mycobacterium tuberculosis* is common. In a review of 52 cases from South Africa (isolates for two other included cases were later reclassified as *Blastomyces percursus*<sup>10</sup>), only one case was diagnosed on the basis of respiratory specimens, from a transbronchial biopsy.<sup>172</sup> In addition to skin and lung disease, other reported sites of disease include involvement of lymph nodes, liver, spleen, and bone marrow.<sup>172</sup>

Half of patients reported in South Africa were diagnosed with emergomycosis after cutaneous lesions appeared in the two months following antiretroviral therapy (ART) initiation.<sup>172</sup> In retrospect, many of these patients sought care because of systemic symptoms such as fever and weight loss. Three-quarters were misdiagnosed with and treated for tuberculosis before the correct diagnosis was made.<sup>172</sup> The appearance of lesions after ART initiation, and the histopathological observation of robust inflammatory responses in such lesions in comparison to patients not on ART,<sup>179</sup> suggest an unmasking immune reconstitution inflammatory syndrome (IRIS).

## ***Diagnosis***

### *Culture and microscopy*

**Evidence** - The diagnosis of emergomycosis is proven by culture of *Emergomyces* spp. from blood or tissue,<sup>172,179,183,188</sup> or occasionally molecular testing of affected tissue.<sup>172,187</sup> *Emergomyces* spp. can sometimes be isolated from blood using standard aerobic blood culture bottles, but the sensitivity might be improved using blood culture bottles that utilize the lysis-centrifugation technique.

*Emergomyces* spp. can grow in culture from clinical samples inoculated onto standard fungal media (e.g. Sabouraud agar, malt extract agar, or potato dextrose agar), incubated at 24-30°C. Growth usually can be observed after 7-30 days.<sup>169</sup> Macroscopically, colonies are typically white to yellowish-white or beige, glabrous at first but becoming powdery or wrinkled over time (Appendix Figure 6D). Microscopically, short, non-branching conidiophores that arise at right angles from hyaline hyphae are slightly swollen at the end and give way to 1-8 short secondary conidiophores each bearing single small (1-2 µm) round conidia (Appendix Figure 6E).<sup>169,179,184</sup> Conversion of mould-to-yeast can be achieved by transfer of the colony onto brain heart infusion or malt extract agar with incubation at 30°C for 7-21 days. Yeast colonies appear cream to grey-brown and microscopically, yeasts are small (2-4 µm in diameter), round to oval, and replicate by budding from a narrow base.<sup>169,179,184</sup>

The identification of *Emergomyces* in culture can be confirmed by DNA sequencing of the ribosomal internal transcribed spacer (ITS) region. Most published data has been generated by PCR amplification of the ITS region, which can enable genus-level identification for most isolates using phylogenetic inference.<sup>10,179,187,192</sup>

Skin lesions are present at the time of diagnosis in 95% of patients diagnosed with emergomycosis, and urgent biopsy for culture and histopathological examination is essential for making a timely diagnosis of invasive fungal disease.<sup>193</sup> In a cross-sectional study of patients with emergomycosis in South Africa, patients who had skin lesions biopsied were diagnosed sooner, more likely to receive antifungal therapy, and had fewer deaths than patients diagnosed only on the basis of positive blood cultures.<sup>172</sup> Examination of skin scrapings with KOH preparations has not been adequately studied for emergomycosis. Histopathological findings of *Emergomyces* in tissue are the appearance of small (3-5 µm), round to oval narrow-budding yeast-like cells in subcutaneous

or other deep tissue (Appendix Figure 6C). These findings are diagnostic for invasive fungal disease but insufficient to allow distinction of *Emergomyces* from other dimorphic fungi with small yeast-like cells, such as *H. capsulatum*.<sup>188</sup> Molecular testing can be performed on fresh tissue or formalin fixed paraffin embedded tissue using published protocols for amplification and sequencing of broad-range fungal targets, such as ITS.<sup>194</sup>

#### *Antigen detection*

*E. africanus* and *E. canadensis* spp. cross react with the *Histoplasma* galactomannan antigen test (IMMY, Norman, OK, USA).<sup>188</sup> However, a negative *Histoplasma* galactomannan antigen test is not sufficient to exclude the diagnosis of emergomycosis. In one series, just 3 of 10 patients with culture proven emergomycosis had a positive *Histoplasma* antigen test.<sup>188</sup> *Emergomyces* spp. do not appear to reliably produce BDG; only 1 of 4 patients with emergomycosis caused by *E. africanus* had an elevated serum BDG.<sup>172</sup>

#### *Molecular methods*

*Emergomyces canadensis* can cross react with a commercially available *Blastomyces* DNA probe (AccuProbe, Hologic Inc., Sunnyvale, CA, USA),<sup>190</sup> so a positive *B. dermatitidis* DNA probe result should be interpreted with caution in situations in which clinical, epidemiological and/or morphological findings are more suggestive of emergomycosis. Several cases of atypical blastomycosis (identified as *B. dermatitidis* based on DNA probe) were later determined to be *E. canadensis*.<sup>190</sup> Definitive identification to the species level requires partial sequencing of *RPB2* or *TUB2* genes. For identification of *E. africanus* in southern Africa, sequencing of ITS is sufficient.<sup>193,195-198</sup> Sequencing of tissue biopsies using a broad-fungal target, such as ITS, or D1-D2 domains of rRNA can also be performed.<sup>195,199,200</sup>

**Emergomycosis Diagnostic Recommendations** - Culture of *Emergomyces* spp. from a patient with compatible symptoms should be considered diagnostic of disease (SoR A, QoE III). When skin lesions are present, punch or incisional biopsies are strongly recommended (SoR A, QoE III). We strongly recommend that tissue be transported in saline to the microbiology laboratory for fungal culture, and tissue additionally sent in formalin to histopathology for fungal stains (GMS and/or PAS stains) (SoR A, QoE III). Cultures may take up to 4 weeks and are not 100% sensitive. Findings of small (2-5 µm), round to oval yeast-like cells with narrow-based budding and an inflammatory reaction in deep tissue is proof of an invasive mycosis, but does not allow differentiation between emergomycosis and other fungal infections. (SoR C, QoE III).

In addition to skin biopsy culture, we recommend that blood should be collected in aerobic bottles and, where available, lysis-centrifugation tubes (SoR B, QoE III). When bone marrow biopsy or aspirate is performed, we strongly recommend that a specimen is obtained for fungal culture, preferably using the lysis-centrifugation system (SoR A, QoE III).

Cultured isolates can be identified morphologically to genus level by experienced mycologists, but most laboratories will need to perform genetic sequencing for definitive identification. Appropriate barcode regions for sequencing analysis are ITS, *RPB2* and *TUB2*.<sup>195,199-201</sup>

We recommend that urine should be sent for *Histoplasma* antigen testing because of clinical and geographic overlap with histoplasmosis, and because this test can sometimes be positive in patients with emergomycosis (SoR C, QoE III). Testing for serum BDG is not useful for diagnosing emergomycosis.

**Table 9. Conventional methods in the diagnosis of emergomycosis.**

Population	Intention	Intervention	SoR	QoE	References and comment
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Advanced HIV or immunosuppressed with skin lesions	Diagnosis	Microscopy of affected tissue	A	III	Kenyon <sup>193</sup> Schwartz <sup>195</sup> Schwartz <sup>196</sup> Crombie <sup>197</sup> by histopathology organism is small (3-5 micron), round to oval yeast-like cells with unipolar budding at narrow-necks, indistinguishable from that of <i>Histoplasma</i> . These can be highlighted with fungal stains, (e.g. PAS and GMS stains).  Fungal culture of skin tissue appears to be the most sensitive way to confirm the diagnosis.
Advanced HIV or immunosuppressed with skin lesions	Diagnosis	Blood culture	A	III	Kenyon <sup>193</sup> Schwartz <sup>195</sup> (sensitivity of blood cultures appears improved with lysis-centrifugation (e.g. Isolator™ or myco-F-lytic™ culture bottles)

Legend: , SoR, Strength of Recommendation; QoE, Quality of Evidence

### ***Treatment rationale and recommendations***

**Evidence** - There have been no trials regarding the treatment of emergomycosis, and all data are observational. The vast majority of reported patients with emergomycosis have been immunocompromised with advanced HIV disease in South Africa.

Several studies have evaluated *in vitro* antifungal susceptibilities of *Emergomyces* spp.<sup>52,189,202</sup> MICs to fluconazole of  $\geq 64$   $\mu\text{g/mL}$  have been reported for most isolates tested, including isolates of *E. africanus*, *E. canadensis*, *E. orientalis*, and *E. pasteurianus*.<sup>189,190,202</sup> Amphotericin B and mould-active triazoles have been consistently active *in vitro*.<sup>189,190,202</sup> MICs to echinocandins have generally been low ( $< 2$   $\mu\text{g/ml}$ ), but clinical evidence of efficacy has not been shown and this class of agents should not be used for treatment.<sup>189,190,202,203</sup>

The optimal dose and duration of antifungal therapy for emergomycosis is not established. Most patients reported in the literature have been treated either with amphotericin B followed by itraconazole or itraconazole alone.<sup>172,188</sup> Amphotericin B and itraconazole result in a rapid clinical response, including defervescence and improvement in cutaneous lesions within days. Clinical

outcome is dependent on the stage of disease at diagnosis. In the largest retrospective series, death occurred in 26/52 (50%) of patients, half of whom were only diagnosed with an invasive fungal disease post-mortem – usually when blood or bone marrow cultures returned, often weeks later.<sup>172</sup> In contrast, a smaller study that used active case finding with skin biopsies of patients with advanced HIV and new onset, generalized skin lesions reported deaths in 3/14 (21%),<sup>188</sup> highlighting the importance of prompt cutaneous biopsy for histopathological examination to enable earlier diagnosis and treatment.

Relapses after months of treatment with itraconazole have been reported.<sup>172,188,204</sup> In a retrospective study, some patients treated with short courses of low doses of fluconazole appeared to improve, but long-term follow up was absent.<sup>172</sup> In these select cases repeat susceptibility testing of isolates may be useful.

In patients who are ART-naïve or on failing ART regimens, the optimal timing of ART initiation or switching has not been established. Paradoxical IRIS is rare in emergomycosis and ART is recommended as soon as possible to facilitate immune reconstitution.<sup>204</sup>

There is a concern about drug-drug interactions between azoles and HIV medication. Certain non-nucleoside reverse transcriptase inhibitors (NNRTIs) typically induce metabolism of azoles resulting in reduced exposure.<sup>205</sup> Conversely, protease inhibitors can increase azole exposures.<sup>205</sup> Unfortunately, in many countries where emergomycosis is endemic, therapeutic drug monitoring (TDM) of azoles is not available.

One paediatric case has been reported, a 3 year-old from South Africa with HIV infection who was diagnosed with emergomycosis complicated by haemophagocytic lymphohistiocytosis.<sup>174</sup> That child was treated successfully with AmB-d (1 mg/kg/day) for 6 weeks, followed by

itraconazole (100 mg daily, in the context of a concurrent protease inhibitor containing ART regimen).<sup>174</sup>

**Emergomycosis Treatment Recommendations** - We strongly recommend that immunocompromised patients with disseminated disease be treated with a lipid formulation of amphotericin B (3-5 mg/kg/day) for 10-14 days (SoR A, QoE III). If unavailable, we strongly recommend an alternative amphotericin B formulation such as AmB-d (0.7-1mg/kg/day) for 10-14 days, as tolerated (SoR A, QoE III). We strongly recommend maintenance treatment with itraconazole (200 mg PO twice daily, however dose adjustment when protease inhibitor-based ART regimens are used may be indicated) for a duration of at least 12 months pending immune reconstitution (CD4  $\geq$ 200 cells/ $\mu$ L with viral load suppressed  $<$ 50 copies/mL) (SoR A, QoE III).

We suggest treatment with itraconazole for at least 12 months pending immune reconstitution in patients who are clinically stable and who are intolerant of amphotericin B (SoR B, QoE III). We recommend against treatment with fluconazole (SoR D, QoE III). Newer triazoles (voriconazole, posaconazole, isavuconazole) are active *in vitro* against *Emergomyces* spp.; however clinical data is scant.<sup>189,202</sup> We recommend against echinocandins (SoR D, QoE III).

For immunocompromised children with disseminated disease, we strongly recommend L-AmB (3-5 mg/kg/day by IV) for 10-14 days (SoR A, QoE III). Where lipid formulations of amphotericin are unavailable, we strongly recommend AmB-d 0.7-1 mg/kg/day for 10-14 days or until definite clinical improvement (SoR A, QoE III). We strongly recommend that amphotericin B should be followed by itraconazole (5-10 mg/kg/day divided in two doses) for at least 12 months (SoR A, QoE III).

We do not recommend routine antifungal susceptibility testing of clinical isolates although this can be considered for patients who appear to fail empiric antifungal treatment and in whom other causes (drug-drug interactions, poor absorption, etc.) are excluded.

The optimal timing of ART initiation in ART-naïve patients is uncertain, but we recommend beginning ART as soon as possible (SoR B, QoE III). In HIV-infected patients already receiving ART, we suggest an NNRTI-based treatment be changed to avoid drug-drug interactions with itraconazole (SoR B, QoE III).<sup>206</sup> Substitution with an integrase inhibitor-based regimen is preferred. Where this is not available, we suggest a protease inhibitor-based regimen but with reduction of itraconazole dosing (to 200 mg PO daily) (SoR B, QoE III).<sup>206</sup> We strongly recommend itraconazole TDM after 1-2 weeks of treatment (SoR A, QoE III).

**Table 10. Treatment of emergomycosis**

Population	Intention	Intervention	SoR	QoE	References and comment
Immunocompromised patients (>18 years-old)	To cure	Amphotericin B (lipid formulation 3-5mg/kg/d preferred, or deoxycholate formulation 0.7-1 mg/kg/d) for 10-14d or until clinically improved followed by itraconazole	A	III	Kenyon <sup>193</sup> (N=13); Schwartz <sup>195</sup> (N=52, demonstrates high fatality rate of infection); Schwartz <sup>196</sup> (N=14); Schwartz <sup>34</sup> (N=1)
Immunocompromised patients (>18 years-old)	To cure	Itraconazole 200 mg PO twice daily	A	III	Kenyon <sup>193</sup> Schwartz <sup>195</sup> Schwartz <sup>196</sup> Schwartz <sup>34</sup> (NNRTI-based ART should be changed to avoid drug-drug interactions. If PI in ART regimen, reduce itraconazole to 200 mg daily. Serum levels should be monitored after 1-2 weeks.)

Immunocompromised patients (>18 years-old)	To cure	Fluconazole Echinocandins	D	III	<b>Do not use fluconazole or echinocandins</b> Maphanga <sup>203</sup> Dukik <sup>52</sup> Schwartz <sup>34</sup> (high MICs in some strains)
Immunocompromised patients (>18 years-old)	To cure	12 months of triazole therapy pending immune reconstitution	B	III	Kenyon <sup>193</sup> Schwartz <sup>195</sup> Schwartz <sup>196</sup> Crombie <sup>197</sup>
Immunocompromised children (<18 years-old)	To cure	Amphotericin B (lipid formulation 3-5mg/kg/d preferred, or deoxycholate formulation 0.7-1 mg/kg/d) for 10-14d or until clinically improved followed by itraconazole for at least 12 months pending immune reconstitution	A	III	Lochan <sup>207</sup> (single case report of emeryngomycosis in child)

**Legend:** , SoR, Strength of Recommendation; QoE, Quality of Evidence; HIV, Human immunodeficiency virus; IRIS, Immune reconstitution inflammatory syndrome; TDM, Therapeutic drug monitoring

## HISTOPLASMOSIS

*Histoplasma* spp. are found in temperate and tropical zones worldwide. Transmission occurs via inhalation of conidia and may occur through a variety of activities including construction, spelunking and bird handling as nitrogen rich soil contaminated with bird or bat droppings is hypothesized to favour sporulation. However, many patients do not have an identifiable activity that predisposes them to infection. Previous guidelines for the diagnosis and treatment of histoplasmosis were published by the IDSA in the year 2000,<sup>208</sup> and updated in 2007.<sup>209</sup> In the last decade, the epidemiology of histoplasmosis has been refined, novel diagnostic methods have been developed and access to modern antifungal drugs has expanded.

### Epidemiology

*Histoplasma* species are commonly present in soil contaminated with bat guano and bird excreta. Studies using skin testing with histoplasmin have revealed that millions of people living in Latin America and the Caribbean have been exposed to the fungus.<sup>210</sup> In North America, histoplasmosis is highly endemic along the St. Lawrence, Mississippi and Ohio River basins, but microfoci exist in the mid-Eastern states and outbreaks of disease are occasionally reported as far northwest as Alberta (Appendix Figure 7).

*Histoplasma* spp. have traditionally been divided into two varieties: *H. capsulatum* var. *capsulatum* (hereafter referred to as *H. capsulatum sensu lato*) and *H. capsulatum* var. *duboisii*. Recent phylogenetic analysis suggests that as many as eleven genetically and geographically distinct clades may exist.<sup>211</sup> The acquisition of infection does not overlap in most cases, although the existence of hybrids has been documented,<sup>212</sup> and renaming of species has been suggested as follows: *H. capsulatum sensu stricto* Darling 1906 (Panama lineage), *H. mississippiense* (former NAm1), *H. ohioense* (former NAm2), *H. suramericanum* (South American histoplasmosis), and an

additional African clade has been postulated but not yet accepted as a novel species. The clinical relevance of species is noted by differential disease manifestations and mortality, with *H. suramericanum* potentially associated with higher mortality and lung pathology.

*Histoplasma capsulatum sensu lato* is found primarily along the Mississippi, Ohio, St. Lawrence Riverways and Latin America but cases have been reported in Europe, Asia and Australia as well. Although histoplasmosis is most frequently documented in the Americas, this condition is a true global disease. In a ECMM study conducted over a five-year period in Europe, 118 cases of histoplasmosis were reported<sup>213</sup> with autochthonous cases reported in Germany, Italy, and Turkey. In Africa, over 400 cases of histoplasmosis have been reported,<sup>214</sup> with the majority of cases secondary to *H. capsulatum* var. *duboisii* in West Africa. In Southern Africa, ~80% of histoplasmosis cases are due to infection with *H. capsulatum sensu lato*.<sup>214</sup> In Asia and Australia, pockets of endemicity also exist, although the frequency of infection may be underestimated due to the scarcity of epidemiological studies.<sup>215-218</sup>

### **Clinical presentation**

Histoplasmosis is acquired via the inhalation of conidia or mycelial fragments. The vast majority of those infected with *H. capsulatum* are asymptomatic; however, with high-inoculum exposure or in the immunocompromised, clinically evident infection is common. Respiratory illness is the most frequent manifestation with bronchopneumonia 1-3 weeks following exposure mimicking community-acquired pneumonia. Fever, chills, night sweats and weight loss are common symptoms of acute pulmonary histoplasmosis.<sup>219</sup> Pericarditis is an uncommon manifestation but was seen in 5-10% of cases in one series,<sup>220</sup> and erythema nodosum has also been described.<sup>221</sup> Life-threatening diffuse pulmonary histoplasmosis may develop with reticulonodular infiltrates and in some cases may produce ARDS. Patients with underlying lung disease are at risk for the

development of chronic pulmonary histoplasmosis with cough, chest pain, fatigue, dyspnoea; fibrotic infiltrates and upper lobe cavitation are the hallmarks of this form of histoplasmosis, which resembles tuberculosis.<sup>222-224</sup> Reactivation of the disease up to 50 years after the initial infection has been described, as well as transmission of infection by transplant donors to recipients.<sup>61</sup>

Broncholithiasis is an infrequent, albeit relatively unique, manifestation of histoplasmosis. Years after initial infection, calcified granulomas in lymph nodes or the pulmonary space may erode into surrounding structures causing cough, wheezing, fever, chills and/or sputum production.<sup>225</sup> Patients may expectorate small gritty material (“stones”) and broncholiths can be removed surgically or by bronchoscopy if indicated.<sup>226</sup>

Granulomatous mediastinitis occurs following acute pulmonary histoplasmosis when involved mediastinal lymph nodes coalesce forming a mass that can press on adjacent structures causing symptoms of dysphagia, cough, and wheezing. Distinct from this syndrome and much less common, fibrosing mediastinitis develops secondary to an over-exuberant fibrotic response to recent histoplasmosis.<sup>227</sup> This syndrome may result in entrapment of intrathoracic structures including the great vessels, airways, oesophagus and other contiguous structures.

Pulmonary macrophages are readily able to ingest *Histoplasma*; however, killing is inefficient and these macrophages serve to disseminate the organism haematogenously during the first 2-3 weeks of infection.<sup>228</sup> In the immunocompromised host, particularly persons with advanced HIV disease, those receiving anti-TNF- $\alpha$  therapy, and solid-organ transplant recipients, fever, fatigue, hepatosplenomegaly and pancytopenia are frequent manifestations. Sepsis can be seen in highly immunosuppressed patients with shock, hepatic and renal failure, and disseminated intravascular coagulation (DIC).<sup>229</sup> Haemophagocytosis may also be observed in some cases.<sup>230</sup>

Disseminated infection frequently involves the gastrointestinal tract (~70% of cases on autopsy) with oral (Appendix Figure 8A) or ileocecal lesions most frequent.<sup>231,232</sup> However, clinical gastrointestinal manifestations are much less common. Adrenal involvement is frequent, and adrenal insufficiency should be considered in those with disseminated infection (Appendix Figure 8B).<sup>231</sup> Skin and CNS disease are less frequent manifestations, even though Latin American patients have experienced a high frequency of skin involvement in disseminated histoplasmosis, which has been linked to a delayed diagnosis.<sup>233</sup>

Disseminated histoplasmosis may mimic tuberculosis in patients with advanced HIV disease and in some cases may co-occur;<sup>234</sup> however an increase in gamma glutamyl transferase, a reduced platelet count, evidence of dissemination and concurrent opportunistic infection all favour histoplasmosis. The presence of skin papules and oral ulcers also increase the probability of a histoplasmosis diagnosis, in comparison to other conditions in persons with advanced HIV.<sup>235,236</sup> Furthermore, in hyperendemic regions, HIV-infected persons with very low CD4 counts (<50 cells/mm<sup>3</sup>) are more likely to present with histoplasmosis, compared to tuberculosis.<sup>235,237</sup>

## **Diagnosis**

### *Culture and microscopy*

**Evidence** - The sensitivity of diagnostic tests varies by the type of clinical sample, the involved organs, and the underlying conditions. All biopsied tissues should be submitted for either PAS or GMS staining. The sensitivity of tissue examination varies by the burden of disease and is highly dependent on the degree of host immunosuppression.<sup>238</sup> Test sensitivity also varies with the type of infection with the highest rates of positive microscopy findings in those with disseminated infection.

Due to the characteristic appearance of *H. capsulatum* in clinical samples (i.e., intracellular yeast forms in phagocytes or tissue macrophages that may contain a pseudocapsule caused by cytoplasmic shrinkage) (Appendix Figure 8C), cytopathology has been widely used to diagnose the disease. In fact, these findings are sufficient to diagnose proven histoplasmosis, according to the revised EORTC/MSGERC criteria to diagnose invasive fungal diseases.<sup>239</sup> Stained (Giemsa) smears of buffy coat have limited sensitivity (~25%) but may allow for an early diagnosis of disseminated histoplasmosis.<sup>240</sup> *Histoplasma capsulatum* may resemble *Emergomyces*, *Leishmania*, *Sporothrix*, or *Talaromyces* species, and caution is required to interpret such results in areas where these infections are also endemic. Although *Leishmania* spp. are stained by Giemsa, they do not stain with GMS, allowing distinction from *Histoplasma*. *Candida glabrata* may also appear similar microscopically. Cytology may be useful in a number of clinical samples including lymph nodes, blood, bone marrow, skin, lung, and purulent secretions.<sup>241</sup> In contrast, BAL cytology was found useful for detection of *H. capsulatum* in only ~20% of cases.<sup>61</sup> When confirmation is required, a fluorescent in situ hybridization test is available in some reference laboratories.<sup>242</sup>

Conventional blood cultures have a low sensitivity (~50%) in patients with advanced HIV,<sup>243</sup> although improved performance is seen using the lysis-centrifugation method. In one prior report using this method, blood cultures were positive in 71% of patients with disseminated disease and provided the initial diagnosis in 25% of patients.<sup>244</sup> Several weeks of incubation may be required for growth. In other studies evaluating blood, BAL and bone marrow cultures, the sensitivity was found to be 50-80%.<sup>238,241,245-247</sup> In solid-organ transplant recipients, the sensitivity of sputum/BAL cultures was lower (~35%) than blood cultures (Table 11).<sup>61</sup>

On Sabouraud dextrose agar, *H. capsulatum* appears as a tan or white mold (Appendix Figure 8D). Red-brown pigment is observed on Staib agar and can be useful to differentiate *H.*

*capsulatum* from *Cryptococcus* spp. The mould phase contains two types of conidia. Macroconidia are thick walled with a diameter of 8-15 µm and display characteristic tubercles or projections on their surfaces (Appendix Figure 8E). Microconidia, smooth walled (2-4 µm), are the infectious particles.<sup>248,249</sup> Fungal dimorphism of *Histoplasma* species can be demonstrated by incubating samples at two temperatures (i.e., 25°C and 37°C). The type of medium is important for conversion to the yeast form and YPD or BHI works well for most isolates,<sup>250</sup> although other media may be required for conversion and it may take weeks for conversion to occur.<sup>251</sup> The yeast phase develops as small oval budding cells with a diameter of 2-4 µm. The yeast cells of *H. capsulatum* var. *duboisii* are thick-walled and larger (8-15 µm) in diameter. In most laboratories, conversion to the yeast phase is not needed for identification. The previously used DNA probe specific for *H. capsulatum* has been recently discontinued.

**Table 11. Conventional methods in the diagnosis of histoplasmosis.**

Population	Intention	Intervention	SoR	QoE	References and comment
Any	Diagnosis	Histopathology/Cytology	A	IIt	Hage <sup>238</sup> (sensitivity varies by type of sample, involved organ and underlying comorbidity); Mata-Essayag <sup>241</sup> (N=27, biopsy specimens sensitivity 100%; cytology sensitivity 87%); Leimann <sup>247</sup> (N=38, sensitivity 63%)
Any	Diagnosis	Culture (lysis-centrifugation method)	A	IIt	Paya <sup>244</sup> (N=28, Blood cultures positive in 71% and provided initial diagnosis in 25%).

Any	Diagnosis	Culture	A	IIt	Lopez <sup>246</sup> (N=25 – culture 80% sensitivity); Mata-Essayag <sup>241</sup> (N=70, culture of aspirates, CSF, pathologic samples sensitivity=23%); Leimann <sup>247</sup> (N=59, culture sensitivity of tissue and CSF=70%)  Hage <sup>238</sup> (N=48, 71% sensitivity in AIDS; N=75, 76% sensitivity in non-HIV immunocompromised; N=9, 78% sensitivity in non-immunocompromised)
AIDS	Diagnosis	Blood culture	A	IIt	Dantas <sup>243</sup> (N=12, blood culture sensitivity in AIDS=50%)
Solid organ and haematopoietic cell transplant recipients	Diagnosis	Culture	A	IIt	Kauffman <sup>61</sup> (Sensitivity: sputum/BAL (35%), lung biopsy (8%), blood cultures: 50%); Assi <sup>252</sup> (sensitivity: in patients with pulmonary and disseminated histoplasmosis was 17/29 (59%) 87/118 (74%), respectively); Cuellar-Rodriguez <sup>245</sup> (Specimens: blood, BAL, bone marrow, sensitivity: 7/14 (50%)).
Solid organ transplant recipients	Diagnosis	Histopathology	A	IIt	Cuellar-Rodriguez <sup>245</sup> (Tissue, sensitivity: 7/14 (50%); Kauffman <sup>61</sup> (Cytological examination of BAL was more useful for <i>H. capsulatum</i> [20% of cases]. Histopathology showed 2-10% sensitivity based on different specimens; Assi <sup>252</sup> (Specimens: Lung, respiratory, sensitivity: in patients with pulmonary and disseminated histoplasmosis was 20/24 (83%) and 65/83 (78%), respectively).

**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence

### Serology

**Evidence** – The immunodiffusion and complement fixation tests are the most widely used diagnostic tests to detect antibodies against *Histoplasma* spp. Two precipitin lines (H and M bands) can be seen with this test. The M band appears early in acute histoplasmosis and is observed in most patients with histoplasmosis, but may be positive from prior infection. Conversely, the H band appears later, is present in only ~20% of cases (primarily disseminated infection, chronic cavitary disease or severe acute pulmonary histoplasmosis) and indicates active disease.<sup>253,254</sup> Patients with acute histoplasmosis may be negative by immunodiffusion as antibodies have not yet been produced (Table 12).

Complement fixation testing is slightly more sensitive but less specific than immunodiffusion.<sup>255</sup> Titers  $\geq 1:32$  suggest acute infection although titers of 1:8 or 1:16 may also be seen in those with active disease. Positive results may, however, represent prior infection, or represent cross-reactivity with coccidioidomycosis or blastomycosis.

Serologic testing is most useful for patients with chronic pulmonary histoplasmosis and may not be helpful in those with severe immunosuppression. For instance, immunodiffusion detected *Histoplasma* antibodies in only 20/35 (57.1%) AIDS patients with culture-confirmed histoplasmosis.<sup>247</sup> Specificity ranges from 70-100%.<sup>256</sup> Others have observed similar results with immunodiffusion positive in only 25% of cases (n=20) although specificity was 100% (10 controls).<sup>243</sup> Alternative methods to detect *Histoplasma* antibodies include a newly developed EIA method that has shown reduced sensitivity compared to the complement fixation test, and a newer EIA for both IgM and IgG antibodies that may be more sensitive.<sup>256-258</sup>

**Table 12. Role of serology in the diagnosis of histoplasmosis.**

Popula- tion	Intention	Intervent ion	SoR	QoE	References and comment
Immu- nocom- petent	Diagnosis	Serology	B	Iiu	Wheat <sup>255</sup> (N=276, CF testing sensitivity 95%, ID 80% - less than 1 percent of residents in endemic areas are seropositive by ID or CF and background seropositivity is thus not a major limitation). Picardi <sup>253</sup> (ID has greater specificity than CF, most patients develop M band and H band seen in only 20%); Richer <sup>258</sup> (EIA has reduced sensitivity compared to CF testing)
AIDS	Diagnosis	Immuno- diffusion	D	Iiu	Dantas <sup>243</sup> (N=20, ID detected only 25% of cases); Lei- mann <sup>247</sup> (N=35, sensitivity 57.1%, specificity 70- 100%).

AIDS	Diagnosis	ELISA	D	III	Torres <sup>259</sup> (N=3); Guimaraes <sup>257</sup> (N=14, sensitivity 85.7%).
AIDS	Diagnosis	Complement fixation	D	III	Torres <sup>259</sup> (N=3); Falci <sup>256</sup> (specificity ranges between 70-80%)

**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence; ELISA, Enzyme-Linked Immunosorbent Assay; ID, immunodiffusion; CF, Complement Fixation

### *Antigen detection*

**Evidence** – The detection of *Histoplasma* antigen in urine or serum is helpful in making a rapid diagnosis of probable histoplasmosis. Antigen assays are most useful in patients who have disseminated histoplasmosis and acute pulmonary histoplasmosis, but are less useful in localized pulmonary infection and chronic cavitary pulmonary histoplasmosis (Table 13).

The initial *Histoplasma* antigen test exhibited a sensitivity of 90% (MiraVista), and test specificity has since improved with methodologic refinement.<sup>260,261</sup> More recently IMMY developed an EIA *Histoplasma* antigen test with a 98% concordance with the MiraVista test in one study,<sup>262</sup> but a poor correlation was observed in a separately conducted study.<sup>263</sup> Antigen testing has recently been evaluated by a lateral flow device and found to have a high sensitivity and specificity.<sup>264</sup>

One of the major limitations with use of the MiraVista tests is that it is not available outside the U.S. The Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) also developed a polyclonal antigen-capture enzyme-linked immunosorbent assay that had 81% sensitivity and 95% specificity, in a cohort of culture-proven histoplasmosis cases but this test is no longer available (since there were already companies producing *Histoplasma* antigen in the market).<sup>265</sup> A

systematic review recently compared the CDC and IMMY tests and found the latter test to be superior with a sensitivity of 98% and specificity of 97%.<sup>266</sup> Overall the recent IMMY monoclonal test performed better than the CDC test in a similar Latin American context.<sup>266</sup> The IMMY monoclonal antigen detection sensitivity was 98% and specificity 97%.<sup>267</sup> The IMMY test has the advantage of being commercially available, although it is not registered in many countries in which histoplasmosis is endemic. *Histoplasma* antigen testing has also been evaluated in serum samples and in view of the elevated degree of concordance between urine and serum (and considering the facility to obtain urine samples), some feel that concomitant testing of serum and urine appears redundant.<sup>268</sup>

The *Aspergillus* galactomannan EIA cross-reacts with *Histoplasma* antigen in patients who have a high burden of organisms and in some locations the test has been used to diagnose histoplasmosis, but the low sensitivity of ~12% for histoplasmosis makes this assay less useful.<sup>256,269</sup>

**Table 13. Performance of *Histoplasma* antigen tests in patients with disseminated histoplasmosis.**

Population	Intention	Intervention	SoR	QoE	References and comment
AIDS	Diagnosis	Antigen detection (ELISA)	A	Ilu	Wheat <sup>260</sup> (N=61, MiraVista test sensitivity 90% in urine samples); Wheat <sup>261</sup> ; Theel <sup>262</sup> (high agreement between MiraVista and the commercially-available IMMY test [97.6%]); Libert <sup>268</sup> (agreement between antigen detection in urine and serum ~98%)
AIDS	Diagnosis	<i>Aspergillus</i> Platelia Galactomannan ELISA	D	Ilu	Falci <sup>256</sup> (cross-reaction occurs between <i>Aspergillus</i> and <i>Histoplasma</i> in the <i>Aspergillus</i> Platelia GM test. Test sensitivity in serum was 12.5% only (n=78).

**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence; ELISA, Enzyme-linked immunosorbent assay; GM, Galactomannan; IMMY, Immuno-Mycological Inc. (Norman, OK, USA); MiraVista (Indianapolis, IN, USA).

## **Molecular methods**

**Evidence** – Molecular testing in blood samples to diagnose histoplasmosis is based on PCR amplification targeting the Hc100 or ITS regions (SoR B, QoE IIu). The test sensitivity is variable and whole blood is preferred (54-70%, depending on target) to sera (18-65%), likely secondary to the intracellular infection of *Histoplasma*.<sup>270</sup> Targeting the ITS region (65-70%) rather than Hc100 (18-54%) improves sensitivity.<sup>243</sup> Specificity ranges from 80-100%.<sup>271,272</sup> PCR can also be performed with DNA from paraffin embedded tissues and other samples with good sensitivity (up to 100%).<sup>212,271,273,274</sup>

### *Susceptibility testing*

**Evidence** – Guidelines for the *in vitro* susceptibility testing of *Histoplasma* spp. do not exist although *in vitro* susceptibility testing has been proposed to guide treatment, particularly in the HIV population.<sup>275</sup> In a study involving 138 isolates of *H. capsulatum* in its filamentous form, the proposed epidemiological cut-offs value (ECV) were (µg/ml): amphotericin B 0.5; itraconazole 0.03; fluconazole 128; voriconazole 0.5; and caspofungin 16.<sup>275</sup> Lower minimum inhibitory concentrations were observed for the yeast forms (n=20) of *H. capsulatum*, in comparison to mycelia. Echinocandins usually demonstrate a poor activity against *H. capsulatum*, especially in the yeast form.<sup>238,276</sup> There is not clear evidence MIC testing is helpful to guide therapy during the treatment of histoplasmosis.

## ***Imaging***

**Evidence** – Radiographic manifestations of disease vary significantly by the severity of exposure and underlying immune status of the host. Computed tomography (CT) is preferred over chest X-ray, and may reveal diffuse infiltrates, nodules or micronodules, opacities, cavities, mediastinal mass and lymph node enlargement.<sup>277</sup> Nodules may be difficult to distinguish from malignancy if no prior films are available. In cases of disseminated disease, abdominal CT may show diffuse lymph node enlargement, hepatosplenomegaly, gastrointestinal mass, adrenal involvement, or bowel wall thickening.<sup>278</sup> Histoplasmosis involving the CNS may appear as mass lesions, ventricular enlargement, or areas of T2/flair or infarcts, or meningeal enhancement on MRI examination. One review noted that 28% of patients had no abnormalities detected on MRI.<sup>279</sup>

**Histoplasmosis Diagnostic Recommendations** – Whenever possible, tissue should be obtained for the histopathological diagnosis of histoplasmosis, using fungal stains (i.e., silver or PAS staining) and fungal culture (SoR A, QoE II). Due to the typical appearance of *Histoplasma* species in biological fluids, cytology should always be examined in the evaluation for possible histoplasmosis. This procedure is cheap and easy to perform (SoR A, QoE II). Patients with suspected disseminated histoplasmosis should have blood cultures performed, preferably using lysis-centrifugation methods (SoR A, QoE II), bearing in mind that *H. capsulatum* may take 2-5 weeks to grow. Buffy coat may also be used for culture, and these samples may reveal *H. capsulatum* by direct microscopy when viewed by experienced technicians. All biopsy samples from patients with suspected histoplasmosis should be sent for fungal cultures (SoR A, QoE II). Additional samples that should be considered for fungal culture include sputum, bronchoalveolar lavage, and bone marrow aspirates. Most clinical labs identify isolates using a DNA probe, although in some locations the conversion from mycelial phase to yeast is still performed.

Antifungal susceptibility testing for histoplasmosis is not routinely recommended, since data on correlation with antifungal treatment response are lacking.

Antibody detection is not recommended for patients who are highly immunocompromised (SoR D, QoE III). These tests should be used primarily for patients with pulmonary histoplasmosis (SoR C, QoE). *Histoplasma* antigen detection in urine and serum samples is the preferred method to diagnose disseminated histoplasmosis, particularly in AIDS patients (SoR A, QoE Iir). PCR may be useful for countries in which *Histoplasma* antigen detection is not available.

Chest, abdominal and CNS imaging should be performed according to the clinical scenario. Imaging tests are useful to guide further investigation, including bronchoscopy and biopsies (SoR A, QoE Iiu).

### ***Treatment rationale and recommendations***

**Evidence** – In a randomized clinical trial, L-AmB at 3 mg/kg daily was shown to provide a survival benefit in comparison with AmB-d (Table 14), in advanced HIV patients with disseminated histoplasmosis.<sup>280</sup> Itraconazole is highly active against *H. capsulatum*, with success rates as high as 85% in AIDS patients as step-down therapy.<sup>65</sup> Patients on long-term therapy with itraconazole benefit from TDM, as itraconazole absorption is often erratic.<sup>281</sup> In those with less severe diseases, fluconazole has a lower success rate than itraconazole (SoR C, QoE III),<sup>282,283</sup> and emergence of fluconazole resistance has been reported in patients on therapy.<sup>284</sup> Voriconazole is not routinely recommended.<sup>285,286</sup> Posaconazole has been used successfully as step-down therapy and is recommended in select cases (SoR B, QoE III).<sup>283,287</sup>

After successful induction therapy during treatment of disseminated infection, itraconazole 200 mg twice daily is usually given for at least one year (SoR A, QoE Iiu).<sup>209</sup> A study using

itraconazole for secondary prophylaxis for 12 weeks in persons with advanced HIV showed a 95% relapse free rate (SoR A, QoE IIu).<sup>288</sup>

**Histoplasmosis Treatment Recommendations** – Liposomal amphotericin B is the drug of choice for induction therapy for advanced HIV patients with moderate to severe histoplasmosis (SoR A, QoE I). Other amphotericin B formulations are acceptable alternatives when L-AmB is not available (SoR C, QoE III). Itraconazole is an alternative induction therapy for patients with less severe infection. In many countries in which histoplasmosis is endemic, itraconazole solution (which is better absorbed than itraconazole capsules) is not available. TDM is recommended when itraconazole is prescribed (SoR A, QoE III). SUBA-itraconazole is a new formulation with enhanced bioavailability, and there are no significant food or gastric acid effects on absorption.<sup>289</sup> Fluconazole and voriconazole are not recommended in the treatment of histoplasmosis (itraconazole is preferred) due to their higher failure rate compared to other options (SoR D, QoE III).

Induction therapy with an amphotericin B formulation should be followed by oral therapy with itraconazole (SoR A, QoE I). HIV-infected patients with resolution of clinical symptoms and immune reconstitution may have therapy discontinued after 12 months. Some physicians would discontinue secondary prophylaxis earlier, at the time the patient has received at least 3-6 months of ART, negative results of fungal cultures, negative urine *Histoplasma* antigen levels, and a sustained CD<sup>4+</sup> T-cell count of >150 cells/ $\mu$ L (SoR A, QoE IIu). Life-long suppressive therapy may be needed in HIV-infected patients who do not manifest immune recovery despite ART. HIV-infected children should be treated in similar fashion as adults.

Histoplasmosis secondary to TNF- $\alpha$  inhibitor therapy may require discontinuation of the TNF- $\alpha$  blocker during antifungal therapy. Following a clinical response to treatment,

pharmacologic immunosuppression may be reinstated if antifungal treatment is administered for ~12 months and the *Histoplasma* antigen has been negative.<sup>290</sup>

Antifungal treatment in the non-immunosuppressed population is suggested for at least six months, although the severity and site of disease need to be taken into account before determining the optimal duration of therapy.

**Table 14. Recommendations for treatment of histoplasmosis.**

Population	Intention	Intervention	SoR	QoE	References and comment
HIV+, moderate to severe histoplasmosis	Induction	L-AMB vs AmB-d	A	I	Johnson <sup>280</sup> (N=81, superior mortality outcome with L-AmB, clinical success in 88% vs 64%)
Culture or histopathology proven histoplasmosis	To treat	Itraconazole	B	Iiu	Dismukes <sup>65</sup> (N=37, ~85% success rate; 29% of patients with side effects)
AIDS	Efficacy of itraconazole	Itraconazole	A	Iiu	Wheat <sup>291</sup> (N=59, ~85% success rate)
AIDS patients with histoplasmosis (post 12 weeks itraconazole induction therapy)	Secondary prophylaxis	Itraconazole	A	Iiu	Hecht <sup>288</sup> (N=46, 95% relapse free)

Legend: AmB-d, amphotericin B deoxycholate; L-AmB, Liposomal amphotericin B; HIV, human immunodeficiency virus

**Table 15. Duration of therapy for patients with disseminated histoplasmosis.**

Population	Intention	Intervention	SoR	QoE	References and comment
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AIDS patients (disseminated disease)	To cure	Induction therapy with L-AmB 3-5 mg/kg daily for 1-2 weeks	A	I	Johnson <sup>280</sup> (induction therapy should be followed by long-term oral therapy with itraconazole. L-AmB has survival benefit over AmB-d)
AIDS patients (disseminated disease)	To cure	Induction therapy with AmB-d (0.7–1.0 mg/kg daily) for 1-2 weeks	C	III	Johnson <sup>280</sup> Wheat <sup>209</sup> (alternative to L-AmB for resource-poor healthcare settings. Induction therapy should be followed by oral treatment with itraconazole)
AIDS patients (disseminated disease)	To cure	Itraconazole 200 mg twice daily for at least 12 months	A	IIu	Goldman <sup>292</sup> (N=32, after induction therapy, oral treatment should be given for at least 1 year)

**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence; AmB-d, amphotericin B deoxycholate; AIDS,

Acquired immunodeficiency syndrome; L-AmB, Liposomal amphotericin B.

## PARACOCCIDIOIDOMYCOSIS

Paracoccidioidomycosis (PCM) is caused by the endemic fungi *Paracoccidioides brasiliensis* and the newly recognized species *P. lutzii*, *P. americana*, *P. restrepiensis*, and *P. venezuelensis*.<sup>293,294</sup>

The Brazilian guidelines on PCM were originally published in 2006 and updated in 2017.<sup>295,296</sup>

Continued advances in the ecology, taxonomy and diagnosis of *Paracoccidioides* have since occurred, although randomized comparative antifungal studies evaluating the efficacy of treatment have yet to be performed.

### Epidemiology

*Paracoccidioides* species are a soil inhabiting fungus although our understanding of their precise environmental habitat remains limited. The primary infection usually occurs during the first two decades of life in individuals living within the endemic regions of Latin America and who have diverse activities related to the management of soil or soil products. This systemic mycosis occurs in the subtropical humid areas of most of the Latin America countries, with higher incidence in Argentina, Brazil, Colombia, Ecuador, and Venezuela. Infection has been rarely observed in Grenada, Guadeloupe, and Trinidad (Appendix Figure 9). Thus far, no cases have been reported in Chile or Nicaragua. The prevalence of this infection is known to vary greatly between different endemic regions. However, skin testing has revealed up to 50-75% of those within an endemic region have been infected.<sup>297</sup> The incidence of symptomatic infection ranges from one to three cases per 100,000 inhabitants in endemic areas although in hyperendemic regions of Brazil the incidence may range from 9-40 cases/100,000.<sup>298</sup> The acute/subacute clinical forms, representing 10% of the clinical cases, are prevalent in children and adolescents, affecting both genders equally. The chronic form is prevalent in adults with a male to female ratio of 20:1 and this difference may be secondary to oestrogen's inhibition of mycelial to yeast conversion.<sup>299</sup> Non-autochthonous

cases have been observed in Asia, some European countries, and the U.S.<sup>300</sup> All affected individuals came from endemic Latin American areas and clinical manifestations occurred years to decades after visiting the endemic areas. Therefore, PCM should be also regarded as a disease of travelers who have lived in endemic areas for extended times.

### **Clinical Presentation**

The vast majority of patients exposed to *Paracoccidioides* spp. do not manifest symptoms of infection. Approximately 5% of patients develop symptoms of disease that may evolve into one of two patterns: the acute/subacute form, or the chronic form which represents reactivation of the primary infection.<sup>297</sup>

The acute/subacute form is also known as juvenile PCM as it is more frequent in children, adolescents and adults <30 years of age (~10% of all cases).<sup>300</sup> This form does not exhibit an epidemiologic difference between males and females (since these are prepubertal patients).<sup>301</sup> As stated earlier, the chronic form of infection is much more frequent in men.

Symptoms such as fever, chills and weight loss are common in cases of disseminated infection (Appendix Figure 10A and B). In these cases, involvement of the reticuloendothelial system with lymphadenopathy (cervical, axillary, or inguinal) and hepatosplenomegaly is observed and in some cases bone marrow dysfunction with severe anemia is seen. Osseous and cutaneous involvement may be seen in some cases.<sup>300</sup>

Similar to other systemic endemic mycoses, the chronic manifestations result from the reactivation of pulmonary latent foci. In chronic cases, pulmonary infiltrates and/or upper respiratory mucosal lesions are frequently seen. Lung involvement may present as a dry cough, sputum production, or haemoptysis. Extensive lung disease with fibrosis at the time of initial presentation can be seen (Appendix Figure 10C). Mucosal disease is seen in >50% of case with the mouth and

larynx the most frequent sites of infection.<sup>302-304</sup> A painful ulcer with ragged borders and small areas of haemorrhage is typically observed.<sup>305</sup> Hematologic, lymphatic or contiguous dissemination may occur with infection resulting in the skin, lymph nodes or adrenal glands, which may cause adrenal insufficiency. The CNS may also be involved.<sup>306</sup> The frequency of neuroinvolvement in this disease may range from 1 to 27%.<sup>307</sup> In a series of 173 PCM cases, 14% of the patients presented neuroparacoccidioidomycosis. The genital and osteoarticular systems may be also less frequently involved. (Appendix Figure 10D).<sup>308-311</sup>

Laboratory manifestations of disease include anemia, hypergammaglobulinemia, eosinophilia, hypoalbuminemia, and hyperbilirubinemia. Mild transaminase elevation is also found.<sup>301</sup>

## **Diagnosis**

### *Culture and microscopy*

**Evidence** – Microscopy enables a proven diagnosis of PCM to be made (SoR A, QoE IIu). Rounded thick-walled yeast cells (diameter 15-30  $\mu\text{m}$  – up to 60  $\mu\text{m}$  in some cases) with multiple buds (“ship’s wheel”, “pilot wheel” or “Mickey Mouse” cells) (Appendix Figure 10E) are diagnostic features and are frequently seen in lymph node aspirates and/or affected tissues, but fungal elements are less common in bronchoalveolar lavage and sputum samples (Table 16). Other forms including chains, single buds and elongated forms require mycologic expertise for diagnosis when these forms are observed.<sup>300</sup>

Cultures should be inoculated and incubated at 25-30°C and also at 35-37 °C, for 4-8 weeks although may be negative depending on the site and burden of infection (Appendix Figure 10F and 10G). Incubation at two temperatures (i.e., 25°C and 37°C) is recommended to demonstrate fungal dimorphism.<sup>300,312,313</sup> In countries in which PCM is not endemic, all suspected cultures should be safely dispatched to a reference laboratory for confirmation of identification. The diagnosis of

PCM can also be made based on the microscopic visualization of fungal elements consistent with *Paracoccidioides* spp. in tissue samples.

Samples are most frequently obtained in patients with PCM from mucosal tissue, skin, lung, lymph nodes, and respiratory secretions. Tissue from any suspected site of involvement should be examined using standard fungal stains such as H&E, PAS and GMS. Immunohistochemistry can also be performed but no commercial assays are available.<sup>314,315</sup> The tissue reaction is similar to those exhibited by other systemic mycoses: granulomatous or mixed granulomatous and suppurative inflammatory infiltrate with yeast elements and best observed with H&E staining.<sup>296</sup>

**Table 16. Conventional methods in the diagnosis of paracoccidioidomycosis.**

Popula- tion	Intention	Interventio n	SoR	QoE	References and comment
Any	Diagnosis	Direct microscopy	A	Iu	Brummer <sup>300</sup> (Optical brighteners preferred)
Any	Diagnosis	Culture	A	III	Restrepo <sup>312</sup> Teles <sup>313</sup> (Samples should be cultured for 4-8 weeks)
Any	Diagnosis	Histopathol- ogy	A	III	Guarner <sup>314</sup> Brummer <sup>300</sup> Schelenz <sup>316</sup> (yeast cell diameter 15-30 μm [up to 60 μm in some cases] - GMS staining most sensitive).
Any	Diagnosis	Immuno-his- tochemistry	C	Iu	de Freitas <sup>315</sup> Guarner <sup>314</sup> In-house, no commercial assay available.

**Legend:** , SoR, Strength of Recommendation; QoE, Quality of Evidence; GMS, Gomori methenamine-silver staining

### *Serology*

**Evidence** – The majority of patients are diagnosed using non-invasive testing such as serology. Immunodiffusion is the most widely used reference assay (IMMY, Norman, OK, USA).<sup>295,317,318</sup> This assay is inexpensive, and has a high specificity (>95%), and sensitivity (~80%) although may

not be widely available in all countries. Additional methods have been used to detect antibodies, including counter immunoelectrophoresis (similar performance to immunodiffusion), ELISA (more sensitive, less specific than immunodiffusion), complement fixation (also more sensitive, less specific than immunodiffusion), and Western Blot (more sensitive and specific than immunodiffusion),<sup>317,318</sup> and in house testing has been developed by a number of centers.<sup>319</sup> Quantitative antibody titres are higher in patients with acute and more severe disease forms. Table 17 summarizes the guidelines for the use of antibody detection in the diagnosis of PCM. Serology can also be used to assess the response to treatment with a reduction in titre a favorable sign.<sup>295</sup>

**Table 17. Antibody and antigen detection in patients with paracoccidioidomycosis.**

Popula-tion	Intention	Intervention	SoR	QoE	References and comment
Any	Diagno-sis	Immunodiffu-sion	A	Iir	Shikanai-Yasuda <sup>295</sup> Perenha-Viana <sup>317</sup> de Camargo <sup>318</sup> (High specificity (>95%), sensitivity ~80%)
Any	Diagno-sis	Counter im-muno electro-phoresis	A	Iir	Shikanai-Yasuda <sup>295</sup> de Camargo <sup>318</sup> (similar performance characteristics to ID)
Any	Diagno-sis	ELISA	A	Iir	de Camargo <sup>318</sup> (more sensitive, less specific than ID)
Any	Diagno-sis	CF	B	Iiu	de Camargo <sup>318</sup> (more sensitive, less specific than ID)
Any	Diagno-sis	WB	B	Iir	Perenha-Viana <sup>317</sup> (more sensitive and specific than ID - allows detection of specific antibodies - not widely available)
Any	Diagno-sis	Antigen detec-tion	B	Iiu	de Camargo <sup>318</sup> Gomez <sup>320</sup> Marques da Silva <sup>321</sup> Marques da Silva <sup>322</sup> (87Kd, gp43 and gp70 assays give similar sensitivity and specificity. Useful in immunocompromised patients. Not widely available)

Any	To monitor treatment	Antigen detection	C	Iiu	Shikanai-Yasuda <sup>295</sup> de Camargo <sup>318</sup> (May be of some value in determining end of treatment along with clinical features).
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**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence; CF, Complement- Fixation; ELISA, Enzyme-linked immunosorbent assay; ID, immunodiffusion; WB, Western blot.

### *Antigen detection*

**Evidence** – Assays detecting 87Kd, gp43 and gp70 give similar sensitivity and specificity. However, gp43 does not appear to be detected in infections caused by *P. lutzii*.<sup>323</sup> Antigen detection has the potential to be useful in immunocompromised patients, and also to diagnose neuroPCM. The detection of of gp43 and gp70 was found in 10 of 11 patients with neurologic involvement.<sup>324</sup> However antigen detection for the diagnosis of PCM is not commercially available.<sup>318,320,322</sup>

### *Molecular methods*

**Evidence** – Several in-house conventional PCR tests have been developed, targeting gp43 or ITS regions.<sup>46,325,326</sup> Sensitivity varies from 91-100%, in samples that include sputum, BAL, and tissue biopsies (SoR B, QoE Iiu). Reduced sensitivity has been demonstrated for serum samples.<sup>327</sup> In-house qPCR tests have also been developed, targeting ITS or Pb27. Sensitivity is generally higher (94-100%) and includes blood and biopsy samples.<sup>328,329</sup> In-house molecular tests based on loop mediated isothermal amplification (LAMP) have also been developed, targeting gp43. Sensitivity is 61% (11/18) in sputum samples.<sup>330</sup>

Molecular identification of *P. brasiliensis (sensu lato)* can be performed with PCR amplification of the ITS region, followed by DNA sequencing and Taqman probe targeting gp43 or LAMP method (SoR B, QoE Iiu).<sup>331-333</sup> The differentiation between *P. brasiliensis* and *P. lutzii* can be determined by DNA sequencing (gp43 and HSp 70), probes targeting ITS, and by their MALDI-TOF protein profile.<sup>334,335</sup>

### *Susceptibility testing*

**Evidence** – A limited number of studies have evaluated the susceptibility of *Paracoccidioides* species to antifungal drugs.<sup>336-339</sup> Studies have generally included only a small number of isolates and there are currently no standardized methods nor consensus breakpoints. Highly active drugs include amphotericin B and itraconazole. Higher MICs were observed for fluconazole. Echinocandins have little *in vitro* activity against *Paracoccidioides* species. Collaborative efforts are ongoing to determine optimal methodologies for *Paracoccidioides* susceptibility testing.

### **Imaging**

**Evidence** – Image findings from X-rays, CT and MRI are non-specific to diagnose this disease (SoR A, QoE III).<sup>307,340,341</sup> “Butterfly or bat wing” images observed in conventional radiology are very suggestive, as are pleural plaques.<sup>342,343</sup> In the acute/subacute clinical forms pulmonary images mainly show lymphatic involvement. Cavitory lung disease and/or fibrosis may be a late manifestation of PCM. The reverse halo sign may also be seen.<sup>344</sup> Neuro images, like CT, MRI with contrast and spectroscopy are frequent but non-specific in PCM patients.<sup>306</sup> Single lesions can be found in 47% of the patients, two image lesions in 23% and multiple lesions in 30% of a case series.<sup>341</sup> Pseudotumoral lesions, consisting of granulomas can be detected by radiological images in the cerebral hemispheres in 67%, cerebellum and brainstem in 25% and spinal cord, in 4%, respectively.<sup>343</sup>

### **Paracoccidioidomycosis Diagnostic Recommendations**

Due to the characteristic appearances of *Paracoccidioides* species in clinical samples, microscopy has an important role in the diagnosis of PCM (SoR A, QoE IIu). Microscopy should preferably be performed using optical brighteners. Fungal culture is also important in the diagnosis of PCM,

and clinical specimens including sputum, BAL and tissue (mostly lymph nodes and skin/mucosal lesions) should be submitted (SoR A, QoE III). Physicians should be aware that *Paracoccidioides* may take 2-8 weeks to grow in standard fungal culture media. Histopathology may reveal typical fungal morphology, usually with a background of granulomatous inflammation, and may mimic malignancy and tuberculosis (SoR A, QoE III).

PCM is mainly a chronic condition and antibodies can be detected in the vast majority of infected patients. However, accuracy of serologic assays for the diagnosis of PCM are dependent on the quality and performance of the antigen preparation used. It is recommended that serology only be performed by reference laboratories, using reagents with known and published performance characteristics (SoR A, QoE IIr). Mixed antigen preparations are recommended in order to improve the detection of *P. lutzii* infection. Antigen detection in PCM is not yet applicable to clinical practice, due to the lack of standardized or commercially-available tests. Molecular based tests are useful particularly in countries where PCM is not endemic. The differentiation between the two species, *P. brasiliensis* and *P. lutzii* is usually performed with molecular tests although infrequently needed. MALDI-TOF is promising, but (in-house) databases are limited. Antifungal susceptibility tests for *Paracoccidioides* species are not yet standardized and should not be used in clinical practice.

## **Treatment**

**Evidence** – Itraconazole has largely been used for patients with mild to moderate clinical forms of the disease (Table 18).<sup>345-347</sup> Itraconazole has been shown in a single-center noncomparative study to exhibit an efficacy rate of 91% (median duration of treatment six months).<sup>346</sup> Itraconazole has many drug-drug interactions and requires TDM. Comparison of itraconazole to trimethoprim/sulfamethoxazole (TMP-SMX) has found itraconazole to be superior (86.4% success rate

vs 51.3%).<sup>348</sup> Another study showed a significantly shorter time to serologic cure in the itraconazole group compared with the TMP/SMX group (105 vs 159 days).<sup>347</sup> Voriconazole 6 mg/kg/day for 6-12 months is similarly efficacious in PCM (SoR A, QoE III) and is useful in cases with CNS involvement.<sup>345</sup> TMP/SMX 160 mg/800 mg daily has a fungistatic effect; however a long duration of treatment (18-24 months) is required.<sup>296</sup> AmB-d at 0.7-1 mg/kg daily for 2-4 weeks is indicated for severe and disseminated clinical forms of the disease. Induction therapy with L-AmB is equally effective, and it is associated with fewer side effects than AmB-d.<sup>349,350</sup> Amphotericin B is also recommended for immunocompromised patients (i.e., advanced HIV disease). After induction therapy with an AmB formulation, maintenance treatment with an azole derivative or TMP/SMX is required. Intravenous cotrimoxazole is also useful for children with disseminated disease.<sup>296</sup>

Posaconazole (300 mg daily using delayed release tablets) voriconazole (400 mg daily), and isavuconazole (400 mg daily) may be useful as salvage therapy for 6-12 months.<sup>345,351</sup> Only a few patients were included in studies evaluating these medications and additional data are needed.

PCM has been associated with tuberculosis in 2-20% of the cases.<sup>296,342,352</sup> Caution should be taken due to significant drug-drug interactions with rifamycins and triazoles when concurrent treatment is required.

**Table 18. First-line options for primary treatment of paracoccidioidomycosis.**

Population	Intention	Intervention	SoR	QoE	References and comment
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Adults	To cure	Itraconazole, 200 mg /day/9- 12 mo	A	I	Queiroz-Telles <sup>345</sup> (N=18, 94% satisfactory response); Naranjo <sup>346</sup> (N=47, success in 91%); Cavalcante <sup>347</sup> (N=117, quasi-experimental study itraconazole vs TMP/SMX – itraconazole with shorter time to serologic cure); Borges <sup>348</sup> (N=200, itraconazole [86%] superior to TMP/SMX [51%]); Shikanai-Yasuda <sup>353</sup> (N=42, compared itraconazole, ketoconazole, sulfadiazine)  Itraconazole may present several drug-drug interactions and unpredictable serum drug levels
All	To cure	Voriconazole 400 mg/day/06-12 mo	A	I	Queiroz-Telles <sup>345</sup> (N=35, 89% satisfactory response). Potential use in neuroparacoccidioidomycosis
All	To cure	TMP/SMX 160 mg/800 mg/day	B	Ilu	Cavalcante <sup>347</sup> (N=117, quasi-experimental study itraconazole vs TMP/SMX – Fungistatic effect. Long duration of TMP/SMX treatment is required)
Adults and children	Induction therapy	AmB-d 0.7-1mg/kg/2- 4 weeks	A	III	Dillon <sup>354</sup> de Campos <sup>355</sup> (N=47) (AmB-d is indicated for severe and disseminated disease and in immunocompromised)
Adults and children	Induction therapy	ABLC at 3-5 mg/kg/day/02- 04 weeks	B	Ilu	Pecanha <sup>349</sup> (N=28, 100% cure rate)  L-AmB is alternative
For severe clinical forms  (children)	To cure	Intravenous TMP/SMX	B	III	Shikanai-Yasuda <sup>296</sup> (This is particularly useful for children with disseminated disease if amphotericin B formulations not available)

**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence; AmB-d, Amphotericin B deoxycholate; ABLC, Amphotericin B Lipid complex; L-AmB, liposomal amphotericin B; TMP/SMX, trimethoprim/sulfamethoxazole

**Paracoccidioidomycosis Treatment Recommendations** – Itraconazole 200 mg/daily for 9-12 months is the therapy of choice for patients with mild to moderate forms of PCM (SoR A, QoE I), with TMP/SMX (for 18-24 months) being the main therapeutic alternative to itraconazole (SoR B, QoE Ilu) (Table 19). A short (2-4 weeks) induction therapy with amphotericin B is reserved for

severe cases (SoR A, QoE III), or in immunocompromised patients. Induction therapy with amphotericin B should be followed by 400-600 mg of itraconazole (SoR A, QoE III). For neuro PCM, long durations of therapy with TMP/SMX are suggested (eg 2-5 years). Cotrimoxazole at the daily dose of 8-10 mg/kg, is also useful for the acute/subacute clinical form, especially in young children due to its tolerability, intravenous and oral solution presentation. TMP/SMX may be useful especially with coincident *Pneumocystis* infection. For patients with AIDS associated infection, care must be taken in view of itraconazole interactions with antiretroviral therapy. If possible, therapy of PCM should be started before antiretrovirals in AIDS patients, to avoid IRIS. Patients with severe PCM who are at an increased risk for kidney toxicity may benefit from induction therapy with L-AmB. Some patients with severe clinical forms presenting intense inflammatory response may receive corticosteroids associated to antifungal therapy. The use of corticosteroids are indicated in the presence of severe pneumonia with respiratory insufficiency, extensive tracheal involvement, and mechanic compression by ganglia and neurological mass effect. Itraconazole and TMP/SMX may have teratogenic effects and use during pregnancy must be discussed with the patient regarding potential risks; amphotericin B is the preferred drug during pregnancy (SoR A, QoE III).<sup>296,356</sup> In cases refractory or intolerant to these agents posaconazole (SoR B, QoE III), voriconazole (SoR B, QoE I), or isavuconazole (SoR B, QoE III) can be used as salvage therapy for 6-12 months.

## SPOROTRICHOSIS

Sporotrichosis is a subacute to chronic infection caused by the dimorphic saprotrophic fungal genus *Sporothrix* of which only a few species are known to infect humans and animals.<sup>357</sup> These organisms are found globally in temperate to tropical climates (Appendix Figure 11). The initial IDSA guidelines for *Sporothrix*-infections were written in 2000<sup>358</sup> and later updated in 2007.<sup>359</sup> Since these guidelines were developed, significant advances in our understanding of the ecology, taxonomy, and treatment of *Sporothrix* species have occurred.

### Epidemiology

The majority of infections are caused by cutaneous inoculation by plant material, insect bites, animal bites or scratches.<sup>360</sup> The estimated prevalence of sporotrichosis is between 0.1-0.5% although the number of cases is rapidly increasing in Brazil. Until recently it was believed that only members of the pathogenic clade of *Sporothrix schenckii* were able to cause disease, but a taxonomic revision revealed the presence of novel medically relevant species: *S. brasiliensis*, *S. globosa*, *S. mexicana*, and *S. pallida*.<sup>361-363</sup> Other species have only been rarely reported from the clinic, and include *S. cyanescens*,<sup>364</sup> *S. luriei*,<sup>365</sup> and *S. chilensis*.<sup>366,367</sup> A consensus was not reached on the guideline panel on whether these novel species are uniformly able to cause disease. The clinical presentation of *S. schenckii* infection is typically a benign chronic subcutaneous mycosis. *S. globosa* most commonly causes a benign, fixed or lymphocutaneous infection, while infections caused by *S. brasiliensis* are often more severe.<sup>357</sup>

The first report of a large outbreak occurred between 1938-1947 and affected approximately 3,300 South African mine-workers. Several decades later, 87 mine-workers who laboured in the re-opened parts of the same goldmine developed lymphocutaneous sporotrichosis caused by *S. schenckii* sensu stricto.<sup>368</sup> Together with *S. globosa*, this species is found globally and

the cause of infections resulting from contact with decomposing material. However the expansion and potential adaptation of *S. brasiliensis* to a zoonotic niche has seen a correspondingly large increase in the number of cases (human and feline) within Brazil.<sup>357,362,369,370</sup>

### **Clinical presentation**

Sporotrichosis develops following inoculation of soil, bark, moss, or other organic material containing the organism into the skin or subcutaneous tissue rather than the inhalational route of most dimorphic fungi. Lymphocutaneous sporotrichosis is the most common form of disease and develops days to weeks after inoculation (Appendix Figure 12A).<sup>371</sup> A papule initially develops that may ulcerate or form a discrete nodule with surrounding erythema (Appendix Figure 12B). Warmth over the site is uncommon and drainage from these lesions is typically odorless.<sup>372</sup> Additional lesions may later develop over lymphatic channels proximal to the initial lesion (“sporotrichoid spread”). Systemic symptoms are uncommon but if left untreated, infection of contiguous osseous structures may occur. Osteoarticular disease is also seen and may occur from local inoculation or from haematogenous spread. Chronic or “fixed” lesions may also be seen primarily on the face. This form of the disease may be plaque-like or ulcerative.

Pulmonary disease is seen in patients with significant alcohol use and with underlying chronic obstructive pulmonary disease.<sup>373</sup> Symptoms are similar to those observed in tuberculosis with fever, chills, night sweats and weight loss associated with cough, chest pain, shortness of breath and sputum production. Dissemination is uncommon but may be seen in the immunocompromised, (particularly advanced HIV patients), with the skin, CNS, spleen, liver, and bone marrow the sites most frequently affected.<sup>374,375</sup>

### **Diagnosis**

#### *Culture and microscopy*

**Evidence** - The standard and most sensitive method for diagnosis of invasive sporotrichosis is culture although may be unrevealing.<sup>376</sup> Material obtained via lesion aspiration, biopsy, sputum or body fluids should be inoculated on Sabouraud dextrose agar and incubated at room temperature. Some isolates have been thought sensitive to temperature specific growth conditions,<sup>377</sup> however these findings have been questioned.<sup>378</sup> Flat, smooth or highly wrinkled with white or creamy colonies that may darken with aging are observed after 1-4 weeks incubated at 25-30°C (Appendix Figure 12C).<sup>379</sup> Conversion from the mould (Appendix Figure 12D) to yeast form can be performed for confirmation using blood agar. Blood cultures are rarely positive.<sup>380</sup>

Histopathology is often unrevealing of the organism even with the use of fungal specific stains, largely due to the small number of organisms that are needed to cause disease. The ovoid yeast cells are 3-5 µm in diameter, oval to cigar-shaped (Appendix Figure 12E and 12F), and eosinophilic projections from the yeast may be present, representing the “asteroid body” associated with *Sporothrix* species (Table 20). The latter histologic feature is thought to be comprised of antigen-antibody complexes or disintegrating neutrophils and is not specific for sporotrichosis. Direct aspirates of lesions/pus can be examined for evidence of sporotrichosis. Using this method asteroid bodies are frequently found (85.7% of cases) and cultures are positive in 95.2% of cases. Yeast cells may be visualized and surrounded by club-shaped hyaline-like structures with refractivity in the necrotic material.<sup>381</sup>

**Table 20. Conventional methods in the diagnosis of sporotrichosis.**

Population	Intention	Intervention	SoR	QoE	References and comment
Cutaneous lesions	Diagnosis	Culture from potentially involved sites	A	III	Queiroz-Telles <sup>382</sup> Bonifaz <sup>383</sup> (Culture is gold standard - yeast cells are observed in a low percentage of biopsies (5-10%).

Any	Diagnosis	Direct microscopy and culture from any clinical samples	C	III	Moreira <sup>384</sup> Bonifaz <sup>383</sup> Orofino-Costa <sup>379</sup> Zhang <sup>385</sup> (Yeast cells are round to oval shape and may be stained with PAS, GMS or calcofluor - The conversion from mould, 25°C, to yeast, 35°C is helpful for confirmation. Large clusters of yeast cells are usually observed in advanced HIV patients)
Any	Diagnosis	Fresh direct examination from pus, observe under light microscope	C	IIIh	Civila <sup>381</sup> (N=42, asteroid bodies found in 86%, cultures positive in 95%)

**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence; AIDS, Acquired immunodeficiency syndrome; GMS, Gomori-Methenamine Silver; PAS, Periodic acid-Schiff

### *Serology*

**Evidence** - Serologic testing is infrequently used in the diagnosis of sporotrichosis despite the availability of a commercial assay. A latex agglutination assay has been used in the past to assist in the diagnosis of meningeal sporotrichosis,<sup>386</sup> but that assay is no longer available and serology has little utility in other clinical situations. Other assays have been developed and have exhibited excellent sensitivity and specificity but are currently limited by availability.<sup>376,386,387</sup> A comprehensive review of these assays has recently been published.<sup>388</sup>

The utility of serology to assess a response to therapy has been recently re-examined and although these tests are in various stages of development thus far they have shown variable correlation with the clinical response.<sup>389,390</sup>

### *Skin testing*

Skin testing with sporotrichin antigen (prepared from the mycelial phase) and a peptide-rhamnomannan antigen (prepared from the yeast phase) have been studied to evaluate disease endemicity and have been found positive in over 90% of those with proven sporotrichosis.<sup>391,392</sup> Patients

with past exposure develop positive skin tests and it is thus a useful test for epidemiologic purposes; however, it is not useful for diagnosis and standardized reagents are not commercially available.

#### *Antigen detection*

Antigen testing has not been evaluated in clinical studies of sporotrichosis.

#### *Molecular methods*

**Evidence** – A number of different PCR assays have been developed for detection of *Sporothrix* species in tissue samples with varying sensitivity (83-92%) and specificity; however none of these are currently commercially available.<sup>393-395</sup>

Identification of *Sporothrix* spp. to the species level is rarely required in clinical circumstances, although high-throughput, sensitive and accurate assays have been published and can be used for epidemiological purposes and if species identification is requested.<sup>363,396</sup>

#### *Susceptibility testing*

**Evidence** – Guidelines for the *in vitro* antifungal susceptibility testing of *Sporothrix* spp. do not exist. *In vitro* antifungal susceptibility testing is not commonly performed during clinical care; however, a recent examination of *S. brasiliensis* isolates has found increasing amphotericin B and itraconazole MICs over time, although fortunately terbinafine MICs have remained low over this same time period.<sup>397,398</sup> *S. schenckii* isolates also have variable MICs to amphotericin B *in vitro*.<sup>399</sup> Voriconazole and fluconazole are not effective agents *in vitro*. No breakpoints are available and the clinical relevance of susceptibility testing merits further study.

### **Table 21. Role of non-invasive diagnostics in the diagnosis of sporotrichosis.**

Population	Intention	Intervention	SoR	QoE	References and comment
Any	Diagnosis	Sporotrichin skin testing	C	III	Kusuhara <sup>400</sup> (N=131, sensitivity 89%); Bonifaz <sup>401</sup> (N=138, sensitivity 95%, specificity 95%); Itoh <sup>392</sup> (sensitivity 96%). Helpful for epidemiologic purposes although not standardized preparations.
Any	Diagnosis	Serology	C	IIIt	<b>Numerous assays have been investigated:</b> Penha <sup>402</sup> (N=35, ELISA 100% specificity) Bernardes-Engemann <sup>387</sup> (N=92, ELISA, sensitivity of 90%) Bernardes-Engemann <sup>395</sup> (N=177, ELISA – sensitivity 89%, ROC AUC 0.92)
Any	To monitor treatment	Diagnosis and response to treatment	C	IIIt	Macedo <sup>389</sup> (N=102, serologic improvement correlated with clinical efficacy); Orofino-Costa <sup>390</sup> (N=5); Orofino-Costa <sup>403</sup> (N=1); Costa <sup>404</sup> (N=1) (serologic methods must correlate with the species responsible for infection)
CNS infection	Diagnosis	Serology in CSF	C	IIu	Scott <sup>386</sup> (N=7, detection of <i>schenckii</i> - CSF is frequently culture negative)

**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence; ELISA, Enzyme-linked immunosorbent assay.

## Sporotrichosis Diagnostic Recommendations

We recommend culture and histopathologic evaluation of skin or tissue aspirates, biopsies, or BAL fluid when the diagnosis of sporotrichosis is considered (SoR A, QoE IIu). Fungal specific stains should be used to maximize attempts to observe the organism on tissue samples (SoR A, QoE III). Culture on standard fungal media (e.g. Sabouraud dextrose agar) should be performed. The micro- and macroscopic appearance of *Sporothrix* spp is sufficient for the diagnosis in most cases.

Skin testing for sporotrichosis is not recommended for diagnostic purposes (SoR C, QoE IIu). Serologic testing for sporotrichosis exhibits a high sensitivity and specificity and may be useful to monitor during therapy (SoR C, QoE IIIt). Serology is recommended in the diagnosis of suspected CNS infections (SoR C, QoE, IIu). We recommend antifungal susceptibility testing of

*Sporothrix* isolates based on local epidemiologic patterns or in cases refractory to therapy (SoR A, QoE III).

### ***Imaging***

**Evidence** - Skin and soft tissue sporotrichosis rarely warrants imaging and in these cases only if concern exists for spread to contiguous bone or deeper structures. In cases of pulmonary sporotrichosis manifestations are similar to those seen in tuberculosis with unilateral or bilateral upper lobe cavities; in those with disseminated infection scattered nodules or fibrosis may also be seen.<sup>405,406</sup> Response to treatment may be observed with sequential imaging. Radiographic manifestations of joint or CNS involvement are non-specific but may be helpful to determine the extent of infection and response to therapy.<sup>407-409</sup>

### **Sporotrichosis Imaging Recommendations**

We recommend imaging in soft tissue infection only if there is concern for a foreign body or extension of infection to the contiguous tissue (SoR B, LoE III). We recommend pulmonary imaging to determine the extent of disease and response to therapy in cases of pulmonary sporotrichosis (SoR B, LoE III).

### ***Treatment rationale and recommendations***

**Evidence** – For cutaneous and lymphocutaneous sporotrichosis, therapy with itraconazole is associated with response rates of 80-100% (Table 22).<sup>410-412</sup> A variety of different itraconazole doses have been studied with no clear difference observed between doses prescribed.<sup>410</sup> The type of disease was associated with the duration of therapy required with lymphocutaneous disease requiring longer courses of therapy. Itraconazole 100-200 mg/day orally is recommended for 2-4 weeks after all lesions have resolved (typically 3-6 months required). Patients with suboptimal responses to itraconazole 200mg/day should receive itraconazole 200 mg orally twice daily, terbinafine 500

mg twice daily, saturated solution potassium iodide (SSKI), or fluconazole 400-800 mg daily. SSKI was the standard treatment until the 1990s and in areas where other antifungals are not available it is still used. The initial dose is five drops in juice/milk three times daily, increasing weekly, as tolerated, to a maximum of 40 to 50 drops in juice or milk three times daily.<sup>371,413</sup> Side effects include nausea, rash, metallic taste, fever, and salivary gland swelling.

In a cohort study comparing terbinafine (250 mg daily) and itraconazole 100 mg daily, there was no difference in outcomes (92% vs 92.7%) and no difference in adverse events between groups.<sup>412</sup> Patients benefit from TDM, as absorption of itraconazole is frequently inadequate. Terbinafine for cutaneous and lymphocutaneous sporotrichosis has been associated with response rates between 70-93%.<sup>412,414</sup> A comparison of sporotrichosis treatment demonstrated increased efficacy (and no relapses) in patients receiving terbinafine 1000 mg daily compared to those receiving 500 mg daily.<sup>414</sup>

SSKI has long been used for treatment of cutaneous sporotrichosis, with response rates between 70 and 89%.<sup>389,413</sup> Although this option is efficacious and cost-effective, alternative agents are preferred due to the difficulty of this regimen for patients (dysgeusia, gastrointestinal intolerance and acneiform eruptions).<sup>389</sup>

Fluconazole has been used in some cases and in those with lymphocutaneous disease cure has been seen in 71% of patients, however more advanced disease (osteoarticular or visceral sporotrichosis) exhibits low cure rates (31%).<sup>415</sup> For this reason fluconazole should be used only if other options are not available.

Heat therapy (local hyperthermia) has been used successfully in cases of cutaneous sporotrichosis and may be an option in pregnancy or in those intolerant of other options.<sup>416,417</sup>

Osteoarticular infections respond less well (approximately 70%) to itraconazole and may require initial therapy with amphotericin B preparations.<sup>418-420</sup> Treatment for these infections should continue for at least 12 months<sup>359</sup>

Amphotericin B preparations have been used with some success in patients with disseminated or severe pulmonary sporotrichosis.<sup>375,386,421-425</sup> Limited data are available for newer azole agents used in single or combination salvage therapy, and high MICs have been observed in vitro with voriconazole and isavuconazole suggesting they are not effective.<sup>426</sup> Posaconazole has been used successfully in several cases although large scale studies have not been performed.<sup>426,427</sup>

Treatment in the HIV population should commence as soon as possible and antifungal therapy maintained until resolution of clinical signs and symptoms of disease and improvement in the CD4 cell count to >200 cells/ $\mu$ L. Cases of immune reconstitution inflammatory syndrome are uncommon with sporotrichosis, but have been reported.<sup>428</sup>

**Sporotrichosis Treatment Recommendations** – For cutaneous and lymphocutaneous sporotrichosis, itraconazole 200 mg orally daily is recommended for 2-4 weeks after resolution of lesions, usually for a 3-6 month duration of therapy (SoR A, QoE IIu) (Table 22). The dose of itraconazole may need to be adjusted with the use of TDM given concerns with oral bioavailability.

Alternative therapies include terbinafine 500 mg orally twice daily (SoR B, QoE I); or increasing the itraconazole dose to 200 mg orally twice daily (SoR A, QoE IIu). SSKI 40-50 drops three times daily has been historically used, although other options are preferred if available (SoR C, QoE I). Fluconazole should be used only in the absence of other options (SoR C, QoE IIu). We recommend against voriconazole or isavuconazole (SoR D, QoE III).

For osteoarticular sporotrichosis, itraconazole 200 mg orally twice daily for at least 12 months is recommended (SoR B, QoE III). The dose of orally administered itraconazole may need

to be adjusted with the use of TDM given concern with oral bioavailability. Lipid preparations of amphotericin B (3-5 mg/kg daily) or AmB-d (0.7-1.0 mg/kg/d) may be used for initial therapy with a change to oral itraconazole once a favourable response has occurred (SoR B, QoE III).

For disseminated or severe pulmonary sporotrichosis, a lipid preparation of amphotericin B (3-5mg/kg daily) is recommended (SoR B, QoE III) or alternatively AmB-d (0.7-1.0 mg/kg/d) (SoR B, QoE III). After the patient has shown a favourable response to treatment, therapy can be changed to itraconazole 200 mg orally twice daily for a duration of at least 12 months (SoR B, QoE III). Patients on itraconazole benefit from TDM.

**Table 22. Treatment of sporotrichosis**

Population	Intention	Intervention	SoR	QoE	References and comment
Cutaneous/ lymphocutaneous (localised)	To cure	Itraconazole 100-200mg/day	A	I	Francesconi <sup>412</sup> (N=304, itraconazole vs terbinafine observed cure in 92% in both groups); Conti Diaz <sup>411</sup> (N=18, 100% cure rate after median of 44 days)
Localised / lymphocutaneous/ disseminated cutaneous	To cure	Itraconazole 100-400mg /day	A	Iu	de Lima Barros <sup>410</sup> (N=645, multiple different itraconazole doses assessed without clear difference – overall cure in 95%)
Cutaneous and lymphocutaneous sporotrichosis	To cure	Terbinafine	B	I	Chapman <sup>414</sup> (N=63, terbinafine 1000mg/day vs 500mg/day – cure rate 87 vs 52% and no relapses in higher terbinafine dosing group) Francesconi (N=55, terbinafine 250mg/day vs itraconazole 100mg/day with cure in 92% in both groups)
Osteoarticular, immunocompetent	To cure	Itraconazole 200mg for 24 months	B	III	Badley <sup>418</sup> (N=2); Lesperance <sup>420</sup> (N=1); Sharkey-Mathis <sup>419</sup> (N=12 with articular/osseous disease, Response rate 77%. 33% disease-free at 12-18 months)
Cutaneous Disease	To cure	Saturated solution of potassium iodide (SSKI)	C	I	Cabezas <sup>413</sup> (N=57, comparison of SSKI daily or 3x daily – response rates of 89% in both groups); Macedo <sup>389</sup> (N=102 comparison of different SSKI doses – cure rates 71-84%) Alternative options preferred to SSKI unless not available

Severe or disseminated	To cure	Amphotericin B	B	III	Rojas <sup>421</sup> (AmB-d); Hassan <sup>422</sup> (L-AmB); Freitas <sup>375</sup> (N=8 HIV+ patients treated with AmB-d – response in 7/8)
SOT	To cure	Amphotericin B	B	III	Gullberg <sup>423</sup> (N=1, AmB-d); Bahr <sup>425</sup> (N=1, L-AmB)
CNS	To cure	Amphotericin B	B	IIu	Scott <sup>386</sup> (N=7, AmB-d); Mialski <sup>424</sup> (N=2, AmB-d); Galhardo <sup>429</sup> (N=1, AmB-d) Poor outcomes common
CNS	To cure	Posaconazole	C	III	Paixão <sup>426</sup> (N=1, Post-treatment CSF culture was sterile)

**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence; AmB-d, Amphotericin B deoxycholate; L-

AmB, liposomal amphotericin B; SSKI, Saturated solution of potassium iodide.

## TALAROMYCOSIS

Talaromycosis is an invasive fungal infection caused by the thermally dimorphic fungus *Talaromyces marneffe* and is endemic throughout Southeast Asia and is highly endemic in northern Thailand, Vietnam, Myanmar, Hong Kong, Taiwan, southern China, and northeastern India (Appendix Figure 13).<sup>430</sup> *T. marneffe* was originally named *Penicillium marneffe* and classified under the *Penicillium* subgenus *Biverticillium*. In 2011 the *Penicillium* subgenus *Biverticillium* were taxonomically unified with the *Talaromyces* genus based on accumulated genetic sequencing data showing that together they form a monophyletic group that is distinct from *Penicillium*.<sup>431</sup> Hence, *P. marneffe* was changed to *T. marneffe*, and the disease penicilliosis is now called talaromycosis.

### **Epidemiology in subpopulations**

HIV is a major risk factor for talaromycosis. In just over two decades, the HIV epidemic has transformed talaromycosis from a rare infection to a leading HIV-associated opportunistic infection in Southeast Asia, accounting for up to 16% of HIV-hospital admissions,<sup>432-434</sup> and is the second leading cause of HIV-associated bloodstream infections and death in Vietnam and southern China with a mortality of up to 28%.<sup>435-437</sup> Infection occurs in patients with advanced HIV disease who have a CD4 count <100 cells/ $\mu$ L.<sup>430,434,437</sup> Recently, an increase in both the number and type of *Talaromyces* infections has been observed in individuals with a primary immunocompromising condition (e.g., idiopathic CD4 lymphopenia, anti-interferon-gamma autoantibody-associated immunodeficiency, conditions due to mutations in CYBB, CD40L, or gain-of-function mutation in the STAT1/STAT3 pathways) and in individuals with secondary immunodeficiency conditions other than HIV (e.g., autoimmune diseases requiring corticosteroids and/or other immunosuppressive therapy, solid or haematological malignancies, solid organ or haematopoietic stem cell transplantation, and novel target therapies such as monoclonal antibodies against CD20 and kinase inhibitors).<sup>438</sup> Children aged 3 months to 16 years account for 6.3% of the total cases in non-HIV

patients in one study, and the vast majority have an underlying primary immune defect.<sup>439</sup> Talaromycosis is also increasingly diagnosed in immigrants and returning travellers from Southeast Asia and has been reported in Australia, Belgium, France, Germany, Japan, the Netherlands, Oman, Sweden, Switzerland, the United Kingdom, and the United States.<sup>440,441</sup> There is some evidence to suggest that the region of endemicity is expanding, with reports of autochthonous cases in areas not previously known to be endemic including northern and eastern China (Beijing and Shanghai) and in the Assam state in northeastern India<sup>442-444</sup>.

### *Ecology and Transmission*

The wild bamboo rat living in the highland areas in Southeast Asia is the only known enzootic reservoir of *T. marneffeii*.<sup>445</sup> However, occupational exposure to plants and farming animals, rather than exposure to, or consumption of, bamboo rats, is the major risk factor for disease acquisition.<sup>446,447</sup> Incidence increases 30-50% during the rainy season, and is associated with humidity but not precipitation.<sup>434,448,449</sup> *T. marneffeii* has been isolated from soil samples collected deep within bamboo rat burrows but not from other soil or environmental samples.<sup>445</sup> These findings suggest the bamboo rat may be important in the maintenance of *T. marneffeii* in the environment, and higher humidity facilitates environmental expansion of *T. marneffeii* increasing the risk for acute infection during the rainy months. Transmission is presumed secondary to inhalation of airborne conidia from a soil-related environment source. Reactivation of latent infection can occur many years after travel to the endemic region.<sup>440</sup> A presumed case of laboratory-acquired infection was reported at the Pasteur Institute in Paris in an HIV-infected man with no travel history to Southeast Asia;<sup>450</sup> however, laboratory-acquired contamination or infection has never been reported in biosafety level 2 clinical laboratories within the endemic region. Donor-acquired transmission has been reported in a single lung-transplant recipient who lived in Belgium.<sup>451</sup>

## **Clinical Presentation**

Patients with advanced HIV disease develop a disseminated infection involving multiple organs including the lung, skin, oropharyngeal mucosa, lymph nodes, liver, spleen, gastrointestinal tract, bloodstream, and bone marrow.<sup>434</sup> Symptoms are non-specific and develop over weeks to months, including fevers, cough, weight loss, fatigue, abdominal distension, diarrhoea and lymph node enlargement – often difficult to differentiate from tuberculosis, salmonellosis, or other invasive mycoses. Skin lesions, typically appear as central-necrotic papules on the face, trunk, and extremities, and are the most specific manifestation of talaromycosis (Appendix Figure 14A). However, skin lesions appear late in the disease course, and are present in 40-70% of patients.<sup>430,434</sup> Central nervous system involvement occurs in <1% of patients and has a rapid disease course with a mortality of ~80%.<sup>452</sup> Common laboratory findings include anemia (which can be profound), thrombocytopenia, elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The median CD4 count is typically <50 cells/ $\mu$ L. Chest CT scan or x-ray findings are broad and include interstitial, reticulonodular, or alveolar infiltrates. Abdominal ultrasounds frequently show markedly enlarged liver and spleen and intra-abdominal lymph node enlargement.<sup>430,434</sup>

Non-HIV patients tend to be older, have longer duration of illness, are less likely to have fevers, splenomegaly, skin lesions, and positive blood cultures, but are more likely to have localized disease and osteoarticular involvement.<sup>453</sup> The time to diagnosis is longer for non-HIV patients (median 60 days vs 30 days), likely due to the lack of knowledge of disease in non-HIV patients. The mortality is also higher in non-HIV patients compared with HIV-infected patients (30% versus 21%).<sup>453</sup>

## **Diagnosis**

### *Culture and microscopy*

**Evidence** - A presumptive diagnosis of talaromycosis is made based on the microscopic examination of skin lesion scrapings, lymph node or bone marrow aspirates, or based on the histopathological examination of tissue sections (Table 23). Occasionally *T. marneffe* can be seen on the peripheral blood smear of patients with fungemia. Skin lesions are present in 70% of HIV-infected patients and in 40% of non-HIV-infected patients, and microscopy of skin lesions is 95% sensitive for the diagnosis of talaromycosis.<sup>434,453</sup> Identification of a transverse septum in a dividing yeast cell, 3-6  $\mu\text{m}$  in diameter, round to oval in size, extracellular and within macrophages (Appendix Figure 14B), is characteristic of *T. marneffe* and differentiates it from closely resembling fungi such as *Candida* or *Histoplasma* species.<sup>454,455</sup>

A definitive diagnosis of talaromycosis is made by culture isolation of *T. marneffe* from clinical specimens, which can take 5-14 days to grow and the demonstration of temperature-regulated dimorphism, although culture is not 100% sensitive. *T. marneffe* grows as a mould at temperatures from 25°C to 30°C, producing yellow green colonies with sulcate folds and a distinctive bright red diffusible pigment in the media. Microscopically, filamentous hyphae with characteristic conidiophores and conidia can be seen. The red pigment should be an early indication that an isolate may be *T. marneffe*, although it may be produced in a non-pathogenic *Penicillium* and *Talaromyces* spp. (eg *Talaromyces atroroseus*). At temperatures from 32–37°C, *T. marneffe* can convert to the yeast phase, producing tan colonies without red pigment. The *in vivo* yeast phase conversion is complete within 1–2 days, producing round to oval yeast cells similar to those that are seen in clinical samples.<sup>456</sup> However, the yeast phase conversion is not completed *in vitro*, forming instead transitional sausage-shaped cells (Appendix Figure 14C). Culture yield is highest from bone marrow (100%), followed by skin lesions (90%), and blood (70%).<sup>454</sup>

### *Antigen detection*

Antigen detection is highly accurate, inexpensive, does not require sophisticated equipment, and is particularly well suited for patients with advanced HIV disease and high fungal burden in the blood (Table 24). The commercial Platelia *Aspergillus* galactomannan assay cross reacts with *T. marneffeii* and has a sensitivity ranging from 73-81% and a specificity of 91% at a cut-off index of 0.5.<sup>457,458</sup> However, this test also cross-reacts with other endemic fungi and *Cryptococcus* species.<sup>459</sup>, is not widely available in Asia, and has not been tested in larger clinical studies.

The monoclonal-antibody-based Mp1p antigen detection ELISA developed in Hong Kong does not cross react with other clinically relevant fungi.<sup>460</sup> It is more sensitive than blood cultures, 86% versus 74% and has a specificity of 98%.<sup>461</sup> In a recent prospective study of 521 symptomatic hospitalized persons with advanced HIV followed over six months in Vietnam, the assay detected infection up to 16 weeks prior to cultures turning positive, was superior to cultures when performed in a combination of serum, plasma, and urine samples from the same patients, yielding a combined sensitivity of 98% (compared to cultures of 84% in 80 talaromycosis patients), and had a specificity of 96% (in 441 non-talaromycosis patients). Urine samples yielded the highest sensitivity and specificity, followed by plasma, and serum samples.<sup>462</sup> The same assay detected Mp1p antigen in 9.4% of 8,131 banked serum samples from HIV patients in clinics in southern China<sup>463</sup> and in 4.2% of 1,081 banked plasma samples from HIV patients in 22 clinics across Vietnam,<sup>464</sup> indicating a high disease burden in these countries. In the Vietnam cohort, Mp1p antigenemia was found to be independently associated with 12-month mortality.<sup>464</sup> These data demonstrate that the Mp1p ELISA is highly accurate as a rapid diagnostic tool, and has the potential as a screening tool to identify sub-clinical infections for early treatment to prevent talaromycosis. A commercial Mp1p ELISA (Wantai Beijing, Beijing, China) was approved in 2018 in China for clinical use.

The 4D1 monoclonal-antibody-based antigen detection ELISA developed in Thailand also appears to be highly accurate in a small study.<sup>465</sup> A point-of-care immunochromatographic platform has been developed for testing in urine samples which has a sensitivity of 88% (in 66 blood-culture-positive advanced HIV patients) and a specificity of 100% (in 112 controls).<sup>466</sup>

#### *Antibody detection*

The Mp1p antibody detection ELISA has a variable reported sensitivity, ranging from 30% to 80%, in HIV patients with talaromycosis. Testing for both Mp1p antibody and Mp1p antigen resulted in higher sensitivities compared to testing each assay individually.<sup>460,467</sup> However, the Mp1p antibody test has not been validated in larger clinical studies and has not yet been utilized to study disease exposure or latent infection in populations.

#### *Molecular detection*

A number of qPCR assays based on specific *T. marneffeii* regions within the fungal ribosomal ITS, 5.8S rRNA, 18S rRNA, and Mp1p have been developed. These assays performed in whole blood or plasma samples have high specificities (100%), but sensitivity ranges from 70% to 86%.<sup>458,468,469</sup> These PCR assays have the potential as rapid “rule-in” tests; however, they have been tested in small sample sizes, and none has been prospectively validated or commercially developed for clinical use.

#### *Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF)*

MALDI-TOF has recently been used for identification of *Talaromyces* to the species level from cultured specimens based on either an in-house database generated from an institution’s *T. marneffeii* clinical strain collection<sup>456,470</sup> or from the comprehensive NIH MDL Mold Library.<sup>471</sup> MALDI-TOF represents a rapid and reliable tool for downstream fungal identification,

eliminating the need to demonstrate thermal dimorphism; however, the current costs of this methodology limits its use in resource-limited settings.

### *Susceptibility testing*

While methods for antifungal susceptibility testing and clinical interpretive MIC breakpoints for *T. marneffei* have not been established, the MICs reported in multiple studies using microdilution methods or E-test are consistently low for itraconazole ( $\leq 0.008$   $\mu\text{g/ml}$ ), intermediate for amphotericin B (0.25-1.0  $\mu\text{g/ml}$ ), and intermediate to high for fluconazole (0.3-7.9  $\mu\text{g/ml}$ ). MICs generally correlate with efficacy during treatment of talaromycosis with itraconazole and amphotericin B and poor efficacy for fluconazole.<sup>472</sup> More recent studies report low MICs for voriconazole ( $< 0.063$   $\mu\text{g/ml}$ ) and posaconazole ( $< 0.002$   $\mu\text{g/ml}$ ), and intermediate to high MICs for anidulafungin (2-8  $\mu\text{g/ml}$ ), suggesting that the newer azoles are promising drugs, whereas echinocandins may be less effective against *T. marneffei*.<sup>473,474</sup>

### **Talaromycosis Diagnostic Recommendations**

In patients with a clinical suspicion of talaromycosis, we strongly recommend patient specimens, including skin smears or biopsy, blood, sputum or BAL, aspiration samples of lymph nodes, pus, bone marrow, pleural fluid, ascites, and CSF should be sent for direct microscopy and fungal cultures (SoR A, QoE III). Microscopy of skin lesions provides a high diagnostic yield and is essential for making a rapid diagnosis enabling early therapy in patients presenting with skin lesions. For microscopy, we recommend using Grocott's Methenamine Silver (GMS), PAS, Giemsa, Wright stains, or optical brighteners (calcofluor white/blankophor), as *T. marneffei* cells are not seen well with Gram- or H&E stains.

**Table 23. Conventional methods in the diagnosis of talaromycosis**

Popu-lation	Intention	Intervention	SoR	QoE	References and comment
Any	Diagnosis	Direct microscopy by Giemsa, Wright, GMS, PAS stains, or optical brighteners (calcofluor white, blankophor)	A	III	Vanittanakom <sup>454</sup> Supparatpinyo <sup>475</sup> (intracellular and extracellular 3-6 μ round to oval yeast cells with characteristic transverse septum in macrophages or histiocytes)
Any	Diagnosis	Cultures using conventional aerobic bacteria media (eg, BACTEC) or selected fungal media (eg, Mycolytic/F, or Lysis-centrifugation).	A	III	Vanittanakom <sup>454</sup> Supparatpinyo <sup>475</sup> Culture takes up to 14 days in BACTEC, MycoF/Lytic, or Sabouraud dextrose agar.  At 25°C, produce yellow green colonies with a red diffusible pigment in media. Must demonstrate yeast to mould conversion (or vice versa) going from 37°C to 25°C.  Blood culture positive 70% Skin culture positive 90% Bone marrow culture positive 100%

**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence; GMS, Gomori-Methenamine Silver; PAS, Periodic acid-Schiff

Identification of a transverse septum on microscopy establishes a presumptive diagnosis. Culture is the gold standard for diagnosis of talaromycosis and should be observed for up to 14 days (SoR A, QoE III).

Given the demonstrated superior sensitivity compared to culture, high specificity (>95%), and rapid turn-around-time in large clinical studies, we strongly recommend routine Mp1p antigen testing in addition to microscopy and cultures where a commercial or in-house test is available, and we recommend antigen testing in plasma and urine specimens (SoR A, QoE IIu). All patients with a positive Mp1p antigen test should be further investigated for occult infection, and antifungal therapy should be initiated while waiting for confirmatory culture results (SoR B, QoE III).

In settings where the Mp1p test is not available, but there is a high clinical suspicion in patients without typical skin lesions, we recommend qPCR testing in whole blood or plasma using a validated in-house assay as a rapid diagnostic. Our recommendation is based on these assays' high specificity (100%) and fast turn-around-time (SoR B, QoE III).

We recommend investigation for underlying immunodeficiency conditions in all patients with a diagnosis of talaromycosis.

**Table 24. Role of non-invasive diagnostics in talaromycosis.**

<b>Antigen detection</b>					
Any	Diagnosis	Antigen detection in urine using PAb-based dot blot ELISA, latex agglutination, and conventional ELISA	A	Ilu	<b>Numerous assays have been investigated:</b> Desakorn <sup>476</sup> (Sensitivities: 97.3%, 94.6%, and 100% in urine of 37 blood culture-positive samples. Specificities: 98%, 97.3%, and 99.3% in 300 controls) Wang <sup>460</sup> (Sensitivity 75% - specificity 99%) Prakit <sup>465</sup> (Sensitivity 100% - specificity 100%) Thu <sup>461</sup> (sensitivity 86% compared to blood culture sensitivity of 74% - specificity 98%) Pruksaphon <sup>466</sup> (Sensitivity 88% - specificity 100%) Ly <sup>462</sup> (Evaluation in advanced HIV patients; combined sensitivity in plasma, sera, and urine samples from same patients of 98% [sensitivity of cultures 84%] - specificity 96% (in 441 of patients who did not develop talaromycosis))
Any	Diagnosis	Platelia Aspergillus Galactomannan antigen detection test in sera (Bio-Rad)	C	III	Li <sup>477</sup> (N=36, cross reacts with other endemic fungi and <i>Cryptococcus</i> . Sensitivity for talaromycosis 73%-81%, specificity 91%). Huang <sup>478</sup> (N=3)
<b>Antibody detection</b>					
Any	Diagnosis	Serology - ELISA	C	III	Wang <sup>460</sup> (N=20); Cao <sup>467</sup> (N=17) (sensitivities range from 30% to 80% in HIV patients)
<b>Realtime PCR assays</b>					
Any	Diagnosis	qPCR assays using 5.8S rRNA, <i>MPI</i> , or ITS locus	B	III	Li <sup>477</sup> (N=92); Pornprasert <sup>468</sup> (N=20); Hien <sup>479</sup> (N=50) Sensitivities range from 70% to 86%. Specificity 100%, but small sample sizes

**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence; ELISA, Enzyme-linked immunosorbent assay; GMS, Gomori-methenamine silver. MAb, Monoclonal antibody; MALDI-TOF, Matrix Assisted Laser

Desorption/Ionization Time of Flight Mass Spectrometry; NIH, National Institute of Health (USA); PAb, Polyclonal antibody; PAS, Periodic-acid Schiff; PCR, Polymerase chain reaction.

## ***Treatment rationale and recommendations***

### **Evidence**

#### *Induction and consolidation therapy*

Disseminated talaromycosis is fatal if untreated, and the mortality rate approaches 30% even with antifungal therapy.<sup>430,434,437</sup> Treatment should be given promptly to all immunocompromised patients with a compatible clinical syndrome and a positive result of any of the following tests: microscopy, cultures, histopathology, Mp1p antigen test, or a qPCR test.

Similar to the approach in cryptococcosis, antifungal therapy is divided into induction, consolidation, and maintenance phases and is guided by observational studies and one randomized controlled trial performed in patients with HIV infection (Table 25).<sup>480</sup> Itraconazole capsules and amphotericin B deoxycholate (AmB-d) are available in Southeast Asia and are the most commonly used antifungals. Voriconazole and lipid amphotericin B formulations are also effective drugs against talaromycosis, but are expensive and are currently not widely available in Southeast Asia.

In one early non-comparative study of 74 patients in Thailand, induction therapy with AmB-d was highly effective with a treatment success rate (defined by negative blood culture and resolution of fevers and skin lesions) at 12 weeks of 97%.<sup>481</sup> In a multi-center randomized controlled trial in Vietnam, induction therapy with AmB-d was shown to be superior to itraconazole with respect to mortality, blood fungal clearance, disease relapse, and IRIS.<sup>480</sup> The mortality after six months was 21% in the itraconazole group and 11% in the AmB-d group (hazard ratio of death

was 1.88, 95% confidence interval: 1.15-3.09,  $P=0.012$ ). Importantly, the mortality difference was not dependent on disease severity (as indicated by antiretroviral therapy status, intravenous drug use, lower CD4 counts, positive blood culture, higher fungal burden, or requirement for oxygen support at presentation;<sup>480</sup> therefore, amphotericin B should be the first choice of induction therapy for all talaromycosis patients, regardless of disease severity. Where available, L-AmB or an alternative lipid formulation is preferred over AmB-d as it is less nephrotoxic, allowing much higher doses to be given safely, and has excellent tissue penetration and a long tissue half-life.<sup>482</sup> Itraconazole is still frequently used in some locations as AmB-d is not widely available and requires skilled nursing for drug administration and monitoring. Voriconazole has high oral bioavailability, is also used for induction therapy in patients who do not tolerate AmB-d, and has favourable clinical and microbiological outcomes in small case series: 8/9 adults in Thailand,<sup>483</sup> 10/14 adults in China,<sup>484</sup> and 7/10 children in China.<sup>485</sup> Voriconazole is therefore a potential induction therapy option for patients who are unable to tolerate AmB-d; however, clinical trials comparing AmB-d and voriconazole have not been performed.

#### *Maintenance therapy and when to stop*

A double-blind, placebo-controlled trial in Thailand showed that maintenance therapy with itraconazole 200 mg daily in patients with advanced HIV disease reduced relapse rate from 57% to 0% ( $P < 0.001$ ).<sup>486</sup> Retrospective cohort studies reported no disease relapse after discontinuation of itraconazole maintenance therapy in patients receiving ART with a CD4 count of  $>100$  cells/ $\mu$ L for at least 6 months.<sup>487</sup> Data on duration of maintenance therapy in non-HIV-infected patients are lacking.

#### *Primary prophylaxis*

Primary prophylaxis with itraconazole 200 mg PO daily has been shown to reduce incidence of invasive fungal infections (talaromycosis, cryptococcosis, and oesophageal candidiasis) in HIV-infected patients with a CD4 count of <200 cells/ $\mu$ L in a randomized controlled trial in Thailand.<sup>488</sup> Another retrospective study in Thailand showed that fluconazole 400 mg PO weekly was as effective as itraconazole for primary prophylaxis.<sup>489</sup> However, these studies were conducted prior to the widespread availability of ART, had small sample sizes, and did not show a mortality benefit. Therefore, primary prophylaxis has not been widely adopted.

### **Talaromycosis Treatment Recommendations**

We strongly recommend induction therapy with amphotericin B (SoR A, QoE I). L-AmB is preferred over AmB-d where available. L-AmB can be given at 3 to 5 mg/kg/day IV, and AmB-d can be given at 0.7 mg/kg/day IV, both for 10 to 14 days, followed by consolidation therapy with itraconazole 200 mg PO twice daily for 10 weeks, followed by maintenance therapy with itraconazole 200 mg PO daily for all adults and children (SoR A, QoE I).

Where amphotericin B is not available, we recommend induction therapy with voriconazole 6 mg/kg IV q12h on day 1 (loading dose), and then 4 mg/kg IV q12h or with voriconazole 600 mg PO q12h on day 1 (loading dose) and then 400 mg PO q12h for 10 to 14 days (SoR B, QoE IIu). Dosing of voriconazole should be higher in children aged 2 to 14 at 9 mg/kg q12h on day 1 (loading dose), and then 8 mg/kg IV q12h or 9mg/kg PO q12h for 10 to 14 days.

We strongly recommend against using itraconazole for induction therapy, regardless of disease severity. However, in settings where neither amphotericin nor voriconazole is available, itraconazole induction therapy should be dosed at 200 mg PO q8h for 3 days (loading dose), and then 200 mg PO q12h for 2 weeks for all patients (SoR C, QoE I).

Induction therapy should be followed by consolidation therapy with itraconazole 200 mg PO twice daily for 10 weeks, followed by maintenance therapy with itraconazole 200 mg PO daily (SoR A, QoE I). Voriconazole can be used in lieu of itraconazole at 400 mg PO q12h (or 4-9 mg/kg q12h in children aged 2-14) (SoR B, QoE III).

#### *Discontinuation of maintenance therapy*

For HIV-associated talaromycosis, we recommend that maintenance itraconazole can be stopped when the CD4 count is  $>100$  cells/ $\mu$ L for at least 6 months in response to ART (SoR B, QoE IIu), or with virologic suppression for  $\geq 6$  months in response to ART (SoR B, QoE III). ART should be initiated within one week after the induction therapy with amphotericin (SoR B, QoE IIu).

In non-HIV-associated talaromycosis, we recommend maintenance itraconazole be continued for at least 12 months. Maintenance therapy may safely be withdrawn in patients where immunosuppressive therapy can be similarly reduced or withdrawn, and we recommend careful monitoring for relapse.

#### *Primary prophylaxis*

We currently do not recommend primary prophylaxis for HIV-infected patients with a CD4 count of  $<200$  cells/ $\mu$ L who live or travel in Southeast Asia due to lack of current data of benefit and the concerns about long-term toxicity, drug-drug interactions, and costs (SoR C, QoE IIu).

#### *Therapeutic drug monitoring*

Given variable absorption and interindividual variability of itraconazole, non-linear pharmacokinetics of voriconazole, and known drug interactions with antiretroviral drugs, we recommend

TDM when available, keeping concentrations of  $>1 \mu\text{g/ml}$  to  $4 \mu\text{g/ml}$  for itraconazole and trough concentrations of  $1\text{-}5.5 \mu\text{g/ml}$  for voriconazole<sup>490</sup> (SoR B, QoE III). Samples for voriconazole TDM should be taken within 5-7 days of starting therapy and at about 2 weeks for itraconazole.

**Table 25: Treatment options for talaromycosis**

Popula- tion	Intention	Intervention	SoR	QoE	References and comment
<b>Induction and consolidation therapy (initial 10-14 days)</b>					
All	To cure	Amphotericin B IV	A	I	Le <sup>480</sup> (N=440, mortality at 6 months: 11% in the AmB-d arm versus 21% in the itraconazole arm); Sirisanthana <sup>481</sup> (N=74)  L-AmB 3-5 mg/kg/d preferred, or AmB-d 0.7-1 mg/kg/d x 10-14d
All	To cure (when amphotericin B is not available)	Voriconazole	B	III	Supparatpinyo <sup>483</sup> (N=11, efficacy in 89%); Ouyang <sup>484</sup> (N=17, efficacy in 71%); Guo <sup>485</sup> (N=10, paediatric study-complete response in 70%)
All	To cure (when amphotericin B or voriconazole are not available)	Itraconazole	C	I	Hu <sup>433</sup> (N=586); Le <sup>433</sup> (N=795); Le <sup>480</sup> (N=440) – itraconazole 200 mg q8h x 3 days (loading dose), followed by 200mg q12h x 12 weeks
<b>Consolidation therapy (10 weeks after induction therapy)</b>					
All	To cure	Itraconazole	A	I	Le <sup>480</sup> Sirisanthana <sup>481</sup> – itraconazole PO 200 mg q12h x 10 weeks
All	To cure	Voriconazole	B	III	Supparatpinyo <sup>483</sup> (N=11); Ouyang <sup>484</sup> (N=17, efficacy in 71%) – voriconazole PO 400 mg Q12h x 10 weeks Guo <sup>485</sup> (N=11, HIV-negative paediatric study)
<b>Maintenance therapy to prevent relapse (or secondary prophylaxis)</b>					
All	To prevent relapse	Itraconazole PO 200mg q24h until CD4 count of >100 cells/mm <sup>3</sup> for at least 6 months	A	I	Supparatpinyo <sup>486</sup> (N=72, RCT [itraconazole vs. placebo]: 0/36 (0%) in itraconazole arm and 20/35 (57%) in placebo arm had relapse 24 weeks after completion of consolidation therapy)
HIV-in- fected	When to stop maintenance therapy	CD4 count of >100 cells/mm <sup>3</sup> for at least 6 months	A	III	Chaiwarith <sup>487</sup> (N=33, no relapses if CD4 count of >100 cells); Chien-Ching <sup>491</sup> (N=9, no relapses)
<b>Primary prophylaxis to prevent infection</b>					
All HIV- infected	To prevent infection	Itraconazole PO 200 mg q24h or fluconazole PO 400 mg weekly	C	Ilu	Chariyalertsak <sup>488</sup> (N=63, RCT, itraconazole versus placebo: 1.6% vs 16.7% developed systemic fungal infections); Chaiwarith <sup>489</sup> (N=308, no difference between fluconazole and itraconazole for primary prophylaxis of fungal infections in Thailand AIDS population)

**Legend:** SoR, Strength of Recommendation; QoE, Quality of Evidence; ART, Antiretroviral therapy; HIV: Human immunodeficient virus; IFI; Invasive fungal infection; RCT: Randomized controlled trial.

## **Future directions and unmet needs**

Significant questions persist in the field of the endemic mycoses. The fungal kingdom has undergone substantial taxonomic revision and new species have recently been proposed. The significance of these cryptic species has yet to be determined although emerging data suggests antifungal susceptibility and host differences. In addition to cryptic species – which are distinguished only by genetic but not phenotypic differences - several morphologically and clinically distinct fungi, such as *S. brasiliensis*, several new species of *Blastomyces*, and the new genus *Emergomyces*, have been recognized. New diagnostics focused on non-invasive methods (serologic, antigen capture, breath testing, and imaging) are also under active investigation and merit further study. In vitro susceptibility testing and its correlation to clinical response will also need to be further evaluated. As many diagnostics require the expertise of specialized reference laboratories improvements in this area would be a welcome advance and may reduce the time to diagnosis and initiation of treatment. A number of novel antifungal agents are in various stages of development, and several have significant activity in the treatment of endemic mycoses and may be able to alter the time course of disease.<sup>492,493</sup> Improvements in our understanding of these diverse mycoses requires the input and collaboration of a wide range of investigators and through collaborative efforts we hope to continue to advance the field of endemic mycoses.

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## **Conflicts of Interest**

GRT has received research support and served as consultant for Amplyx, Astellas, Basilea, Cidara, F2G, Immy, Mayne, Scynexis, and served as a consultant for Pfizer.

MJB is a founding partner and holds shares of Micología Molecular S.L, she has received grant support from the Instituto de Salud Carlos III and has been paid for talks on behalf of United Medical LTDA.

AAI has received research grants or honoraria as a speaker or advisor from Gilead Sciences, MSD, Pfizer, F2G and Scynexis

DAJ holds share options in Pulmocide and has received grant support from Pulmocide, Astellas, Pfizer and Gilead. He has received lecture honoraria from Astellas, Pfizer, Gilead and Astra-Zeneca.

JWB has served as a consultant for Pfizer

JFWC has received travel grants from Pfizer Corporation Hong Kong and Astellas Pharma Hong Kong Corporation Limited, and was an invited speaker for Gilead Sciences Hong Kong Limited and Luminex Corporation. The other authors declared no conflict of interests.

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NPG no conflicts of interest

FH no conflicts of interest

NK has received research grants or honoraria as a speaker or advisor from Astellas, Gilead, MSD, and Pfizer, outside the submitted work DCMK has sat on advisory boards for Becton Dickinson Ltd and MSD, and received financial/travel support unrelated to the current work from MSD.

MHM has received research support and served as a consultant for Astellas and Scynexis

PMR no conflicts of interest

IS has served as an advisor to AVIR Pharma

AS has received grant funding from Astellas, and has consulted for Scynexis, Minnetronix, Mayne, and Viamet.

JPG has received research grant support from Pfizer.

FQT has received research grants from Astellas, MSD, Pfizer and also received payments for presentations and continued medical education from MSD, Pfizer, United Medical and TEVA Brazil.

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ACP has received research grant support on behalf of Pfizer, Gilead, MSD, and IMMY. He has also given paid talks or participated in the medical board of Pfizer, United Medical, Gilead, MSD, Astellas and IMMY.

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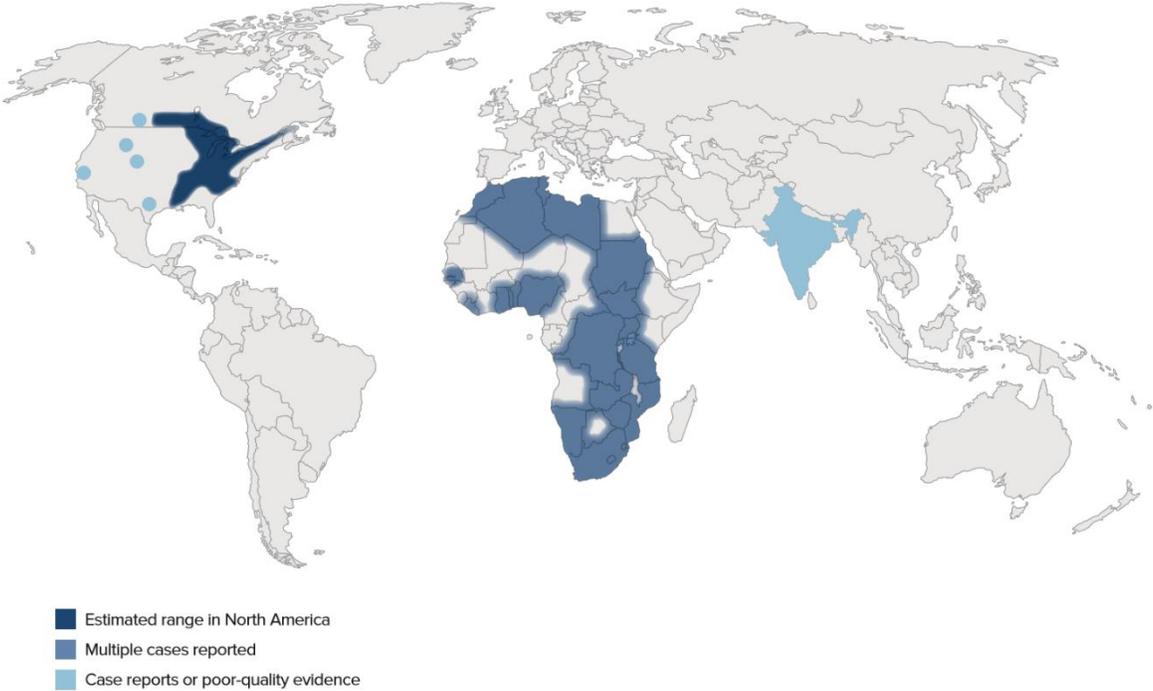
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**Figure 1. Map of endemic region for blastomycosis**



**Figure 2. Clinical and microbiologic findings of blastomycosis**



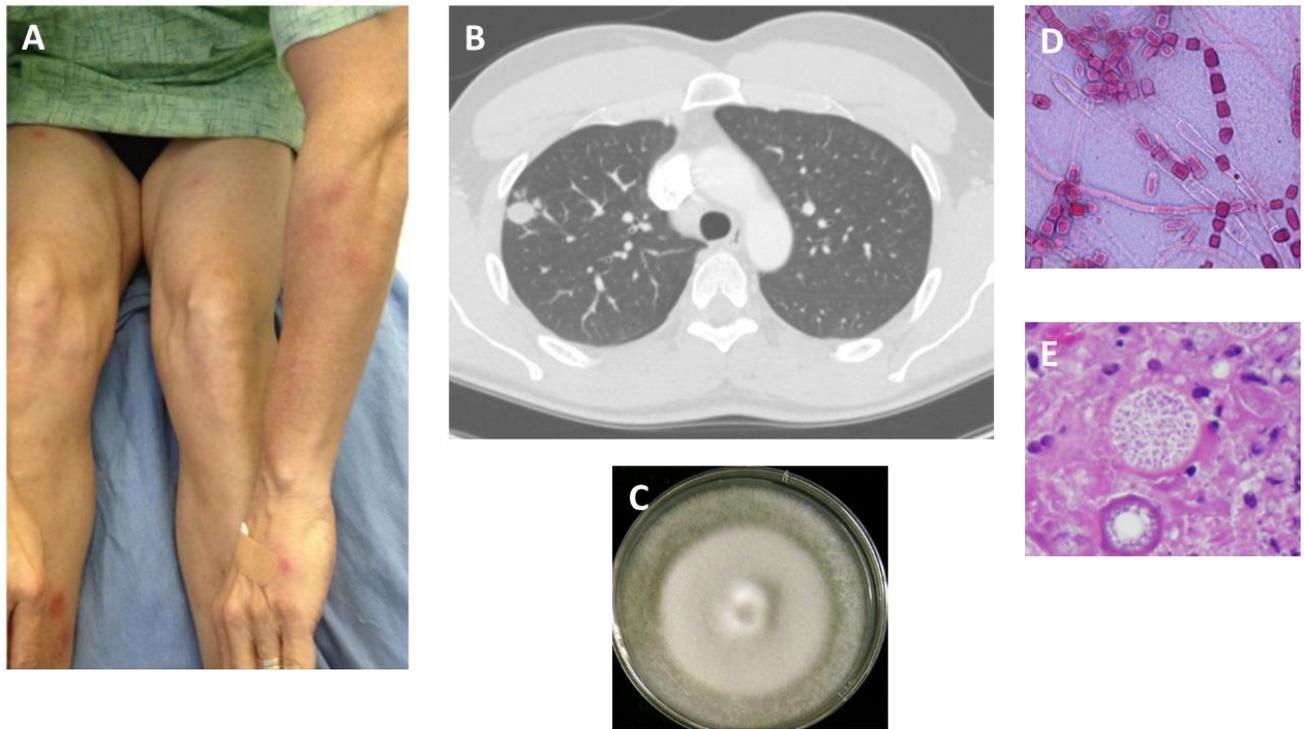
**Figure 2 (A&B) Cutaneous lesions typical for blastomycosis, (C) pulmonary infiltrates seen on chest X-ray, (D) *Blastomyces* yeast seen on GMS stain of a tissue sample with broad-based budding apparent in center (400x magnification), (E) Typical appearance of *Blastomyces* with single-celled conidia at the tip of conidiophores (400x)**

**Courtesy of Dr. John Baddley and Dr. Carol Kauffman and Yuri Amatnieks**

**Figure 3. Map of endemic region for coccidioidomycosis**



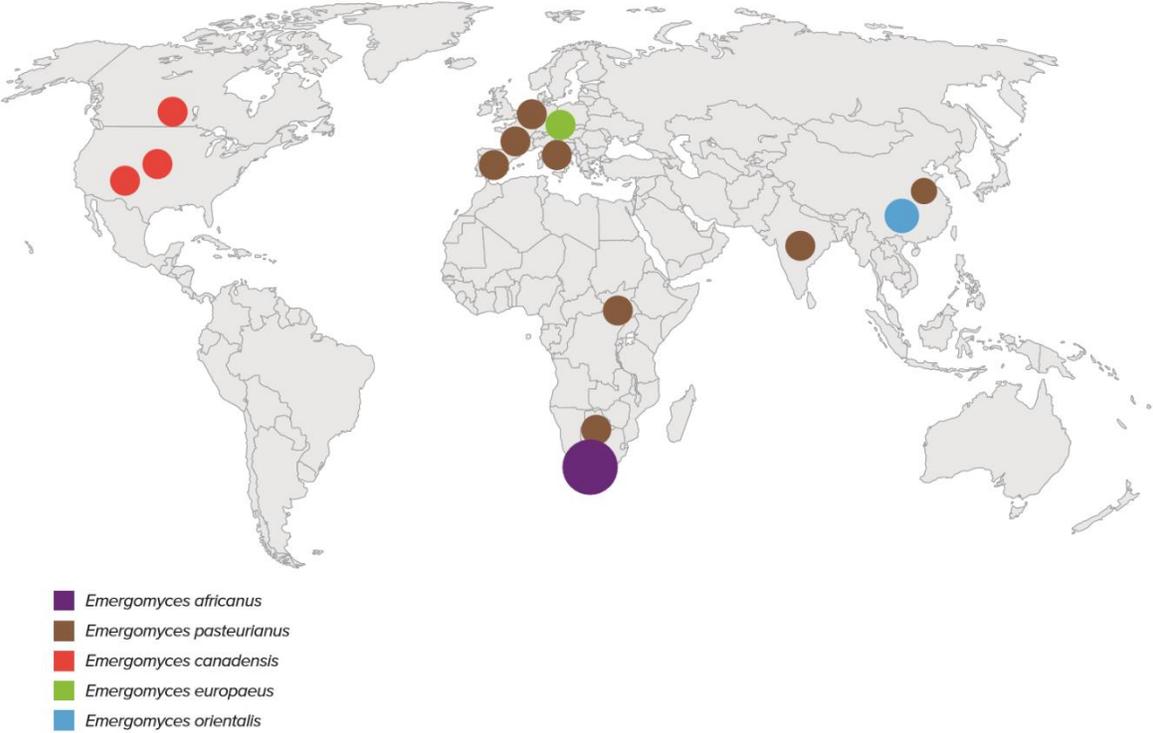
**Figure 4. Clinical and microbiologic findings of coccidioidomycosis**



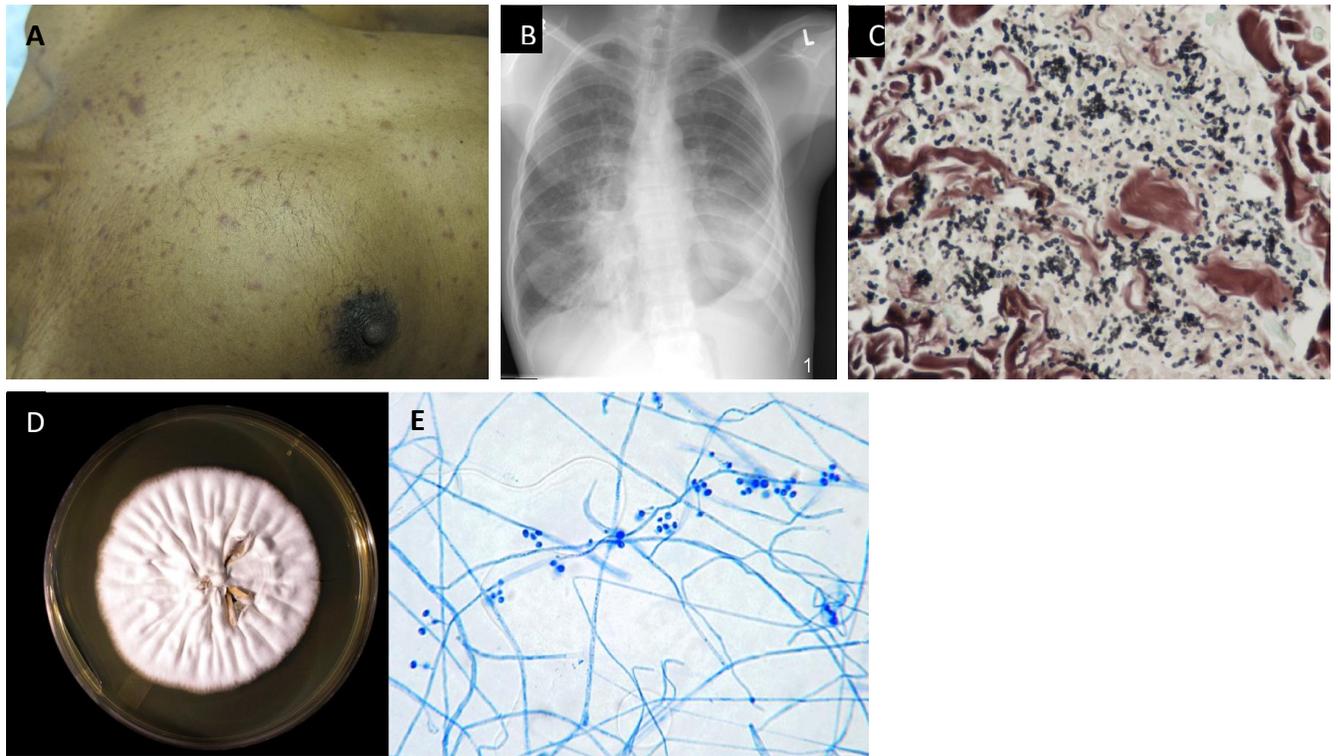
**Figure 4 (A) Typical erythema nodosum skin lesions over forearms and anterior lower extremity associated with coccidioidomycosis, (B) Residual pulmonary nodule from antecedent pulmonary coccidioidomycosis, (C) Macroscopic appearance of *Coccidioides* on routine fungal media, (D) arthroconidia visible with lactofuchsin staining (40x), (E) *Coccidioides* spherule containing numerous endospores (40x).**

**Courtesy of Dr. George Thompson and Dr. Bridget Barker**

Figure 5. Map of endemic region for emergomyces



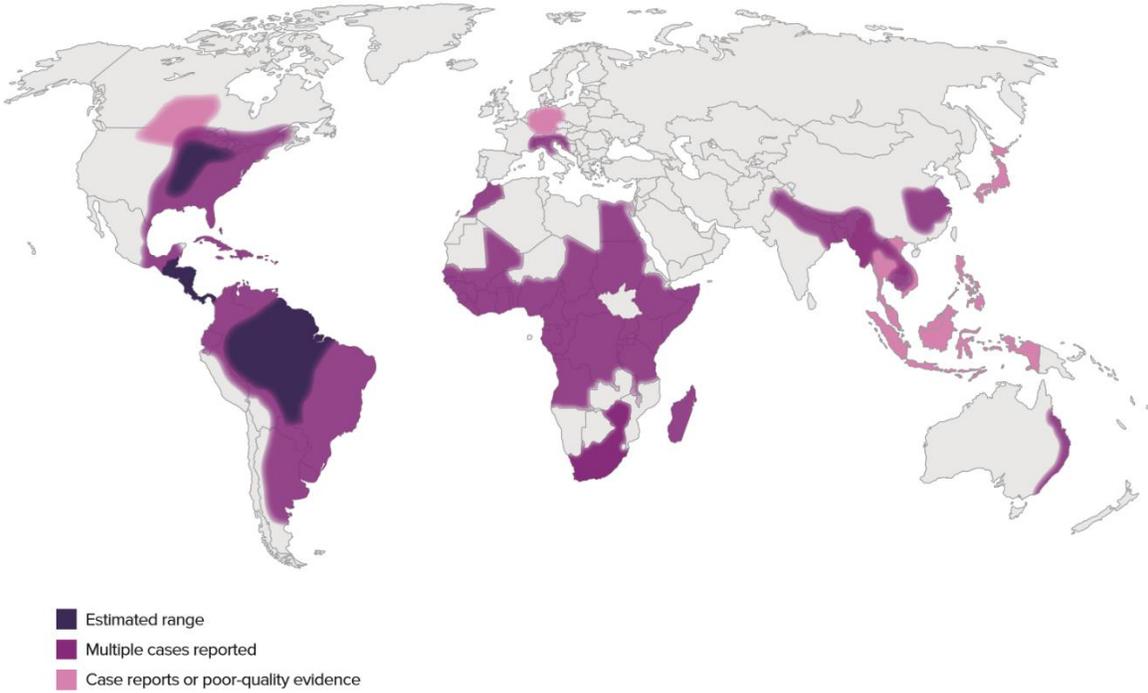
**Figure 6. Clinical and microbiologic findings of emergomycosis**



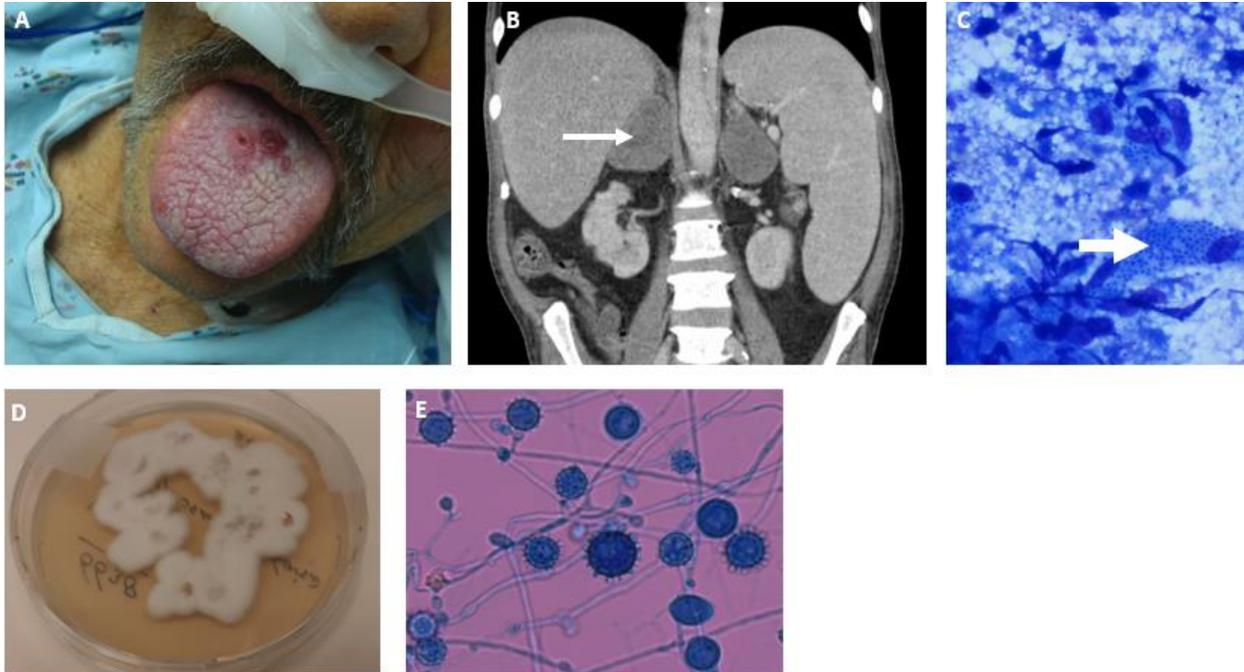
**Figure 6. (A) Erythematous papules from disseminated emergomycosis, (B) Chest X-ray findings in pulmonary emergomycosis, (C) Grocott silver methenamine staining of tissue biopsy (40x), (D) *Emergomyces pasteurianus* cultured on Sabouraud dextrose at 30°C after 4 weeks, (E) Typical appearance of florets of conidia on short secondary conidiaphores (*E. pasteurianus*) (400x)**

Courtesy of Dr. Ilan Schwartz and Dr. Yuri Amatnieks

**Figure 7. Map of endemic region for histoplasmosis**



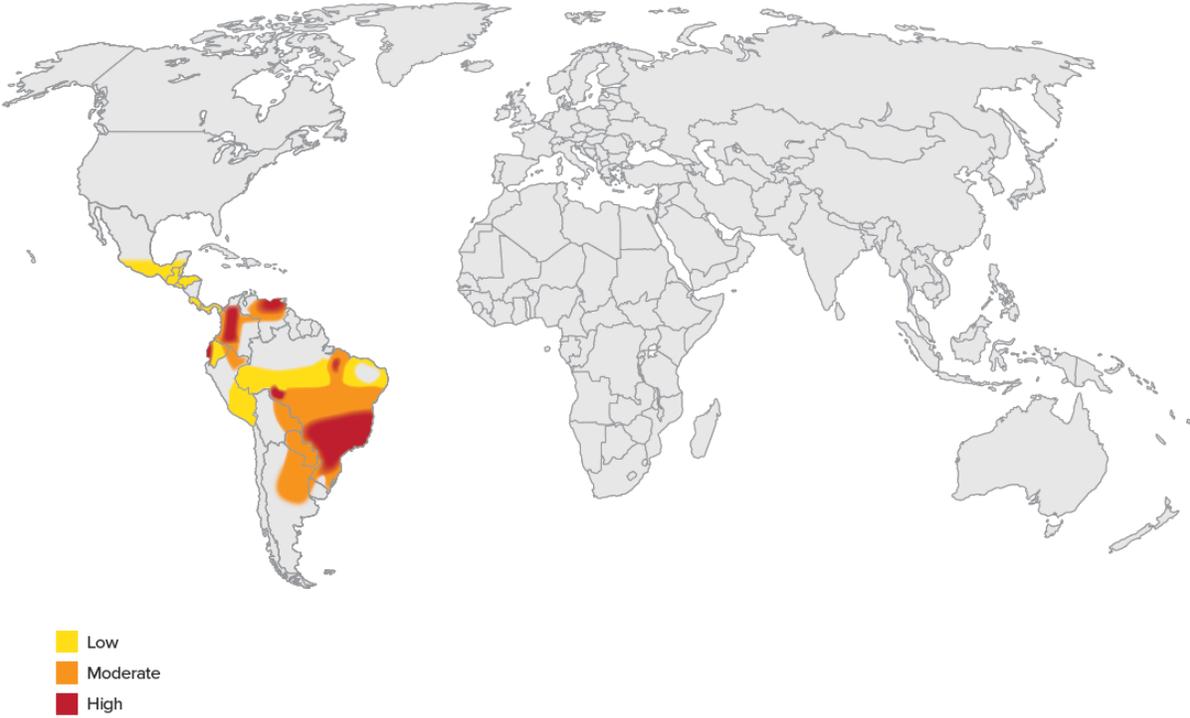
**Figure 8. Clinical and microbiologic findings of histoplasmosis**



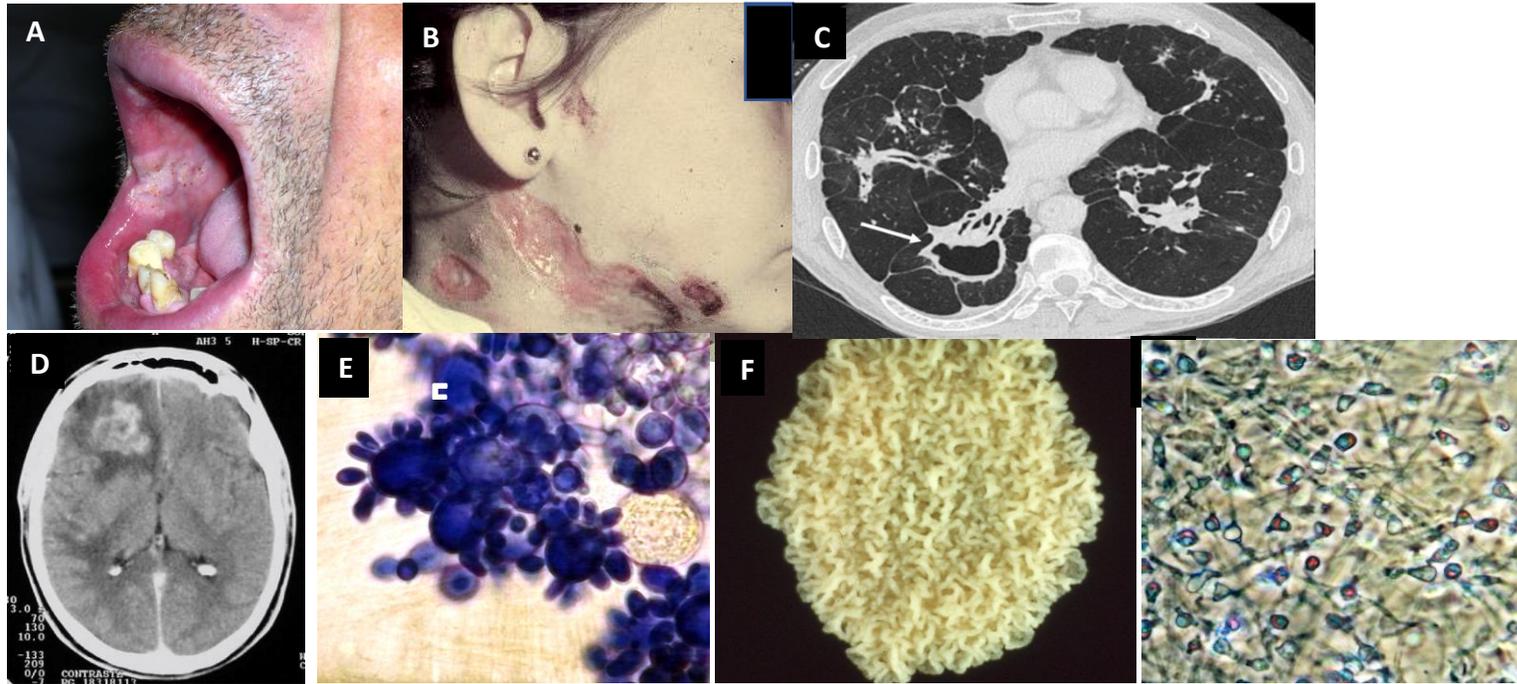
**Figure 8 (A) Typical oral ulcerations of histoplasmosis (tongue lesion), (B) Adrenal enlargement (thin white arrow) seen in disseminated histoplasmosis, (C) Morphologic features of *Histoplasma* in tissue (10x), (D) on routine fungal media, (E) and macro- and microconidia (400x).**

**Courtesy of Dr. John Baddley, Dr. Bert Gerrits van den Ende, and Dr. Maria Serna**

**Figure 9. Map of endemic region for paracoccidioidomycosis**



**Figure 10. Clinical and microbiologic findings of paracoccidioidomycosis**

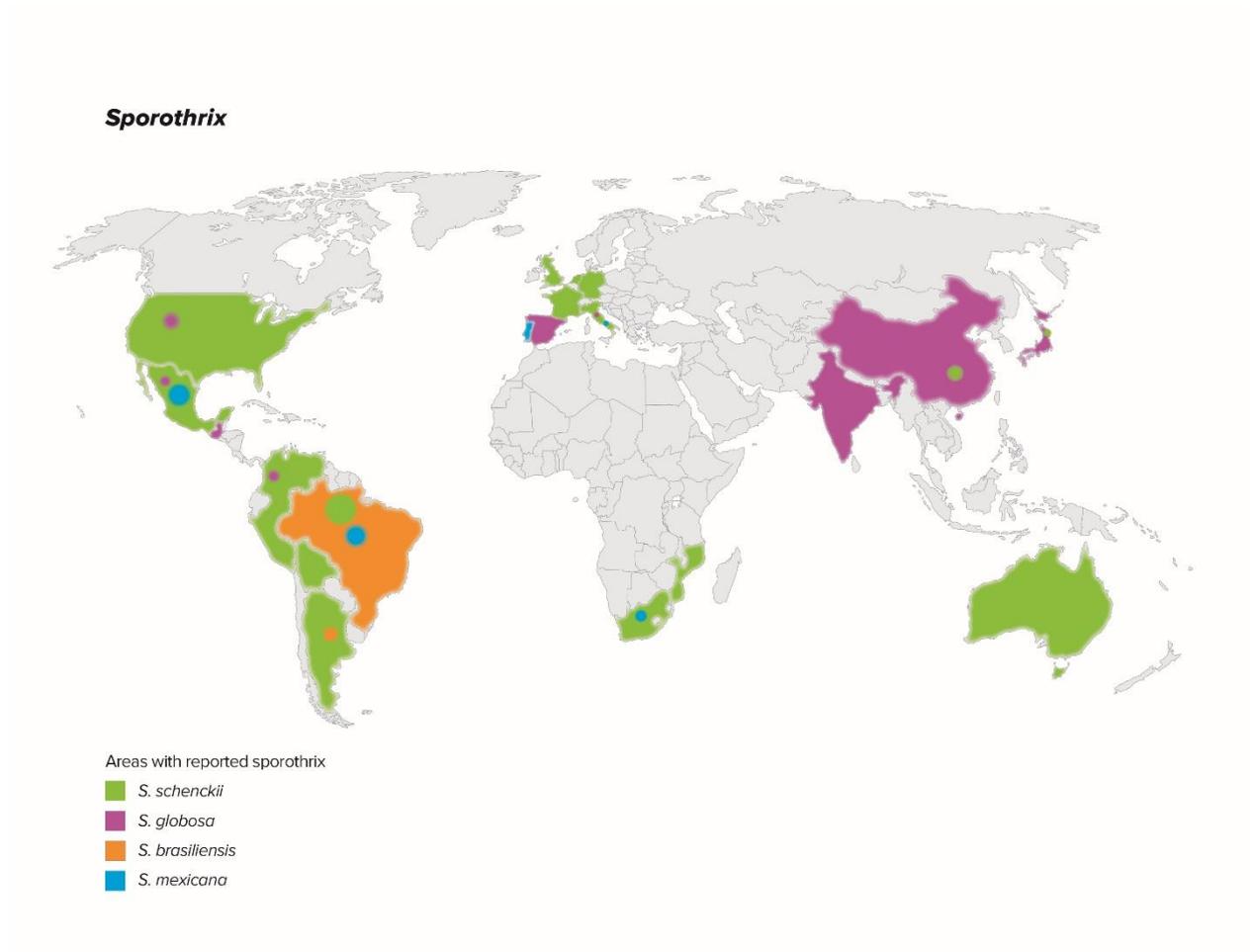


**Figure 10 (A) Typical ulcerative lesions observed in chronic paracoccidioidomycosis, or (B) skin with abscessed lymphatic involvement in a child. (C) Cavitory lung disease seen in some cases of pulmonary paracoccidioidomycosis. (D) CNS lesion secondary to disseminated paracoccidioidomycosis with surrounding cerebral edema. (E) Lactophenol cotton blue staining of a smear obtained from lymph node aspirate from a patient with acute paracoccidioidomycosis (400x) (F) Yeast phase of *Paracoccidioides brasiliensis* with a classic yellow cerebriform colony at 37°C. (G) Mycelial phase of *P. brasiliensis* cultivated in agar soil, showing hyphae and conidia. The sample was collected using adhesive tape and stained with cotton blue (400x).**

Courtesy of Dr. Flavio Telles, Dr. Arnaldo Colombo, and Dr. Eduardo Bagagli

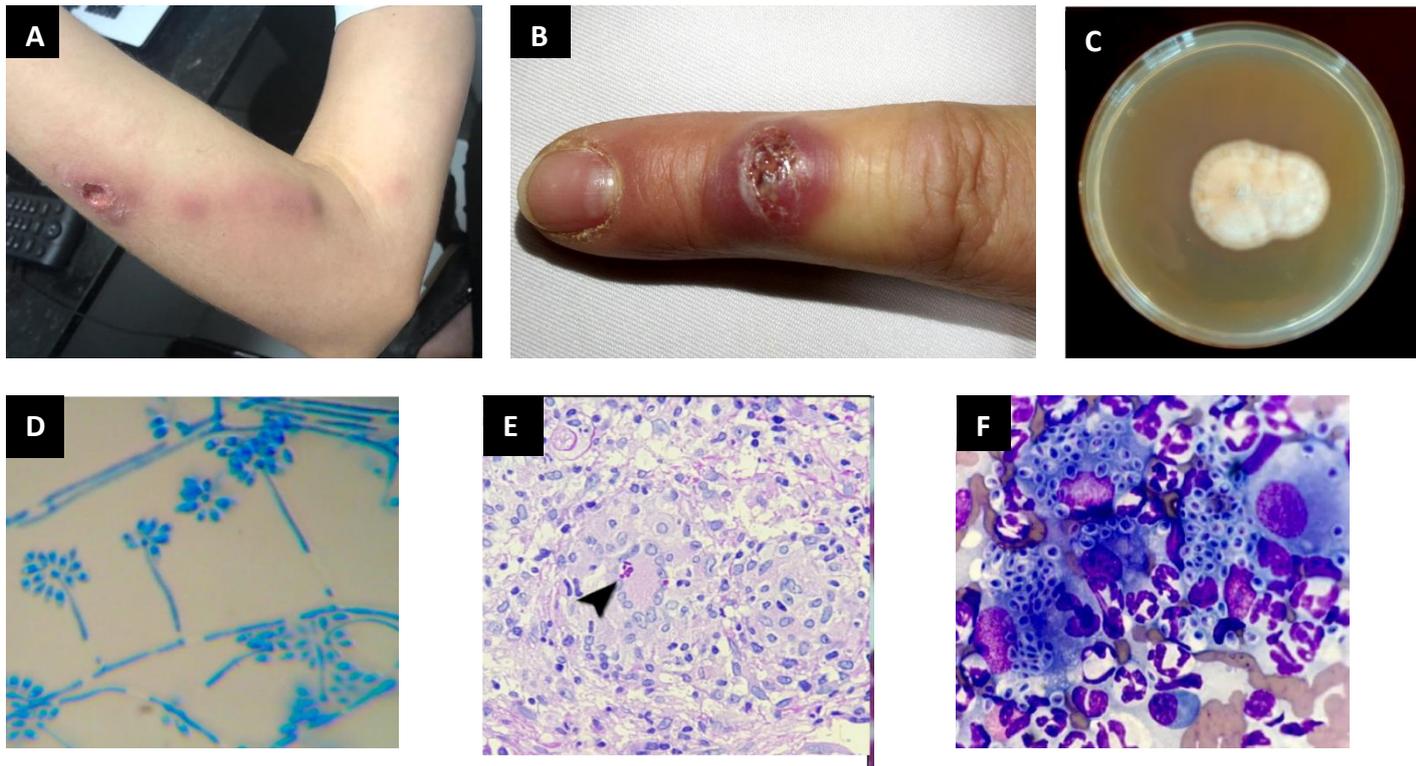


1 **Figure 11. Map of endemic region for sporotrichosis**



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16 **Figure 12. Clinical and microbiologic findings of sporotrichosis**



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18 **Figure 12 (A) Typical cutaneous “sporotrichoid” pattern of spread from a distal lesion,**  
19 **(B) Cutaneous fixed sporotrichosis after a cat scratch infected with *Sporothrix brasili-***  
20 ***ensis*, (C) Mycelial phase of *Sporothrix schenckii* - slow growth of a whitish colony at**  
21 **room temperature on standard fungal media, (D) Slide culture of the mycelial phase of**  
22 **phase of *Sporothrix spp*, showing thin, hyaline, septate hyphae with sympodial conidi-**  
23 **ogenesis, (E) A skin biopsy from a patient with cutaneous sporotrichosis, depicting an**  
24 **exudative and granulomatous infiltrate with cigar shape and round yeast cells (arrow-**  
25 **head), in multinucleate giant cells of the Langerhans type. (Periodic Acid-S, 100x), (F)**  
26 **Direct exam of smear of ulcerated cutaneous lesion depicting a great number of yeast**  
27 **cells of *Sporothrix brasiliensis*, with round to oval and cigar-shaped forms of the fungus**  
28 **(Giemsa stain, 400x)**

29 **Courtesy of Dr. Flavio Telles**

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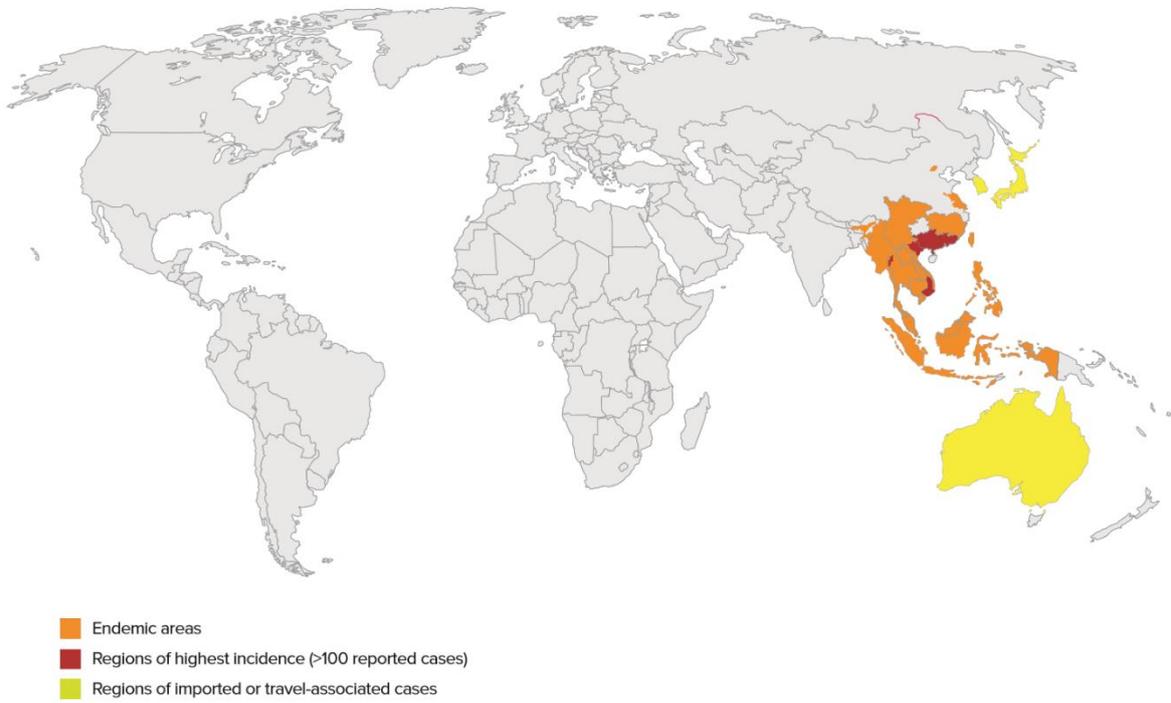
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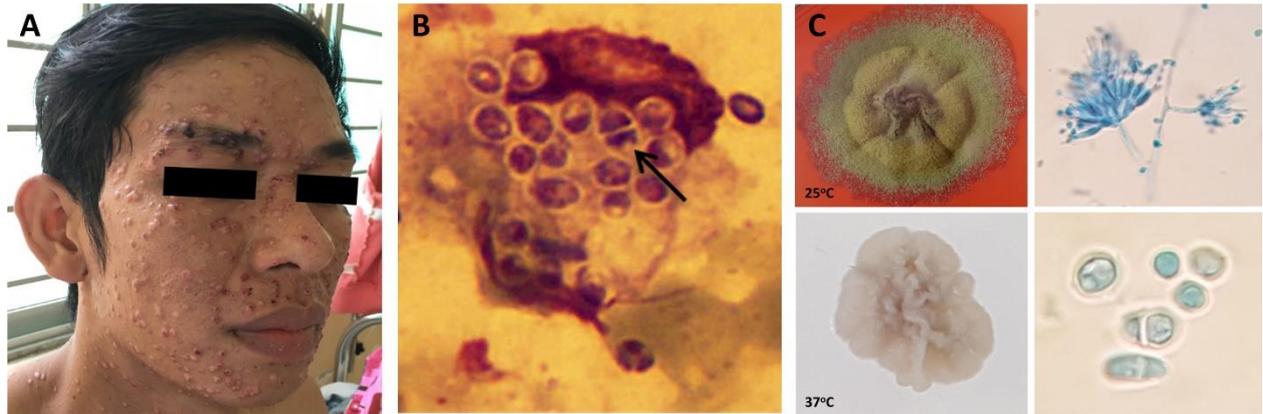
38 **Figure 13. Map of endemic region for talaromyces**



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53 **Figure 14. Clinical and microbiologic findings of talaromycosis**

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57 **Figure 14 (A) Typical central umbilicated skin lesions in a patient with HIV-associated**  
58 **talaromycosis, (B) *Talaromyces marneffii* yeast cells from a Giemsa-stained touch skin**  
59 **smear seen under the microscope (60x). The arrow shows the cross-wall formation seen**  
60 **in a dividing yeast cell. (C) Morphologic and microscopic characteristics of *T. marneffii***  
61 **isolated on Sabouraud dextrose agar. The fungus grows as a mold, producing a charac-**  
62 **teristic deep red diffusible pigment, with spore-bearing structures called conidiophores**  
63 **and conidia at 25°C (40x), and grows as a yeast, producing wrinkled surface, with sin-**  
64 **gle-celled arthroconidia (60x) that divides by binary fission at 37°C (1000x).**

65 **Courtesy of Dr. Thuy Le**

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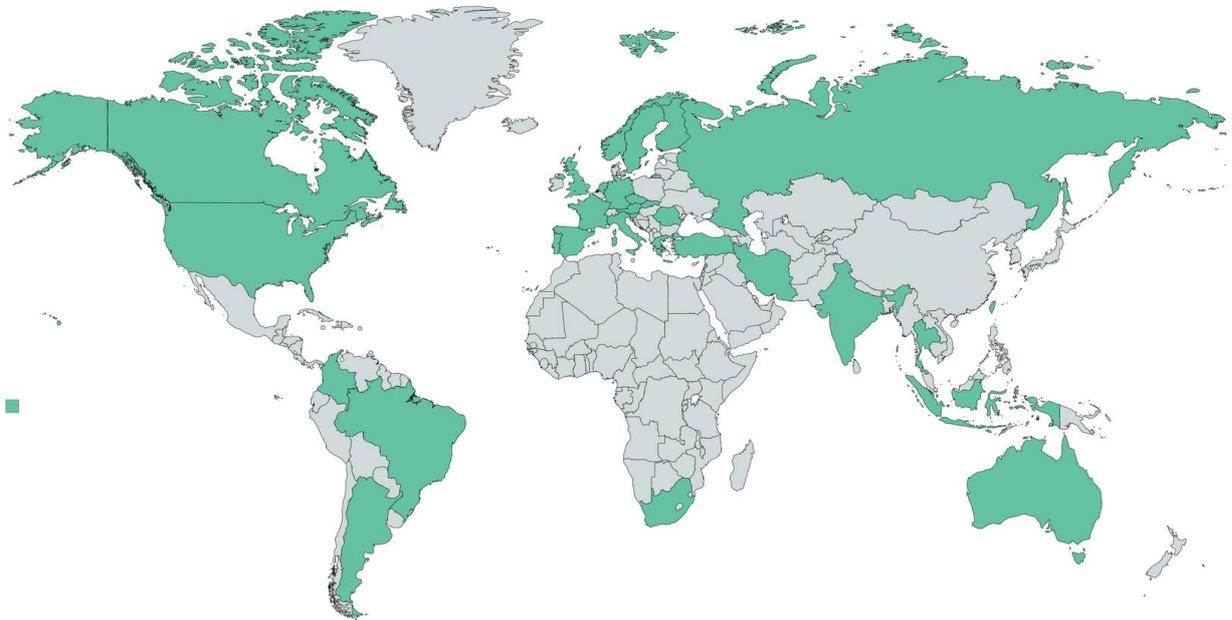
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80 *Supplemental Figure 1. National societies endorsing the Endemic Mycoses Guideline.*

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**Society**

Medical Mycology Society of Nigeria  
Subcomisión de Micología Clínica at the Asociación Argentina de Microbiología  
Indian Society of Medical Mycologist FORMERLY SIHAM Society for Indian Human and Animal Mycology  
ISMM Iranian Society of Medical Mycology  
Medical Mycology and Infectious Diseases Society of Pakistan  
Malaysian Society of Infectious Diseases and Chemotherapy  
Indonesia Society for Medical Mycology  
IDAT Infectious Diseases Society of Thailand with TMMF Thai Medical Mycology Forum  
LSIDCM Lebanese Society of Infectious Diseases and Clinical Microbiology  
Turkish Febrile Neutropenia Society  
Turkish Society of Medical Mycology  
Czech Society for Medical Microbiology (API)  
RSMMM Romanian Society of Medical Mycology and Mycotoxicology  
IACMAC Russian Interregional Association for Clinical Microbiology and Antimicrobial Chemotherapy  
Slovak Society of Chemotherapy  
Finnish Society for Medical Mycology  
Swedish Society for Clinical Mycology  
HSMM Hellenic Society of Medical Mycology  
FIMUA Federazione Italiana Micologia Umana e Animale  
SEIFEM sorveglianza Epidemiologica Infezioni nelle Emopatie  
ASPOMM Portuguese Association of Medical Mycology  
AEM Asociación Española de Micología, Sección de Micología Médica  
ÖGMM Austrian Society for Medical Mycology  
SFMM French Society for Medical Mycology  
NVMy Dutch Society for Medical Mycology  
Asociacion Colombiana de Infectologia-ACIN  
Brazilian Society of Infectious Diseases (Sociedade Brasileira de Infectologia - SBI):  
Asociación Panamericana de Infectología (API):  
Brazilian Society of Microbiology (Sociedade Brasileira de Microbiologia):  
Asociación Iberoamericana de Micología Médica  
INFOCUS  
Euro-Asian Society for Infectious Diseases (EASID)  
Israeli Society for Infectious Diseases